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# Assessing gas exchange, sap flow and water relations using tree canopy spectral reflectance indices in irrigated and rainfed *Olea europaea* L.



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## ABSTRACT

Diurnal and seasonal trends of leaf photosynthesis ( $A$ ), stomatal conductance to water ( $g_s$ ) and water potential ( $\Psi_1$ ), whole-plant transpiration and tree canopy spectral reflectance indices were evaluated in rainfed and well-watered (control) mature olive (*Olea europaea* L., cv. Leccino) trees. The objective was to evaluate whether photochemical reflectance index (PRI), water index (WI) and normalized difference vegetation index (NDVI) could be used for detecting plant functioning in response to seasonal drought. The measurements were made from March to November, repeated every four weeks during the drought period of the growing season. Rainfed trees were subjected to prolonged water deficit with soil water content ranging between ~30% and 50% than that of control. Consequently, there were significant differences in the diurnal trend of  $\Psi_1$ ,  $A$ ,  $g_s$  and sap flux density between treatments. Under severe drought,  $\Psi_1$  ranged between ~-4.5 MPa (predawn) and ~-6.4 MPa (midday),  $A$  ranged between maximum morning values of ~6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and minimum late afternoon values of 2.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $g_s$  was lower than ~0.03  $\text{mol m}^{-2} \text{s}^{-1}$  for most of the daily courses, whereas stem sap flux density reached maximum peaks of 2.1  $\text{g m}^{-2} \text{s}^{-1}$  in rainfed plants. The diurnal trends of all these parameters fully recovered to the control level after autumn rains. PRI, NDVI, and WI of olive tree canopy assessed significantly the effects of drought on rainfed trees and their subsequent recovery. PRI resulted better correlated with  $A$  ( $r^2 = 0.587$ ) than with the other measured parameters, pooling together values measured during the whole growing season. In contrast, NDVI showed a stronger relationship with  $\Psi_1$  ( $r^2 = 0.668$ ) and  $g_s$  ( $r^2 = 0.547$ ) than with  $A$  ( $r^2 = 0.435$ ) and whole-plant transpiration ( $r^2 = 0.416$ ). WI scaled linearly as  $g_s$  and  $\Psi_1$  increased ( $r^2 = 0.597$  and  $r^2 = 0.576$ , respectively) and, even more interestingly, a good correlation was found between WI and whole-plant transpiration ( $r^2 = 0.668$ ) and between WI and  $A$  ( $r^2 = 0.640$ ). Overall PRI and WI ranked better than NDVI for tracking photosynthesis, whereas WI was the most accurate predictive index of plant water status and whole-plant transpiration. This study, which is the first to our knowledge that combines diurnal and seasonal trends of leaf gas-exchange, whole-plant transpiration and reflectance indices, clearly shows that PRI and WI measured at the tree canopy can be used for fast, nonintrusive detection of water stress.

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## 1. Introduction

Water is the most limiting resource in the Mediterranean region, where the climate is typically characterized by high potential evaporation and low and highly variable rainfall during the growing season. The agricultural sector is the largest water consumer accounting for about 70% of all extracted water (Gilbert,

2012). In the Mediterranean regions, agriculture consumes on average about 65% of total water abstraction (Simonet, 2011). Climate change, which is increasing the chronic water scarcity in the Mediterranean basin (Dai, 2010), together with rapidly growing demand of water for industrial and urban uses, is likely to put under unprecedented pressure the limited water resources for agriculture. Therefore, the major need for development of irrigation is to save substantial water through improved irrigation management and increased water productivity (Fereses et al., 2011).

Optimization of irrigation requires the retrieval of real time crop condition and its sensitivity to water stress, which, in turn, results

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from specific physiological status, soil–water availability, climatic conditions (Centritto et al., 2000; Tognetti et al., 2009). Water deficit constraints all the physiological processes involved in plant growth and development. These changes are part of a cascade of responses to drought affecting primary processes including tissue water relations and gas exchange mechanisms (Alvino et al., 1994; Magnani et al., 1996; Aganchich et al., 2009). It is of paramount importance, consequently, to improve non-invasive phenotyping methods to monitor water relations and photosynthetic status in plants experiencing water stress (Loreto and Centritto, 2008; Centritto et al., 2009). Continuous recording of sap flow rate might provide indirect measurements of plant water status, and represent a promising tool for the development of monitoring systems in olive tree plantations to determine irrigation needs in real time, or at least at frequent intervals, and for being integrated with remote sensing techniques in precision irrigation management and control (Fernández et al., 2008).

Major progress has been made with the use of remotely sensed vegetation indices to assess physiological traits associated with plant water status (Peñuelas and Filella, 1998; Sun et al., 2008; Garbulsky et al., 2011). The methodology is based on a number of visible (Vis)- and near infrared (NIR)-based indices as indicators of photosynthetic activity (Gamon et al., 1997) and water status (Sun et al., 2008; Elsayed et al., 2011). The photochemical reflectance index, which was originally developed to estimate rapid changes in the xanthophyll (Gamon et al., 1992), is increasingly used to assess changes in the efficiency of photosynthetic activity (Garbulsky et al., 2011) at different plant scale level (Gamon et al., 1997; Garbulsky et al., 2008; Naumann et al., 2008; Suárez et al., 2008). Relationships between photosynthetic parameters and PRI determined at canopy level have been reported in studies performed on grassland, sunflower, grapevine, and olive (see Garbulsky et al., 2011 for a review). Similarly, the NIR-based water index (WI) is increasingly employed for monitoring plant water status (Peñuelas et al., 1993; Peñuelas and Filella, 1998). Whereas, the normalized difference vegetation index (NDVI), which is based on different radiation absorption by green biomass in Red and NIR wavebands (Rouse et al., 1973), is widely used for the assessment of the green plant biomass at ground, airborne and satellite levels (Peñuelas and Filella, 1998). Further indications about the physiological status of plant can be obtained by the evaluation of the photosynthetic pigment composition. For this purpose, Chlorophyll Index (CI) and Structural Independent Pigment Index (SIPI) were developed to assess chlorophyll concentration and carotenoids/chlorophyll ratio, respectively (Gitelson and Merzlyak, 1994; Peñuelas and Filella, 1998).

Olive (*Olea europaea* L.) is a drought tolerant species which has been traditionally cultivated in agricultural rainfed systems in the Mediterranean basin. However, to promote olive fruit production and its economic competitiveness, there has been a large increase in the amount of irrigation water used in olive farming over the past years. To detect water needs, in order to increase water productivity through the management of precision irrigation (Ferreira et al., 2011), studies have been recently performed on olive potted plants for remote sensing of water stress using rapid spectral reflectance measurements of leaf water status and photosynthetic limitations (Sun et al., 2008; Sun et al., unpublished data). In the present work, photosynthesis, whole-plant transpiration and spectral reflectance indices were measured in mature olive trees under rainfed conditions and in irrigated control. Furthermore, the effects of water deficit on specific leaf area, pigment and nitrogen concentration were evaluated. The aim of this work was to evaluate whether PRI, WI and NDVI could track rapid changes in plant functioning also in field-grown plants subjected to seasonal drought and if CI and SIPI could detect variations occurred in pigment composition.

## 2. Materials and methods

### 2.1. Field conditions and plant material

The experiment was performed at the “Santa Paolina” experimental station of the CNR-IVALSA, located in Follonica, central Italy (42°55′58″ N, 10°45′51″ E, 17 m a.s.l.). The olive orchard used consisted of 10-year-old trees (*O. europaea* L., cv. Leccino) spaced at 4 × 4 m. The soil is sandy-loam (sand 64.2%, silt 16.9% and clay 18.9%). The soil belongs to the Piane del Pecora system and was developed on recent alluvial deposits of river and river-pond nature. The depth of the soil was 3 m. The climate is of Mediterranean type with hot and dry seasons from April to September and cold winters. In the three growing seasons preceding the experiment, all trees were equally irrigated with microsprayers located 30 cm from the trunk irrigating an area of soil with a ray of 1.25 m to guarantee the uniformity of plant development. Two irrigation treatments (rainfed and well-watered control) were applied starting from Mid-May of 2011. The amount of water supplied to the control trees was estimated weekly by calculating reference evapotranspiration according to the Penman-FAO equation and crop evapotranspiration (Doorenbos and Pruitt, 1977) using a crop coefficient of 0.5 as reported by Gucci (2003) for the same area and plantation. The coefficient of ground cover was 0.8, according to tree size. The volume of the irrigation water was then adjusted in order to keep predawn leaf water potential ( $\Psi_1$ ) around  $-0.5$  MPa. The irrigation period lasted from mid-May to late October 2011; each of the control trees received an average of 3900 L of water. During this period the precipitation was 125 mm with a peak on July (51.6 mm).

### 2.2. Meteorological data

Climate data were recorded every 15 min by a standard meteorological digital station placed at 100 m from the orchard (quality-controlled data were supplied by the Laboratory of monitoring and environmental modeling for the sustainable development, Florence, <http://www.lamma.rete.toscana.it/en>). The variables measured were air temperature ( $^{\circ}$ C), precipitations (mm) and relative humidity (RH, %).

### 2.3. Soil water content measurements

Volumetric soil moisture content was measured using Terasense SMT2 soil moisture sensors (model PS-0077-DD, Netsens s.r.l. Florence, Italy) installed in the middle of the irrigation ray at an average distance of 90 cm from the trunk and at 10 cm and 30 cm depths in each plot. Soil moisture content was acquired every 15 min with a Netsens communication platform based on a GPRS integrated main unit, a wireless units and LiveData<sup>®</sup> software for data storage and elaboration.

### 2.4. Sap flow measurements

Granier-type sensors (Granier, 1985) were inserted radially into 20 mm depth of the stem at the height of  $\sim 1.3$  m in six plants. These sensors consisted of a pair of copper-constant thermocouples of the same diameter vertically spaced of  $\sim 15$  cm. The upper probe was continuously heated through a heating wire supplied with a constant power source (120 mA). The temperature difference of the two probes was recorded to obtain the sap flux density, as derived empirically (Granier, 1987; Huang et al., 2009). Sap flux density ( $F_d$ ,  $\text{g H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) was monitored using self-made thermal dissipation probes (SF-L sensor) (Granier, 1987):

$$F_d = \alpha K^{\beta}$$

where  $\alpha$  and  $\beta$  are parameter values, 119 and 1.231, respectively, and  $K$  is the dimensionless sap velocity index:

$$K = \frac{\Delta T_{\max} - \Delta T}{\Delta T}$$

where  $\Delta T_{\max}$  is the temperature difference between the heater and reference probe at zero flow (i.e., measured predawn when  $F_d$  is assumed negligible) and  $\Delta T$  is the temperature difference at any given measurement point.

### 2.5. Gas exchange measurements

Gas exchange measurements were made monthly from March to November 2011. Diurnal courses of photosynthesis ( $A$ ) and stomatal conductance to water ( $g_s$ ) were measured on five leaves for each plant. The measurements were made on the central section of a newly expanded sunny leaf using a LI-6400-40 leaf chamber fluorometer (Li-Cor, Inc., Nebraska, USA). Leaves inside the cuvette were exposed to a flux of ambient air, with  $\text{CO}_2$  concentration fixed at  $385 \mu\text{mol mol}^{-1}$ . Air temperature and relative humidity were maintained close to ambient values. Radiation intensities, provided by a red-blue light diode source, were equal to the photosynthetic photon flux density (PPFD) levels measured at the leaf proximity with the LI-6400 PPFD sensor.

### 2.6. Leaf water potential measurements

In March, and from August to November, daily trends of leaf water potential ( $\Psi_l$ ) were measured in parallel with the gas exchange measurements. From predawn until late afternoon, five sunny-leaves per olive tree were detached from mid canopy and rapidly enclosed in a Scholander-type pressure chamber (SKPM 1400, Skye Instruments, Llandrindod Wells, UK).

### 2.7. Tree spectral reflectance analysis

Simultaneously with the gas exchange measurements, spectral reflectance of individual trees was measured by using a portable spectrometer (ASD FieldSpec 3, Analytical Spectral Devices Inc., USA), operating in the spectral range between 350 and 1025 nm with an average spectral resolution of 3 nm (Full-Width-Half-Maximum) and a sampling interval of 1.4 nm. Measurements were always made in clear weather conditions. Fiber optic cables, providing a field of view edge of  $25^\circ$ , were mounted on movable arms and connected to the spectroradiometer to collect reflectance spectra in nadir direction, from a distance of 1.8 m above the tree. Three measurements, taken from different fixed positions of the movable arm over each tree, were averaged to estimate the spectral response at the whole-tree scale and, thus, of the single tree canopy. The reflectance of a  $1 \times 1$  m polystyrene panel, covered with a mixture of barium sulfate powder and white paint, was taken as white standard before every reflectance measurement (Knighton and Bugbee, 2004). Scaffolds positioned behind the shaded side of the trees were used to approach the top of the canopy to perform the measurements. ViewSpecPro (ASD) software was used to pre-processing reflectance spectra and then reflectance indices were derived.

Water Index (WI) was calculated as  $R_{900}/R_{970}$ , where  $R$  is the reflectance at the wavelength indicated in the sub-indices (Peñuelas and Filella, 1998). The narrow band version of Normalized Difference Vegetation Index (NDVI) defined by Sims and Gamon (2003) was calculated as  $(R_{800} - R_{680})/(R_{800} + R_{680})$ . Photochemical Reflectance Index (PRI) was calculated as  $(R_{531} - R_{570})/(R_{531} + R_{570})$  (Gamon et al., 1992). Whereas Chlorophyll Index (CI) and Structural Independent Pigment Index (SIPI)

were calculated to evaluate differences in chlorophyll concentration and carotenoids/chlorophyll ratio as  $(R_{750} - R_{705})/(R_{750} + R_{705})$  and  $(R_{800} - R_{445})/(R_{800} - R_{680})$ , respectively (Gitelson and Merzlyak, 1994; Peñuelas and Filella, 1998).

### 2.8. Specific leaf area, pigment and nitrogen concentration

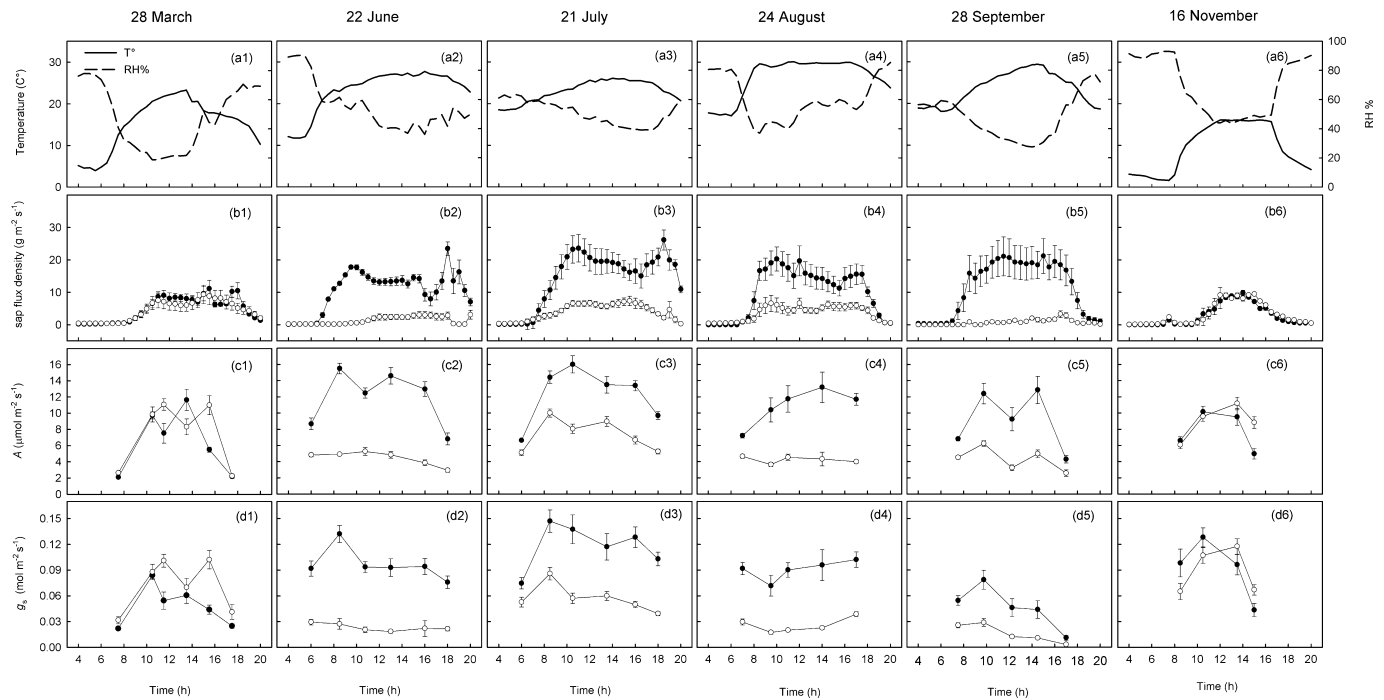
Three leaves per position (higher, medium and lower part of the canopy) and per plant were collected in September, scanned with an image analyser (UTHSCSA Image tool program, University of Texas Health Science Center, San Antonio, USA) to measure leaf area, and then oven-dried at  $80^\circ\text{C}$  for 48 h to determine their dry mass. Specific Leaf Area (SLA,  $\text{m}^2 \text{g}^{-1}$ ) was calculated. Pigments were extracted from  $0.38 \text{ cm}^2$  leaf discs collected with a 7 mm-diameter borer (avoiding veins) in 96% methanol solution in the dark for 96 h (Lichtenthaler, 1987). Chlorophyll *a*, chlorophyll *b* and carotenoids absorbances were determined in the methanol extract at 663, 646 and 470 nm, respectively using a UV-Vis spectrophotometer (Beckman Coulter Inc., DU 640, Indianapolis, USA). The absorbance values were converted to concentration following the procedure proposed by Lichtenthaler (1987). Approximately 1 g of leaf oven-dried and ground samples were prepared in duplicate for nitrogen analysis. N concentration was measured using the Kjeldahl method (Black et al., 1965).

### 2.9. Statistics

The experimental design was a complete randomized block replicated three times. Measurements were performed on three trees (one tree per block) per treatment. All statistical analyses were conducted using Sigma Plot 12.0 software (Systat Inc., San Jose, CA, USA) and SPSS 15.0 (SPSS Inc., Chicago, USA). Data were tested comparing treatment means using *t*-test. Treatment and time effects on pigment concentrations and pigment-based reflectance indices were tested using simple factorial ANOVA and repeated measures analysis of variance. Simple regressions were calculated to analyze relationship between reflectance indices and physiological parameters, determining coefficients of determination and significance levels. Regression curves were fitted to develop empirical models based on linear relationships using repeated random sub-sampling of 80% of each measured gas-exchange and water relation parameter as training data, whereas the remaining sub-sampling of 20% was retained as tested data. Then the root-mean-square error (RMSE) was calculated as a measure of the difference between the observed and the predicted values of the physiological parameters.

## 3. Results

The measurements were made in representative days of the growing season 2011, which was characterized by low amounts of rainfall accompanied by days of high air temperature and low relative humidity (Fig. 1a). The diurnal courses of air temperature and relative humidity changed over the growing season. Maximum air temperature did not exceed  $23^\circ\text{C}$  in March (Fig. 1a1) and  $16^\circ\text{C}$  in November (Fig. 1a6), whereas air temperature reached maximum values in August and September (about  $30^\circ\text{C}$ ). The minimum values of relative humidity ( $\sim 19\%$ ) were recorded at midday in March and September ( $\sim 27\%$ ). In general, the diurnal changes of both temperature and relative humidity indicate that the maximum values of vapour pressure deficit were reached between 9:00 and 17:00 during the summer months. Soil water content was strongly affected by precipitation regime from June to September (Table 1) and, consequently, rainfed olive trees were subjected to prolonged water deficit. In rainfed conditions, the lowest SWC values, i.e.,  $\sim 30\%$  compared to the control treatment, were recorded in June, and remained about 50% lower than those of control over the remaining



**Fig. 1.** Daily course of (a1,6) air temperature and relative humidity, (b1,6) sap flux density, (c1,6) photosynthetic rate (*A*) and (d1,6) stomatal conductance to water (*g<sub>s</sub>*) measured in well-watered (●) and rainfed (○) *Olea europaea* plants during the growing season. Data are mean of three plants per treatment ± 1 SEM; *A* and *g<sub>s</sub>* were measured on 5 sunny leaves per plant.

summer period and at the beginning of autumn. Furthermore, significant differences in SWC at the top layer were also recorded in March and in November, despite no differences were observed between the two irrigation treatments in SWC of deeper soil layers as a result of the rains that occurred before these dates.

There were no significant differences in the diurnal trend of sap flux density between control and rainfed plants at the beginning of spring and in autumn (Fig. 1b1 and b6). Furthermore, the maximum stem sap flux density reached values as low as  $9.5 \text{ g m}^{-2} \text{ s}^{-1}$  in March and November (Fig. 1b1 and b6), suggesting that the weather conditions limited water loss. In contrast, the daily course of sap flux density was significantly higher ( $P < 0.001$ ) in control than in rainfed plants over the summer period and at the onset of autumn (Fig. 1b2–5). In June and July, stem sap flux density started increasing between 6:00 and 7:00 h and peaked at about 9:00–10:00 h in control plants. Then during the next four–five hours, stem sap flux density dropped progressively to minimum values which occurred at 16:00 h. This depression was then followed by a second daily maximum, reached at 18:00 h, as temperature declined and RH increased in the late afternoon. Finally, as light intensity further declined toward the end of the day also stem sap flux density dropped until whole-plant transpiration

approached zero. A similar, although less pronounced trend in stem sap flux density was also recorded in August in control plants. Whereas at the beginning of autumn, stem sap flux density did not vary markedly after reaching the morning peak, indicating that there was no “midday” drop in whole-plant transpiration in control plants. The daily maximum sap flux density recorded over the summer period in control ranged between  $20$  and  $30 \text{ g m}^{-2} \text{ s}^{-1}$ . In rainfed conditions, on the contrary, whole-plant transpiration was very low over the summer period and at the onset of autumn. Stem sap flux density showed minimum diurnal evolution in June and September (with maximum peaks of  $2.6$  and  $2.1 \text{ g m}^{-2} \text{ s}^{-1}$ , respectively) and higher daylight evolutions in July and August, when maximum peaks of  $\sim 7 \text{ g m}^{-2} \text{ s}^{-1}$  were reached.

Similarly to the diurnal trend of sap flux density, overall significant differences in the daylight variation of photosynthesis (*A*) (Fig. 1c) and stomatal conductance to water (*g<sub>s</sub>*) (Fig. 1d) between irrigation treatments were found only under drought conditions (i.e., over the summer period and at the onset of autumn). In March (Fig. 1c1) and November (Fig. 1c6), *A* reached values higher than  $11 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , with little differences between the two irrigation treatments (Fig. 1c1), despite *g<sub>s</sub>* showed higher mean values in rainfed than in control plants in March (Fig. 1d1) and very similar

**Table 1**  
Volumetric soil water content (SWC) of *Olea europaea* under well-watered and rainfed conditions measured at the soil depths of 10 cm and 30 cm during the growing season. Data are mean of four blocks per treatment ± 1 SEM. Asterisks indicate statistically significant differences between the two treatments.

SWC (%)		28 March	22 June	21 July	24 August	28 September	16 November
10 cm soil depth							
Control		26.18 ± 0.35	32.13 ± 0.43	34.24 ± 0.45	34.84 ± 0.47	33.51 ± 0.45	33.73 ± 0.41
Rainfed		18.70 ± 0.25***	9.67 ± 0.13***	18.36 ± 0.24***	16.20 ± 0.22***	14.95 ± 0.20***	30.76 ± 0.45**
30 cm soil depth							
Control		24.21 ± 0.32	33.20 ± 0.44	29.78 ± 0.40	27.74 ± 0.37	20.51 ± 0.27	28.84 ± 0.39
Rainfed		23.43 ± 0.31	10.90 ± 0.44***	15.61 ± 0.21***	13.70 ± 0.18***	12.56 ± 0.17***	29.38 ± 0.38

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

**Table 2**

Pigments concentrations (chlorophyll *a* – Chl *a*, total chlorophyll – Chl tot, carotenoids, carotenoid to chlorophyll *a* ratio – Car/Chl *a*) in well-watered and rainfed *Olea europaea* plants and pigment-based canopy reflectance indices (Chlorophyll Index, CI, and Structure Independent Pigments Index, SIPI) measured in March and in September. Data represent averages of three plants per treatment (nine leaves per plant sampled for pigment analysis)  $\pm$  1 SEM. Letters (a and b) indicate significant differences at  $P < 0.05$  in the same column.

Time	Treatment	Chl <i>a</i> (mg mm <sup>-2</sup> )	Chl tot (mg mm <sup>-2</sup> )	Carotenoid (mg mm <sup>-2</sup> )	Car/Chl <i>a</i>	Chlorophyll index	SIPI
28 March	Control	0.24 $\pm$ 0.14	0.37 $\pm$ 0.23	0.04 $\pm$ 0.02a	0.17 $\pm$ 0.03a	0.400 $\pm$ 0.012ab	1.105 $\pm$ 0.008a
	Rainfed	0.34 $\pm$ 0.16	0.44 $\pm$ 0.12	0.06 $\pm$ 0.01a	0.18 $\pm$ 0.03a	0.436 $\pm$ 0.013b	1.098 $\pm$ 0.005a
28 September	Control	0.46 $\pm$ 0.09	0.56 $\pm$ 0.14	0.13 $\pm$ 0.02b	0.28 $\pm$ 0.02b	0.427 $\pm$ 0.015b	1.111 $\pm$ 0.004a
	Rainfed	0.36 $\pm$ 0.07	0.40 $\pm$ 0.04	0.12 $\pm$ 0.02b	0.33 $\pm$ 0.03b	0.363 $\pm$ 0.020a	1.141 $\pm$ 0.010b

values in November (Fig. 1d6). There were no clear indications of midday depression of both *A* and *g<sub>s</sub>*. Whereas, as expected under drought conditions *A* (Fig. 1c2–5) and *g<sub>s</sub>* (Fig. 1d2–5) dropped dramatically. *A* and *g<sub>s</sub>* registered minimum diurnal trends in June, August and September (when maximum peaks of  $\sim 6 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\sim 0.04 \text{mol m}^{-2} \text{s}^{-1}$  in *A* and *g<sub>s</sub>*, respectively, were recorded) and higher daylight values in July, when *A* and *g<sub>s</sub>* reached maximum values of  $\sim 10 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\sim 0.09 \text{mol m}^{-2} \text{s}^{-1}$ , respectively. In control trees, the maximum values of *A* and *g<sub>s</sub>* ( $\sim 16 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\sim 0.15 \text{mol m}^{-2} \text{s}^{-1}$ , respectively) were recorded at the beginning of summer and in July. Then, *A* slightly declined to maximum daily values of  $\sim 13 \mu\text{mol m}^{-2} \text{s}^{-1}$  in August and September. Whereas a marked decline in maximum *g<sub>s</sub>* values was found in August ( $\sim 0.10 \text{mol m}^{-2} \text{s}^{-1}$ ) and especially in September ( $\sim 0.08 \text{mol m}^{-2} \text{s}^{-1}$ ), in correspondence of rather high diurnal air temperatures and low RH (Fig. 1a4–5), and consequently high VPD values. In general, the diurnal courses of *A* and *g<sub>s</sub>* over the summer period and at the onset of autumn showed a typical maximum in the morning followed by a declining trend in both control and rainfed plants, with the only exception of the values recorded in August which did not show any clear pattern.

The diurnal course of leaf water potential ( $\Psi_1$ ) was significantly affected by time of the year and soil water availability (Fig. 2). In control plants, predawn  $\Psi_1$  ranged between 0.45 and 0.55 MPa over the growing season, whereas midday  $\Psi_1$  decreased on average to a minimum of about  $-1.6$  MPa in March and November (Fig. 2a and d), and to a minimum of  $\sim -2.5$  MPa in August and September (Fig. 2b and c), i.e., when the evaporative demand was higher (Fig. 1a). There were no significant differences in the diurnal trend of  $\Psi_1$  between control and rainfed plants at the beginning of spring (Fig. 2a) and in autumn (Fig. 2d), in absence of water stress. Whereas, the summer drought caused  $\Psi_1$  to decline to very low values from about  $-4.5$  MPa predawn to a minimum of  $\sim -6.4$  MPa around midday in rainfed trees. Similarly, at the beginning of autumn predawn  $\Psi_1$  was about  $-3.7$  MPa in rainfed trees, whereas midday  $\Psi_1$  fell below  $-5.1$  MPa.

Leaf N concentration, which averaged about 2.9% of leaf dry mass, was not affected by irrigation treatment (data not shown). Similarly, no differences were found in SLA in response to irrigation treatment (data not shown).

Leaf pigment concentration were measured in absence of water stress (March), and toward the end of the summer drought period (September). There were no overall effects of irrigation treatment on chlorophyll and carotenoid concentrations as well as on the carotenoid to chlorophyll *a* ratio (Tables 2 and 3). Whereas, carotenoid concentration and, consequently, the carotenoid to chlorophyll *a* ratio resulted significantly higher in September than at the beginning of the growing season. The two pigment-based canopy reflectance indices, CI and SIPI (i.e., a measure of carotenoids to chlorophyll *a* ratio) showed contrasting results. In fact these two indices did not show significant temporal variations (Table 3), whereas in September they resulted significantly affected by water stress (Table 2).

Differently from gas exchange trends, spectral reflectance indices of individual tree canopy did not show any significant

**Table 3**

Repeated measures analysis of variance for pigments concentrations (chlorophyll *a* – Chl *a*, total chlorophyll – Chl tot, carotenoids, carotenoid to chlorophyll *a* ratio – Car/Chl *a*) in well-watered and rainfed *Olea europaea* plants and pigment-based canopy reflectance indices (Chlorophyll Index, CI, and Structure Independent Pigments Index, SIPI) measured in March and in September.

Source	F statistic		
	Time	Treatment	Time $\times$ Treatment
Chl <i>a</i> (mg mm <sup>-2</sup> )	3.176 <sup>ns</sup>	0.0243 <sup>ns</sup>	1.488 <sup>ns</sup>
Chl tot (mg mm <sup>-2</sup> )	0.581 <sup>ns</sup>	0.217 <sup>ns</sup>	1.301 <sup>ns</sup>
Carotenoid (mg mm <sup>-2</sup> )	63.162 <sup>**</sup>	0.0171 <sup>ns</sup>	2.127 <sup>ns</sup>
Car/Chl <i>a</i>	17.844 <sup>*</sup>	0.00705 <sup>ns</sup>	1.359 <sup>ns</sup>
Chlorophyll index	0.566 <sup>ns</sup>	1.723 <sup>ns</sup>	2.308 <sup>ns</sup>
SIPI	3.386 <sup>ns</sup>	2.415 <sup>ns</sup>	0.595 <sup>ns</sup>

\* Significance values are indicated as  $P < 0.05$ .

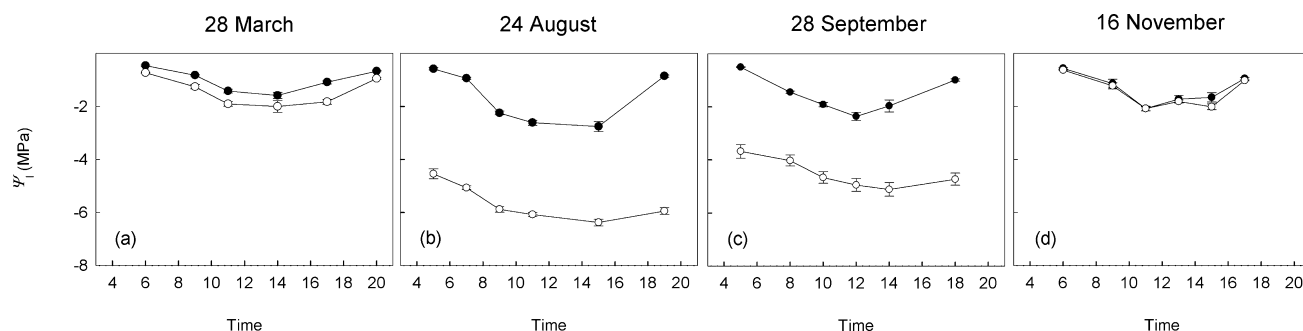
\*\* Significance values are indicated as  $P < 0.01$ .

diurnal variation (data not shown). For this reason, to compare the physiological data with the spectral reflectance indices only data measured during the hours of maximum solar irradiation (i.e., between 10:00 and 16:00 h) were used. There were no differences in PRI, NDVI and WI between irrigation treatments in absence of water stress (March and November) (Table 4). Whereas, these indices were affected by drought, and resulted significantly higher in control than in rainfed trees over the summer period and at the onset of autumn. PRI resulted to be significantly linear related ( $r^2 = 0.587$ ) with photosynthesis after pooling together values measured during the whole growing season (Fig. 3a1). Furthermore, PRI scaled linearly also with *g<sub>s</sub>* and sap flux density (Fig. 3a2 and a4), although this latter relationships were weaker than the former one. In contrast NDVI showed an opposite trend, as NDVI had stronger relationships with stomatal conductance to water ( $r^2 = 0.547$ ) (Fig. 3b2) and above all with leaf water potential ( $r^2 = 0.668$ ) (Fig. 3b2) than with photosynthesis ( $r^2 = 0.435$ ) (Fig. 3b1). It is noteworthy, however, that NDVI values had a high variability in water-stressed plants. Stronger linear relationships were finally found between WI and all measured parameters (Fig. 3c). In fact, WI responded positively as *g<sub>s</sub>* and  $\Psi_1$  increased (Fig. 3c2 and c3), and stronger coefficient of determinations were found between WI and stem sap flux density ( $r^2 = 0.668$ ) (Fig. 3c4). In conclusion, WI assessed better than the other indices the effects of water availability on the photosynthetic activity of olive trees ( $r^2 = 0.640$ ) (Fig. 3c1).

All the physiological parameters estimated using the empirical algorithms obtained, are compared in Fig. 4 with actual measured data. Among the reflectance indices evaluated, WI shows the best accuracy in the estimation of *A*, *g<sub>s</sub>* and mostly of sap flux density (Fig. 4a3, b3 and d3). PRI allowed a sufficiently good estimate of the variation of photosynthetic rate during the growing season (Fig. 4a1).

#### 4. Discussion

We quantified water relations, photosynthesis and whole-plant transpiration in adult olive trees under irrigated or rainfed conditions and used spectral reflectance indices for fast monitoring of olive water status and functioning.



**Fig. 2.** Daily course of leaf water potential ( $\Psi_1$ ) measured in well-watered (●) and rainfed (○) *Olea europaea* plants during the growing season. Data are mean of three plants per treatment (five leaves per tree)  $\pm$  1 SEM.

In our knowledge, only few studies have characterized daily and seasonal gas-exchange and water potential in olive trees in response to water deficit, both on young potted plants (Angelopoulos et al., 1996) and on mature olive trees in field conditions (Moriana et al., 2002; Diaz-Espejo et al., 2007). Our results are overall in keeping with the findings of these previous studies. In fact, we found similar values of  $\Psi_1$ ,  $A$  (Fig. 1c) and  $g_s$  (Fig. 1d) in both well-watered or rainfed conditions. In particular,  $\Psi_1$  (Fig. 2) had a typical pattern in both irrigation treatments, with a maximum values at predawn followed by a continuous decline toward midday and then by a gradual increase toward the end of the afternoon, which reflected the evaporative demand of the atmosphere (Fig. 1a) and was modulated by water deficit (Table 1). In addition, in accordance with previous studies (Angelopoulos et al., 1996; Moriana et al., 2002; Diaz-Espejo et al., 2007), the daily courses of  $A$  (Fig. 1c) and  $g_s$  (Fig. 1d) were affected primarily by SWC (Table 1), and there was also a tendency to reach maximum values in the morning, as PPFD and temperature increased, followed by a midday depression and a slow declining trend in the afternoon in trees subjected to severe drought stress conditions. This is a pattern that typically occurs in trees and crops grown in Mediterranean conditions (Angelopoulos et al., 1996; Moriana et al., 2002). Furthermore, the rainfed values recorded under severe water stress of  $\Psi_1$ , which ranged between  $\sim -4.5$  MPa (predawn) and  $\sim -6.4$  MPa (midday) (Fig. 2), photosynthesis, which ranged between maximum morning values of  $\sim 6 \mu\text{mol m}^{-2} \text{s}^{-1}$  and minimum late afternoon values of  $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 1c) despite  $g_s$  was lower than  $\sim 0.03 \text{ mol m}^{-2} \text{s}^{-1}$  for most of the daily courses (Fig. 1d), are a further indication that olive trees are able to tolerate severe drought stress conditions.

Angelopoulos et al. (1996) showed that the declined in  $A$  under severe water stress was caused by a combination of both stomatal and metabolic limitations. Furthermore, Moriana et al. (2002) found that photosynthesis of severely water-stressed olive trees

did not recover to the control level with the autumn rains, implying that  $A$  was impaired by non-diffusional limitations (Centritto et al., 2005; Diaz-Espejo et al., 2007; Aganchich et al., 2009). Impaired carbon metabolism usually follows  $\text{CO}_2$  transport limitations when environmental stress becomes severe (Cornic, 2000; Centritto et al., 2003). Thus, despite  $A$  of rainfed plants fully recovered to the control level after the autumn rains (Fig. 1c6), it is likely that some degree of metabolic limitations took place under severe drought when  $g_s$  fell well below  $\sim 0.03 \text{ mol m}^{-2} \text{s}^{-1}$  (Fig. 1d2,5), indicating that the amount of soil water available to support plant transpiration was extremely limited (Centritto et al., 2011a).

The good correspondence between  $g_s$ ,  $\Psi_1$  and SWC across treatments suggested that olive trees were able to restrict, at least partially, water loss by closing stomata. The difference between predawn and midday  $\Psi_1$  tended to increase with decreasing soil water availability (and soil water potential) because of a combination of moderate stomatal regulation of transpiration rate and the usually higher transpiration demand in drier periods. Indeed, these olive trees presented anisohydric behavior in relation to soil drought. Anisohydric species tend to occupy more drought-prone habitats compared with isohydric species and have xylem that is more resistant to negative water potential (Centritto et al., 2011b). Olive tree water use was likely to be largely controlled by the diffusion or adiabatic term of the Penman–Monteith equation for evapotranspiration, the transpiration being strongly coupled with the surrounding air (namely VPD) and radiation environment. Diurnal sap flow patterns showed a step morning increase, more evident under good soil moisture conditions, probably related to woody tissues water capacitance (Tognetti et al., 2009). Nevertheless, water stored in woody tissues was not sufficient to maintain transpiration in rainfed trees, and sap flow diurnal patterns showed a pronounced reduction throughout summer (Fig. 1b). This reflected soil water depletion without supplement irrigation, which was, instead, effective in countering water deficit in control trees, at least under these

**Table 4** Photochemical Reflectance Index (PRI), Normalized Difference Reflectance Index (NDVI) and Water Index (WI) of *Olea europaea* under well-watered and rainfed conditions measured during the growing season. Each data represent measurements made between 10:00 and 16:00 h on individual tree canopy (at least 3 per plant) and averaged across three plants per treatment  $\pm$  1 SEM. Asterisks indicate statistically significant differences between the two treatments.

Time	28 March	22 June	21 July	24 August	28 September	16 November
PRI						
Control	$-0.067 \pm 0.002$	$-0.056 \pm 0.002$	$-0.046 \pm 0.004$	$-0.046 \pm 0.001$	$-0.049 \pm 0.006$	$-0.053 \pm 0.003$
Rainfed	$-0.060 \pm 0.003$	$-0.068 \pm 0.002^{***}$	$-0.059 \pm 0.002^{**}$	$-0.062 \pm 0.002^{***}$	$-0.061 \pm 0.002^*$	$-0.056 \pm 0.006$
NDVI						
Control	$0.681 \pm 0.030$	$0.708 \pm 0.012$	$0.693 \pm 0.011$	$0.733 \pm 0.025$	$0.686 \pm 0.026$	$0.695 \pm 0.015$
Rainfed	$0.669 \pm 0.021$	$0.620 \pm 0.037^*$	$0.610 \pm 0.026^*$	$0.607 \pm 0.045^*$	$0.582 \pm 0.039^*$	$0.743 \pm 0.020$
WI						
Control	$1.061 \pm 0.008$	$1.086 \pm 0.002$	$1.093 \pm 0.005$	$1.109 \pm 0.001$	$1.074 \pm 0.008$	$1.072 \pm 0.011$
Rainfed	$1.052 \pm 0.004$	$1.040 \pm 0.005^{***}$	$1.047 \pm 0.010^{**}$	$1.037 \pm 0.007^{***}$	$1.028 \pm 0.011^{**}$	$1.072 \pm 0.009$

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

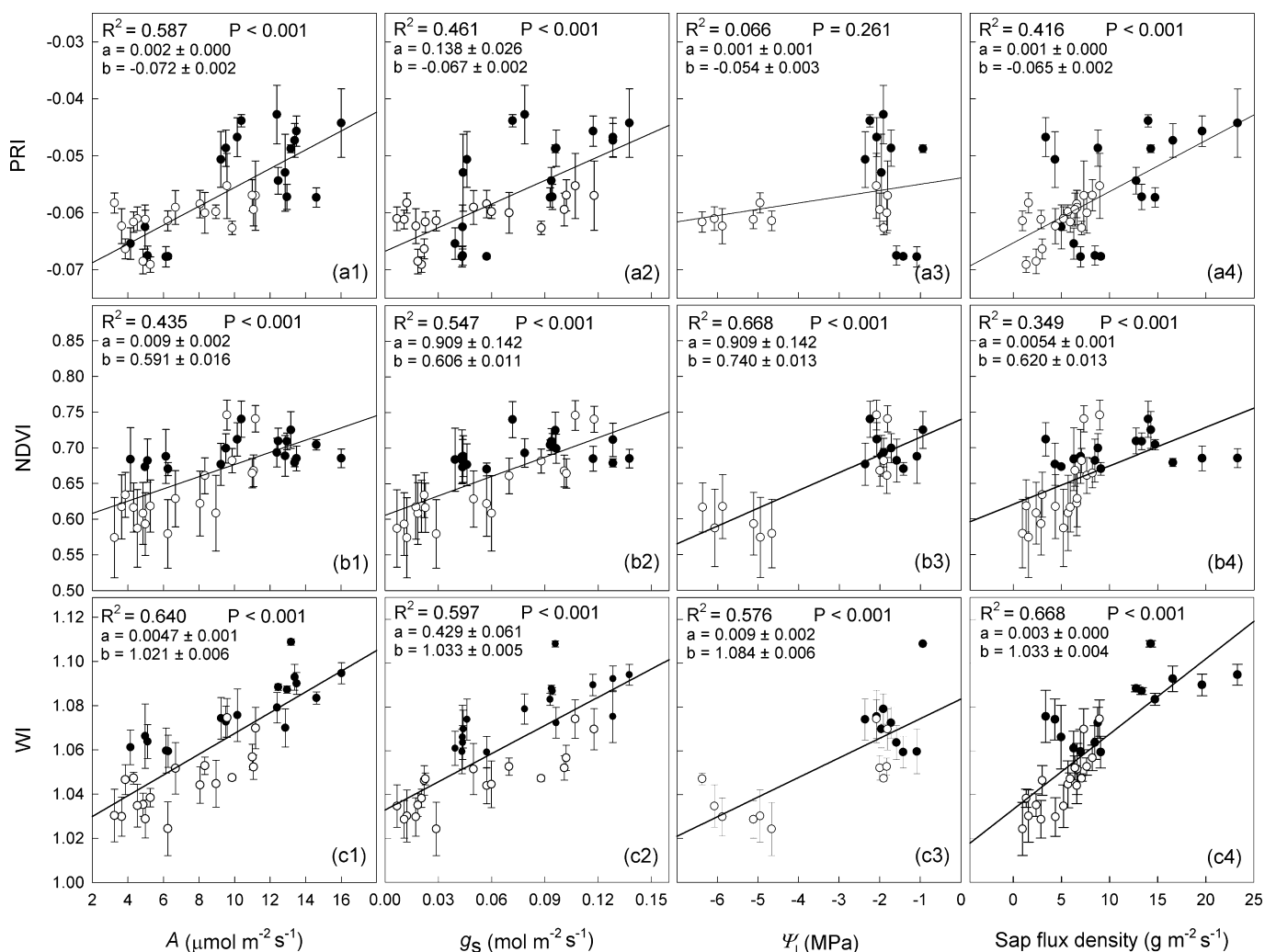
\*\*\*  $P < 0.001$ .

experimental conditions. However, a decline of diurnal sap flow patterns during the season in irrigated trees may be expected due to high water depletion of soil portions explored by roots (Tognetti et al., 2004, 2005; Fernández et al., 2008).

The detection of plant functioning by assessing associated spectral indices has been a major focus of remote sensing (Gamon et al., 1992; Peñuelas and Filella, 1998; Sun et al., 2008; Garbulsky et al., 2011; Tsonev et al., 2013). Sun et al. (2008) found that water stress caused significant changes in the spectral reflectance, in the Vis and NIR regions, of leaves of potted olive plants. They found that a 15-day water stress cycle significantly affected PRI and indices that used algorithms based on the 1455 nm wavelength, whereas WI and SIPI were not significantly affected. PRI, which is a Vis-based index originally elaborated to estimate xanthophyll cycle pigments (Gamon et al., 1992, 1997) and carotenoid/chlorophyll ratios (Sims and Gamon, 2002), has been shown to adequately track photosynthetic activity (see Garbulsky et al., 2011) also under water stress conditions (Sarlikioti et al., 2010; Ripullone et al., 2011; Tsonev et al., 2013). In accordance with these studies, PRI of olive tree canopy was significantly affected by drought in the rainfed treatment (Table 4). Furthermore, NDVI, an index widely used for the assessment of a larger number of plant and ecosystem properties (Ollinger, 2011), and WI, which is extensively used to assess

and leaf water status (Peñuelas et al., 1993; Serrano et al., 2000; Gutierrez et al., 2010), estimated at the canopy level, were significantly modified in rainfed plants subjected to drought stress (Table 4). This is in keeping with a recent study (Serrano et al., 2010) which showed that both NIR-based NDVI and WI are good indicators of vineyard water status. However, our results are in contrast with WI estimated on detached leaves of water-stressed olive saplings grown in pots (Sun et al., 2008). Experimental design and measurement methodology, may account for these conflicting results.

The diurnal courses of PRI, WI, and NDVI of the olive tree canopy were not significant (data not shown). The lack of responsiveness of these indices to the low radiation levels recorded in the early morning and late afternoon hours has been reported to be related to the bi-directional reflectance distribution function (Suárez et al., 2008; Sarlikioti et al., 2010). Thus, to correlate reflectance indices to the gas-exchange and water status parameters, the values obtained from early morning and late afternoon were discarded. The good linear relationship between canopy PRI and leaf photosynthesis (Fig. 3a1) confirms the results found on detached leaves of olive saplings grown in pots under water deficit conditions (Sun et al., unpublished data). There is a growing body of literature showing that PRI is correlated with photosynthetic



**Fig. 3.** Relationships between reflectance indices (PRI, NDVI and WI) and physiological parameters (photosynthetic rate,  $A$ , stomatal conductance to water,  $g_s$ , leaf water potential,  $\Psi_l$ , and sap flux density) in well-watered (●) and rainfed (○) *Olea europaea* plants during the growing season. The measurements were made between 10:00 and 16:00 h. Data are mean of three plants per treatment (five leaves per tree)  $\pm$  1 SEM. The values of the slope and intercept of the linear regression functions are indicated as a and b, respectively.



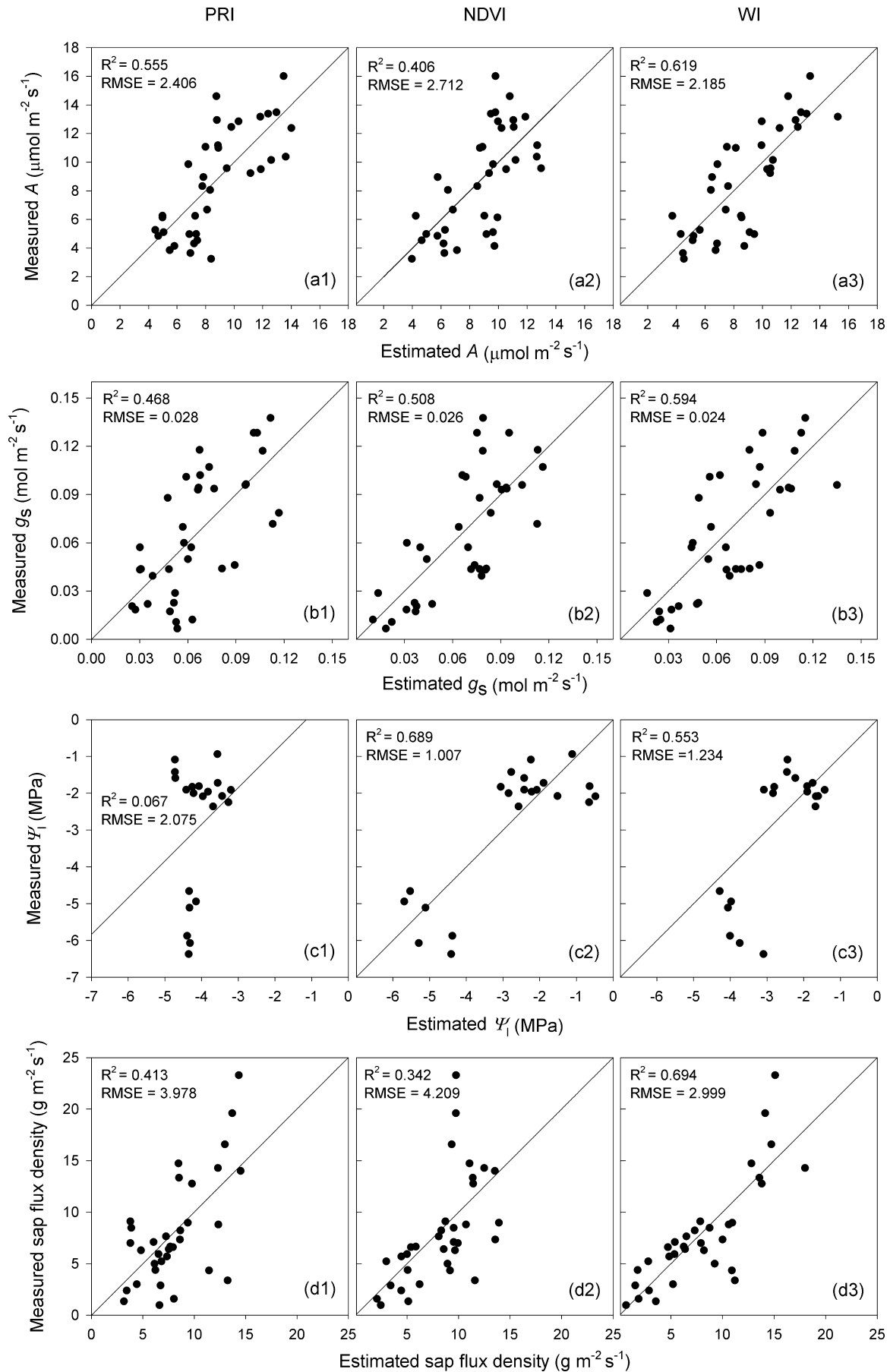


Fig. 4. Observed values of photosynthetic rate ( $A$ ), stomatal conductance to water ( $g_s$ ), leaf water potential ( $\Psi_1$ ), and sap flux density versus the respective predicted values derived from the empirical relationships with PRI, NDVI and WI.

parameters (Garbulsky et al., 2011). Moreover, recent studies have shown significant correlations between PRI and carbon assimilation in different plant species subjected to water stress conditions, for instance in greenhouse-grown *Solanum lycopersicum* (Sarlikioti et al., 2010), and in pot-grown Mediterranean evergreen seedlings of *Quercus ilex* and *Arbutus unedo* (Ripullone et al., 2011), and *Ceratonia siliqua* (Osório et al., 2012). NDVI resulted to be less reliable than PRI to remotely assess the photosynthetic rate in Olive trees (Fig. 4a1 and a2). In fact, NDVI is known to be sensitive to fractional PAR intercepted by green biomass, but it is not always a reliable tool to assess variations in photosynthetic radiation-use efficiency that can occur seasonally without a substantial alteration of canopy greenness (Gamon et al., 1995). On the other hand, PRI is more closely linked to photochemical performances of leaves, revealing short-term changes in de-epoxidation state of xanthophylls, a PSII light-use efficiency indicator (Gamon et al., 1997). A more reliable correlation was found between canopy NDVI and leaf  $g_s$  (Fig. 3b2), and a slight better one emerged between canopy WI and leaf  $g_s$  (Fig. 3c2). These results confirm early findings by Serrano et al. (2010) who showed that in general both NDVI and WI, measured at the canopy level, tracked leaf water status in grapevines, and that WI was in particular well correlated with  $g_s$ . We also found a close correlation between tree canopy WI and A (Fig. 3c1) and, even more interestingly, between tree canopy WI and whole-plant transpiration as assessed by stem sap flux density (Fig. 3c4). Overall, our study clearly shows that the spectral vegetation indices PRI, NDVI and WI measured at the tree canopy are good stress indicators that can be used for fast, noninvasive detection of water stress. Furthermore, these results show that PRI ranks better than NDVI for tracking photosynthesis, whereas WI is the most accurate predictive index of plant water status, whole-plant transpiration and also of the decline of photosynthetic rate in olive trees affected by water deficit. Finally, more intense, daily and seasonally, parallel measurements of physiological parameters and reflectance indices, made during the maximum solar irradiation hours, may further refine the quality of their correlations.

Finally, SIPI and CI, i.e., the pigment-based reflectance indices (Peñuelas and Filella, 1998; Ollinger, 2011), resulted also sensitive to water stress (Table 2). However, CI and SIPI did not track either the response of both chlorophyll concentrations and carotenoid to chlorophyll a ratio to water stress (Table 2) or the temporal variation of carotenoid concentration and carotenoid to chlorophyll ratio (Table 3). These results, consequently, do not provide positive evidence on the reliability of such pigment-based indices. A lack of sensitivity of SIPI for low values of carotenoid/chlorophyll ratio at canopy scale and an increasing variability for higher values of the ratio has been already described (Blackburn, 1998). A wider range of variation of pigment composition and a greater number of observations should be evaluated to better test the sensitivity of these indices.

In conclusion, olive is becoming a strategic crop worldwide, and its plantation is increasingly irrigated to intensify fruit production. In order to save irrigation water and increase its productivity, there is a pressing need to monitor plant water status and functioning to detect signs and degree of water stress. Our study, performed on mature olive plantation in arid Mediterranean environment, it is the first to our knowledge that combines diurnal and seasonal trends of leaf gas-exchange, whole-plant transpiration and tree canopy reflectance indices. Our findings demonstrate that: (a) the seasonal courses of PRI, WI, and NDVI were significantly affected by drought; (b) overall these three indices scaled linearly with gas exchange parameters; (c) PRI resulted better correlated with photosynthesis than NDVI, whereas WI was the most accurate predictive index of A,  $g_s$ ,  $\Psi_1$  and whole-plant transpiration as assessed by stem sap flux density. Thus, this study shows that remotely sensed reflectance indices, in particular PRI and WI, are promising

predictive tools of the impact of drought on photosynthetic activity, water status and whole-plant transpiration.

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