

**Evergreen broadleaf greenness and its relationship with leaf flushing, aging, and water
fluxes**

Authors: Yunpeng Luo^{1*}, Javier Pacheco-Labrador¹, Andrew D. Richardson^{2,3}, Bijan Seyednasrollah^{2,3}, Oscar Perez-Priego⁴, Rosario Gonzalez-Cascon⁵, M. Pilar Martín⁶, Gerardo Moreno⁷, Richard Nair¹, Thomas Wutzler¹, Solveig Franziska Bucher^{8,9}, Arnaud Carrara¹⁰, Edoardo Cremonese¹¹, Tarek S. El-Madany¹, Gianluca Filippa¹¹, Marta Galvagno¹¹, Tiana Hammer¹, Xuanlong Ma^{1,12}, David Martini¹, Qian Zhang^{1,13}, Markus Reichstein^{1,9}, Annette Menzel¹⁴, Christine Römermann^{8,9,15}, Mirco Migliavacca^{1,16*}

Institutional affiliations:

¹ Department for Biogeochemical Integration, Max-Planck-Institute for Biogeochemistry, 07745 Jena, Germany

² School of Informatics, Computing and Cyber Systems, Northern Arizona University, Flagstaff, AZ 86011, USA

³ Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011, USA

⁴ Department of Forest Engineering, ERSAF research group, University of Córdoba, 14071 Córdoba, Spain

⁵ Department of Environment, National Institute for Agriculture and Food Research and Technology (INIA, CSIC), 28040 Madrid, Spain

20 ⁶ Environmental Remote Sensing and Spectroscopy Laboratory (SpecLab), Spanish National
21 Research Council (CSIC), 28037 Madrid, Spain

22 ⁷ Institute for Dehesa Research (INDEHESA), Forestry School, University of Extremadura,
23 10600 Plasencia, Spain

24 ⁸ Institute of Ecology and Evolution, Plant Biodiversity Group, Friedrich Schiller University Jena,
25 07743 Jena, Germany

26 ⁹ Michael-Stifel-Center Jena for Data-driven and Simulation Science, 07743 Jena, Germany

27 ¹⁰ Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), 46980 Paterna, Spain

28 ¹¹ Environmental Protection Agency of Aosta Valley, ARPA Valle d'Aosta, 11020 Aosta, Italy

29 ¹² College of Earth and Environmental Sciences, Lanzhou University, Lanzhou 730000, China

30 ¹³ International Institute for Earth System Sciences, Nanjing University, Nanjing 210023, China

31 ¹⁴ Department of Life Science Systems, TUM School of Life Sciences, Technical University of
32 Munich, Freising, Germany

33 ¹⁵ German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, D-04103
34 Leipzig, Germany

35 ¹⁶ European Commission Joint Research Centre, Via E. Fermi, 2749, 21027 Ispra VA

36

39 Remote sensing capabilities to monitor evergreen broadleaved vegetation are limited by the low
40 temporal variability in the greenness signal. With canopy greenness computed from digital repeat
41 photography (PhenoCam), we investigated how canopy greenness related to seasonal changes in
42 leaf age and traits as well as variation of trees' water fluxes (characterized by sap flow and canopy
43 conductance). The results showed sprouting leaves are mainly responsible for the rapid increase
44 in canopy green chromatic coordinate (GCC) in spring. We found statistically significantly
45 differences in leaf traits and spectral properties between different leaf-age leaves with respect
46 compared: mean GCC of young leaves was 0.385 ± 0.010 (mean $\pm$ SD), while for mature and old
47 leaves was 0.369 ± 0.003 , and 0.376 ± 0.004 , respectively. Thus, the temporal dynamics of canopy
48 GCC can be explained by leaf spectral properties and leaf age. Sap flow and canopy conductance
49 are both well explained by a combination of environmental drivers and greenness (96% and 87%
50 of the variance explained, respectively). In particular, air temperature and vapor pressure deficit
51 (VPD) explained most of sap flow and canopy conductance variance, respectively. Besides, GCC
52 is an important explanatory variable for variation of canopy conductance may because GCC can
53 represent the leaf ontogeny information. We conclude that PhenoCam GCC can be used to identify
54 the new leaf flushing for evergreen broadleaved trees, which carries important information about
55 leaf ontogeny and traits. Thus, it can be helpful for better estimating canopy conductance that can
56 be used to constraining water fluxes.

57 **Key words:** evergreen broadleaved trees; digital repeat photography; PhenoCam; green
58 chromatic coordinate (GCC); leaf age; water fluxes

1. Introduction

Remote and near-surface spectral observations provide Vegetation Indices (VIs) sensitive to canopy greenness (Tucker, 1979; Huete *et al.*, 2002; Richardson *et al.*, 2009), which can be used to monitor canopy development, phenology, and vegetation functioning (Wu *et al.*, 2009; Migliavacca *et al.*, 2011; Toomey *et al.*, 2015). This linkage between the variation of greenness and vegetation functioning is strong in canopies with a pronounced seasonal dynamics of leaf area index (LAI) and chlorophyll content (Myneni *et al.*, 1997; Zhang *et al.*, 2003) like grassland and deciduous forests. By contrast, evergreen forests, especially those with broadleaf trees, exhibit low temporal variability in their canopy greenness (Moore *et al.*, 2016; Moser *et al.*, 2020), which results in a desynchronization between seasonal changes in canopy-scale greenness and seasonality of vegetation functioning such as carbon and water fluxes (Moore *et al.*, 2016).

Evergreen broadleaved forest are widely distributed in the tropics and warm temperate regions (Hengl *et al.*, 2018). Among the distribution area, the tree-grass ecosystem (e.g. open woodlands and savannah ecosystems in the subtropical, semi-arid and/or in Mediterranean areas) is one of the most important ecosystem types that host evergreen trees. They are located in semi-arid regions covering between 16-35% of the global land surface (Friedl *et al.*, 2002; Hanan & Hill, 2012) and play an essential role for the global interannual variability of biosphere-atmosphere carbon and water fluxes (Poulter *et al.*, 2014; Ahlström *et al.*, 2015). However, with co-existence of growth forms featuring different functioning and dynamics (i.e. trees and grasses), it is difficult to explore evergreen trees' functioning-greenness relationship in tree-grass ecosystems because of the necessity to separate the variation in trees' greenness (Liu *et al.*, 2017; Perez-Priego *et al.*, 2017; Luo *et al.*, 2018; El-Madany *et al.*, 2021) and vegetation functioning compared to more homogenous forest ecosystems.

The development of near-surface observations of plants through digital cameras (Richardson *et al.*, 2009), namely PhenoCams, and the establishment of global PhenoCam observation networks facilitates the study of not only canopies but also individual trees (Nasahara & Nagai, 2015; Wingate *et al.*, 2015; Brown *et al.*, 2016; Richardson *et al.*, 2018; Seyednasrollah *et al.*, 2019). PhenoCam can easily discriminate individual tree crowns due to their proximity to the observed objects, minimizing the influence of background and understory optical signals (Richardson *et al.*, 2007). Therefore, this allows us to monitor the changes in individual trees' VIs and facilitate their linkage with crown structure development and functional dynamics.

In recent years, an increasing number of studies have evaluated how PhenoCam green chromatic coordinate (GCC) and other VIs such as camera-based normalized difference vegetation index (CamNDVI) relate to leaf traits (Keenan *et al.*, 2014; Yang *et al.*, 2014) and dynamics of trees' functioning (Luo *et al.*, 2018). These studies sought a biological interpretation of the temporal variability of leaf color changes (Keenan *et al.*, 2014; Yang *et al.*, 2014) or leaf development mainly in deciduous forests and evergreen needle forest (Yang *et al.*, 2017; Filippa *et al.*, 2018). However, despite the PhenoCam network growth, only a handful of studies focused on evergreen broadleaved trees, which have different pattern of VI seasonality compared to colder evergreen needle forest (Zhao *et al.*, 2012; Nasahara & Nagai, 2015; Lopes *et al.*, 2016; Yan *et al.*, 2019). Besides, to our knowledge, only a few studies have investigated the relationship between greenness and leaf traits (e.g. chlorophyll content and nitrogen content) in evergreen broadleaved trees and mainly focused on the leaf-scale in tropics (Chavana-Bryant *et al.*, 2017; Wu *et al.*, 2017a). They demonstrated that the leaf age is a key factor to affect leaf traits and their spectral properties. However, studies still lack a biologically understanding of the seasonality in canopy greenness and

its relation with trees' functioning, such as water fluxes especially with high temporal resolution (e.g., daily).

In this study, we first aimed to identify the reasons for canopy greenness variation (crowns-based) in the evergreen broadleaved trees of a Mediterranean tree-grass ecosystem and, second to explore the relationship between greenness variation and the changes in leaf traits and trees' water fluxes (characterized by sap flow and canopy conductance). To do so, we combined leaf- and canopy-scale greenness measured with field spectrometers and PhenoCams, measurements of leaf traits, tree water fluxes measured with sap flow (SF) meters and derived tree canopy conductance (Gc). Specifically, we intend to answer the following questions:

(1) What does the measured PhenoCam GCC biologically represent (e.g. the GCC variation relates to leaf ontogeny)?

(2) Is the variability in GCC related to leaf traits or other structural properties of the canopy?

(3) Are the variations in greenness (represented by GCC and CamNDVI) temporally related to changes in trees' water fluxes?

2. Materials and Methods

2.1 Study area and environmental variables

The study focuses on a Mediterranean tree-grass ecosystem located in Extremadura, western Spain (Majadas de Tiétar, 39°56'24.68"N, 5°46'28.70"W). This ecosystem, named *dehesa*, consists of a dominant herbaceous (grass) layer combined with approximate 20 tree ha⁻¹ of evergreen oak trees (Bogdanovich *et al.*, 2021), with 98% of trees being Holm oak, i.e. *Quercus ilex* L. (El-Madany *et al.*, 2018; Luo *et al.*, 2020). The average height of trees is 8.9 (±1.0) m, with a mean DBH of 46.4 (± 6.7) cm (El-Madany *et al.*, 2018; Bogdanovich *et al.*, 2021). [The study ecosystem has a typical](#)

Mediterranean climate: hot and dry summer from mid-May to mid-October, and mild and wet winters from late October to February. The mean annual air temperature is 16.7 °C and annual rainfall approximates 700 mm, typically falling from October to May with a dry summer (Perez-Priego *et al.*, 2017; Luo *et al.*, 2018; El-Madany *et al.*, 2021).

Three closely located EC towers measure the carbon, water, and energy fluxes at three sites in the study ecosystem, and their FLUXNET ID abbreviations are ES-LMa, ES-LM1, and ES-LM2 (Luo *et al.*, 2018). Micrometeorological variables were measured at each EC tower (El-Madany *et al.* (2018) for details) with 20 Hz time frequency and processed to obtain half-hourly water and energy flux measurements (El-Madany *et al.*, 2018; El-Madany *et al.*, 2020; El-Madany *et al.*, 2021). Meteorological measurements were measured every 5 minutes and average at half-hourly to match the EC temporal resolution. We measured air temperature (T_a ; °C), shortwave downwelling radiation (SWDR; $W\ m^{-2}$), wind speed (WS; $m\ s^{-1}$), incoming photosynthetically active radiation (PAR; $\mu mol\ m^{-2}\ s^{-1}$), vapor pressure deficit (VPD; hPa), and precipitation (Prec; mm). Volumetric soil water content (SWC) was recorded at 0.05, 0.10, 0.20, 0.30, 0.50, 1.00 m below ground with four replicated profiles of sensors (ML2x, Delta-T Devices Ltd, Cambridge, UK) in the footprint of each EC tower (two under the tree canopy and two in the open grassland). We further classified the measured SWC under the tree canopy into three different soil depth layers: shallow (soil depth smaller than 0.30 m where roots of herbaceous plants are dominant (Rolo & Moreno, 2012)), medium (0.50 m), and deep (1.00 m). We aggregated the soil moisture measurements at a daily scale for each sensor at different soil depths. Then for each soil depth layer (shallow, medium, and deep), we calculated the mean of daily soil moisture from the six soil moisture sensors. Additionally, average longwave downwelling and upwelling radiation (LWDR and LWUR; $W\ m^{-2}$

²) was measured every 15 mins on three individual trees close to three EC towers by radiometric towers and used to calculate the surface temperature (T_s ; °C) of trees (see details in section 2.4).

2.2 Canopy and leaf-level spectral measurements for evergreen broadleaved trees

2.2.1 Tree crowns GCC from PhenoCam

At the top of each EC tower, a north-facing near-infrared-enabled PhenoCam (Stardot NetCam, StarDot Technologies, USA) sampled sequential red, blue, green (RGB), and RGB + near-infrared (NIR) images every half hour (from 10:00 to 14:30 UTC) in JPEG format from March, 2014 (Luo *et al.*, 2018). In the field of view of each camera, we selected six regions of interest (ROIs) corresponding to six tree crowns from each site for further analysis (examples from ES-LM1 site in Figure S1) and for a total of 18 trees in the study area (Figure S1). We limited the ROIs selection to the intermediate distance (approximately 90.3 ± 18.3 m (mean $\pm$ standard deviation (SD))) because of the time series' lower noise (see details in Note S1). The digital numbers (DNs) from blue (B_{DN}), green (G_{DN}), red (R_{DN}), and near-infrared (NIR_{DN}) channels were extracted from pixels in different ROIs from every PhenoCam image and averaged for each ROI (tree crown). GCC was then calculated as the ratio between G_{DN} and the sum of the RGB-DNs for each crown (Eq.1). Daily crown GCC time series were produced from the 90th percentile of GCC at 1-day intervals according to the protocol of PhenoCam Dataset version 2 (Seyednasrollah *et al.*, 2019).

$$GCC = \frac{G_{DN}}{R_{DN} + G_{DN} + B_{DN}} \quad (1)$$

The GCC from different crowns at each site concurred over the seasonal pattern, being highly correlated to each other (e.g., $r > 0.74$; Figure S2). Then we calculated the mean GCC from the different tree crowns as site-level GCC. The variation pattern of GCC from the three sites was also highly consistent. Furthermore, we confirmed that the GCC of the 18 selected trees was

representative of the trees' GCC in the study area (see details in supplementary material Note S2 and Figure S3, S4). Hence, we obtained daily PhenoCam GCC of the whole study area by averaging the daily GCC from the three individual sites (for a total of 18 tree crowns).

We also computed the PhenoCam normalized difference vegetation index (CamNDVI; Eq.2). CamNDVI is considered a good proxy for LAI and biomass (Filippa *et al.*, 2018; Luo *et al.*, 2018). Compared to GCC, it might carry different information regarding leaf development and therefore changes in water fluxes of trees. CamNDVI was computed using the same ROIs as for GCC according to equation 2 using the “phenopix” R package (Filippa *et al.*, 2016):

$$CamNDVI = \frac{NIR_{DN}' - R_{DN}'}{NIR_{DN}' + R_{DN}'} \quad (2)$$

where NIR_{DN}' and R_{DN}' are the exposure-adjusted NIR_{DN} and R_{DN} (Petach *et al.*, 2014). CamNDVI variation was also consistent among different crowns and sites. Hence, daily CamNDVI for the study area was obtained with the same procedures as for GCC. Because absolute values of CamNDVI are not comparable to other direct reflectance measurements (Petach *et al.*, 2014; Filippa *et al.*, 2018; Luo *et al.*, 2018), we rescaled the CamNDVI between 0-1 for a more intuitive interpretation.

2.2.2 Leaf spectral reflectance

Optical measurements at the leaf scale were conducted to support the interpretation of PhenoCam GCC signals at the canopy scale. Holm oak leaves' reflectance factors were measured between 2014 and 2016 in 21 field campaigns, using a plant probe with a leaf clip attached to an ASD spectroradiometer (FieldSpec3, Analytical Spectral Devices Inc., Boulder, CO, USA). Leaves were measured in all the seasons but more intensively in spring when leaves properties change

faster (Pacheco-Labrador *et al.*, 2014). The FieldSpec3 spectral range covers 350-2500 nm. The original spectral resolution and spectral sampling interval are 3.0 and 1.4 nm in the visible (VIS) and 10.0 and 2.2 nm in the NIR and beyond. However, the ASD interpolates the spectra at 1 nm step by default. In each campaign, leaves of two different ages were sampled in each tree: current and old leaves (i.e. younger and older than one year, respectively). In practice, this was determined by the position of the leaves with respect to scars in the branch and leaves' underside color (personal communication with Gerardo Moreno). Twelve leaves were collected for each category in each tree, from the upper part of the crown at two principal orientations (i.e. six north leaves and six south leaves). However, as newly flushed leaves have distinct spectral properties (Pacheco-Labrador *et al.*, 2014; González-Cascón *et al.*, 2016; Wu *et al.*, 2017b), we re-classified the leaves into three categories according to leaf ontogeny: young leaves (less than 3 months from sampling date when flushed leaves were measured for the first time), mature leaves (between 3 months and 1 year), and old leaves. Next, we convolved ASD leaves' spectra according to PhenoCam spectral response function (Figure S5) to compute leaf ASD GCC (GCC_{ASD}) in Eq.1.

2.3 Leaf traits and leaf development

2.3.1 Leaf traits

From 2015 to 2019, we measured leaf traits of the Holm oak trees. We sampled the leaves in different seasons, but more intensively in spring. Leaf chlorophyll content per leaf area (Chl_{lab} , $\mu g\ cm^{-2}$) was estimated by using an empirical relationship established between pigment content and the SPAD chlorophyll meter (SPAD-501, Konica Minolta Inc, Osaka, Japan) at the same site following Gonzalez-Cascon *et al.* (2017). Area-based nitrogen content (N_{area} , $mg\ cm^{-2}$), leaf nitrogen concentration (%N, $mg\ g^{-1}$), and leaf carbon concentration (LCC, $mg\ g^{-1}$) were determined using the dry combustion method with a LECO CN-2000 analyzer (65 °C) and leaf

area. Water content per area (WCA), leaf mass per area (LMA), and leaf dry-matter content (LDMC) were also calculated based on the leaf area and fresh and dry leaf weight (Eq.3-5):

$$WCA = \frac{(W_f - W_d)}{A_{leaf}} \quad (3)$$

$$LMA = \frac{W_d}{A_{leaf}} \quad (4)$$

$$LDMC = \frac{W_d}{W_f} \quad (5)$$

where W_f and W_d are fresh and dry leaf weight, respectively. A_{leaf} is the leaf area for fresh leaves. Chl_{ab} was estimated based on the same 12 leaves per leaf age class in each sampled tree where ASD measurements were obtained. For the other biological traits, we sampled 40 current and 40 old leaves per tree in each field campaign to analyze the leaf traits before 2016. From 2016-2019, apart from 12 leaves sampled in the same trees as Chl_{ab} , we randomly sampled 100 additional leaves in the study area to determine those biological traits. Similarly, we classified the leaves as young, mature, and old as described in section 2.2.2.

2.3.2 Leaf flushing period

Oak leaf flushing episodes generally occur once and occasionally twice per year. Spring flushing contributes the most of new leaves production (Barbeta & Peñuelas, 2016), whereas autumn flushing happens under adverse climate condition or biotic stress such as drought or caterpillar attack (G. Moreno, R. Gonzalez-Cascon, and M.P. Martín, personal communication, and Luo *et al.* (2020)). This study focused on spring flushing because of its importance and the considerable variation of biochemical and biophysical traits during this period.

The timing of spring leaf flushing was evaluated both by visually checking the PhenoCam images and by direct observation in the field. The flushing period was identified as the period between the first and last time new leaves were observed in the PhenoCam images and also considering the first time the leaves were measured with the ASD in annual sampling campaigns. This occurred, on average, when the foliar area of new (young) leaves was larger than 2.7 cm². These two sources of information, used to determine the flushing period, led to consistent estimates (Figure S6a) despite smaller number of trees sampled during field campaigns.

2.3.3 Leaf fraction of different leaf age classes in the canopy

The fraction of each leaf type (current (young and mature) and old leaves) was estimated in the field campaigns between 2015-2019. We empirically fit a 2nd-degree polynomial function to predict the temporal variation of the fraction of current leaves as a function of the day of the year (DOY, see details in Note S3 and Figure S7a). Canopy GCC can be represented by using leaf spectral reflectance and leaf fraction in two leaf types: old and current leaves. The latter were featured by young or mature leaves, depending on the time after the flushing date. Young leaves' spectra and properties represented the current leaves during the first three months after flushing, and were replaced by the mature leaves afterward (Note S3 and Figure S7b). We adopted this simplified approach to represent the leaf fraction of different leaf age classes because: 1) most of the new leaves are flushed in the spring (see 2.3.2); 2) the fraction of young leaves was not directly measured in the field, and mature leaves are more similar to old leaves compared to young leaves regarding the spectral properties (Pacheco-Labrador *et al.*, 2014). The estimated leaf age from the periodical field campaigns confirmed the rationality of the changes in leaves fraction estimated from the polynomial function (Note S3 and Figure S8a).

2.4 Sap flow and canopy conductance

2.4.1 Daily SF fluxes

Sap flow (SF) meters (SFM1, ICT International Pty Ltd, Armidale, Australia) were installed at breast height (1.3m above ground) of six trees in the footprint of each EC tower (18 trees in total). The SFM1 sensor consisted of two 35-mm long temperature probes, each one with two thermocouple junctions at 7.5 mm and 22.5 mm distance from the needle tip that cover variations of the sap flow velocity profile. A heater probe was inserted equidistant (5 mm) from the two temperature probes. Heat pulses, powered with 20 J, were set at the two sampling points at half-hourly intervals and their respective sap velocities were computed from the ratio of the average temperature rise of the upper to lower sensor between 60 and 100 s after each heat pulse. In post-processing, corrections for probe misalignment, wounding effects (when applicable), and sap velocity determination were applied according to Burgess et al. (2001). To integrate the sap velocity profile from the two sampling points, sap flow density (SFD) was weighted by the cross-sectional area of sapwood defined within each annulus around the two-point temperature measurements. Total sapwood area was derived from an empirical relationship between tree sapwood area and the diameter at the breast height determined by destructive sampling (See Perez-Priego et al., 2017 for more details). In each site, six trees with diameters that covered the range of variations were sampled. The ensemble of the six half-hourly SFD values was used to calculate the mean as an integrative quantity of the overstory water fluxes. In turn, the mean value allowed us to minimize the impact of data gaps due to any sensor failure, which accounted for the 25 % of the raw data set. We used the SFD to approximate the variation pattern of trees' transpiration and the SFD are available from February 2015 to March 2019. As the variation pattern of SFD (referred

as SF hereafter) among three sites is consistent, therefore, the SF was averaged across three sites to derived the average daily SF flux in the whole study area.

2.4.2 Tree canopy conductance

Canopy conductance (G_c ; cm s^{-1}) was estimated using the empirical relationship from Phillips and Oren (1998) (Eq. 6):

$$G_c = \frac{(115.8 + 0.4226 \times T_s) \times SF}{VPD} \quad (6)$$

where SF is sap flux density ($\text{kg cm}^{-2} \text{ hour}^{-1}$), and VPD (KPa) is vapor pressure deficit as indicated before. Surface temperature (T_s (°C)) of tree canopy was estimated through Stefan–Boltzmann law (Eq.7):

$$T_s = \sqrt[4]{\frac{LWUR - (1 - \varepsilon)LWDR}{\varepsilon \times \sigma}} - 273.15 \quad (7)$$

where LWDR and LWUR (W m^{-2}) were measured from the radiometric towers, ε is the emissivity (set to 0.98 (Knauer *et al.*, 2018)) and σ is Stefan–Boltzmann constant ($5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$).

2.5 Statistical analyses

To biologically interpret the seasonal changes in PhenoCam GCC and understand how leaves of different leaf-age impact the greenness, we first tested the significant differences of GCC_{ASD} among young, mature, and old leaves with the Tukey’s honest significant difference test (Tukey’s HSD). Age-weighted average GCC_{ASD} was compared with PhenoCam GCC to confirm whether the variation of GCC at the canopy scale could be well represented by considering spectral information from different leaf age classes. Then, we further applied linear regression analysis

between leaf traits and GCC_{ASD} to biologically understand the relationship between the variation of leaf traits and leaf greenness. We conducted this analysis mainly using the available data between 2014 and 2016.

We used Generalized Additive Models (GAM (Hastie & Tibshirani, 1990)) to analyze the relationship between the trees' water fluxes (specifically for Gc and SF) and greenness (PhenoCam GCC and CamNDVI). We used the available data during the period of 2015- 2019 to conduct this analysis. Daily Gc (and SF) were used as the response variables, and we ran different GAMs by using daily VIs and environmental factors as predictors: GCC, CamNDVI, SWDR, Ta, WS, $SWC_{shallow}$, SWC_{medium} , SWC_{deep} , and VPD (only for predicting SF since VPD was used to calculate the Gc). GAM fitting applied penalized regression smoothing splines using "gam ()" function implemented in the *mgcv* R package (Wood, 2017). We also calculated each variable's variance inflation factor (VIF) to measure how much regression variance was inflated due to multicollinearity in the model. We removed any variables with $VIF > 10$, keeping GCC, CamNDVI, SWDR, Ta, WS, $SWC_{shallow}$, and SWC_{deep} in the Gc GAM and GCC, CamNDVI, SWDR, VPD, WS, $SWC_{shallow}$, and SWC_{deep} in the SF model (Table 1).

To understand how VIs contribute to the estimation of Gc and SF, we selected four combinations of predictors listed in Table 1 and compared the AIC value of the fitted models. We run a set of environmental predictors, environmental predictors plus GCC, environmental predictors plus CamNDVI, and environmental predictors plus CamNDVI and GCC. The smoothing functions resulting from the best GAM models were used to evaluate the sensitivity of Gc or SF to each predictor.

Table 1. Four combinations of predictors in the Generalized Additive Models (GAMs: M1-M4) to understand the contribution of VIs to estimate the canopy conductance (Gc) and sap flux density (SF)¹.

	GCC	CamNDVI	SWDR	Ta	WS	VPD	SWC _{shallow}	SWC _{deep}
M1: “No VIs”			X	X	X	X	X	X
M2: “GCC”	X		X	X	X	X	X	X
M3: “CamNDVI”		X	X	X	X	X	X	X
M4: “All VIs”	X	X	X	X	X	X	X	X

¹ The predictors of Gc (grey and orange) and SF (grey and pink) GAM models. Those variables were selected for the different GAM models (M1-M4) were marked as “x” in the table.

3. Results

3.1 Relationship between PhenoCam, GCC_{ASD}, and leaf traits

3.1.1 Relation between PhenoCam GCC (canopy scale) and GCC_{ASD} (leaf scale)

The PhenoCam GCC significantly increased during the leaf flushing period in the spring. Autumn precipitation also led to an increase in tree greenness, even though the amplitude of this increase was much smaller than in spring (Figure 1). At the leaf scale, GCC_{ASD} from different leaf age classes showed that young leaves had larger GCC_{ASD} than mature and old leaves (Figure 2). Field observations confirmed this contrasting leaf color in young and old leaves (Figure S9). GCC_{ASD} was significantly different ($p < 0.001$) among three age classes (Figure 2): mean GCC_{ASD} was 0.385 ± 0.010 (mean $\pm$ SD), 0.369 ± 0.003 , and 0.376 ± 0.004 for young, mature, and old leaves, respectively.

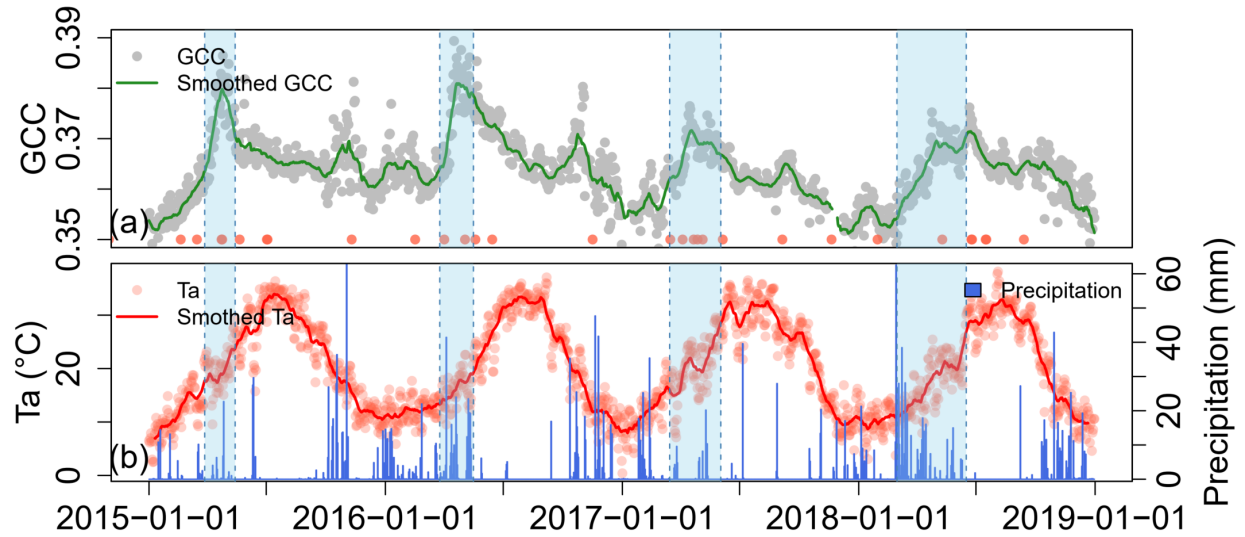


Figure 1. Variation of (a) daily PhenoCam green chromatic coordinate (GCC) for evergreen oak trees, (b) daily mean temperature (Ta), and precipitation in the study area. The red dots in panel a) indicate the dates when leaf traits were measured. GCC and Ta time series were smoothed with a 20 days average moving window.

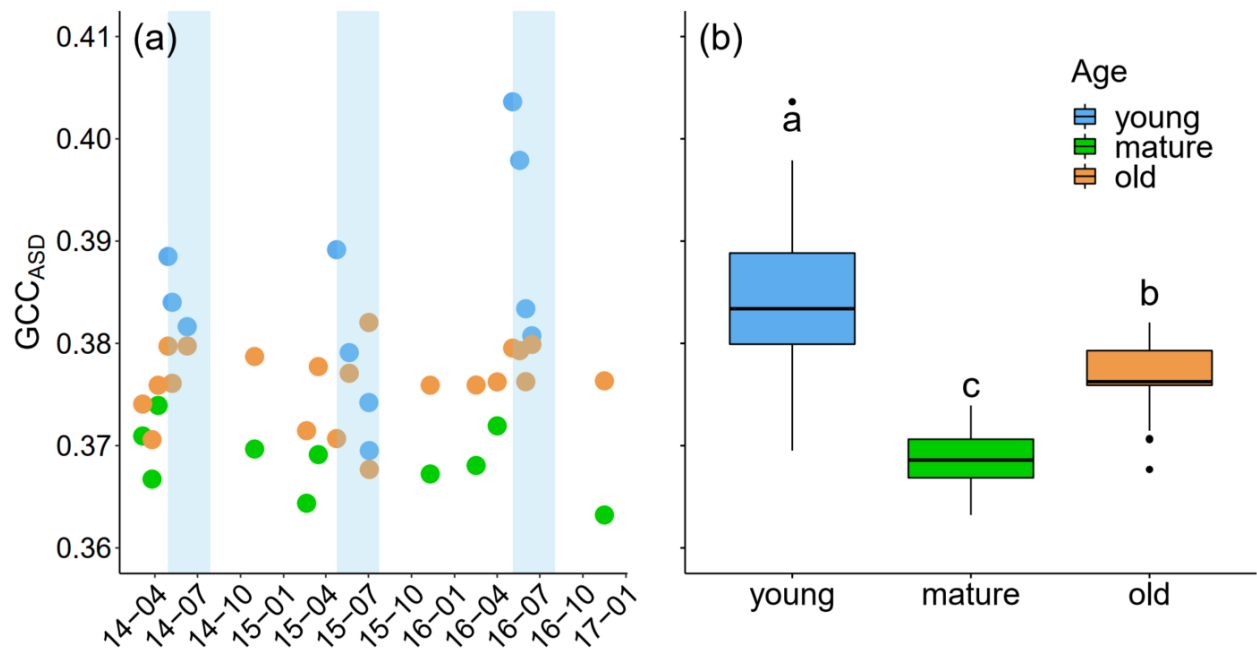


Figure 2. Comparison of green chromatic coordinate (GCC) for leaves of different age classes. (a) GCC_{ASD} (leaf-level GCC measured by an ASD spectroradiometer) was calculated for young (leaf age ≤ 3 months),

mature (3 months < leaf age ≤ 1 year), and old leaves (leaf age > 1 year). The blue shaded areas indicate the leaf flushing periods visually determined using PhenoCam images. (b) Comparison of GCC_{ASD} between young, mature, and old leaves. The length of each box indicates the interquartile range: the horizontal line inside each box the median, and the bottom and top of the box the first and third quartiles, respectively. Tukey's honest significant difference (Tukey's HSD) test evaluated GCC_{ASD} differences among the three age groups, and letters a, b, and c indicate the significant differences ($p < 0.05$).

We compared the PhenoCam GCC and the age-weighted average GCC_{ASD} (Figure 3 and Figure S10). The results show that the weighted average GCC_{ASD} captured the temporal variability of PhenoCam GCC (Figure 3a). They had strongly linear relationship ($R^2=0.72$), although age-weighted average GCC_{ASD} featured higher values (Figure 3c). We found that GCC_{ASD} from current (young and mature) leaves had a much stronger link with PhenoCam GCC compared to old leaves (Figure S10). By considering the fraction of both current and old leaves, the bias in the relationship between the PhenoCam GCC and the weighted average GCC_{ASD} was smaller than in the PhenoCam GCC relationships with current or old leaves GCC_{ASD} (mean absolute error (MAE) was 0.007, 0.008, and 0.009 for weighted mean, current and old leaves, respectively). PhenoCam GCC was generally negatively related to leaf age, especially between leaf flushing and the end of the year (Figure S8b).

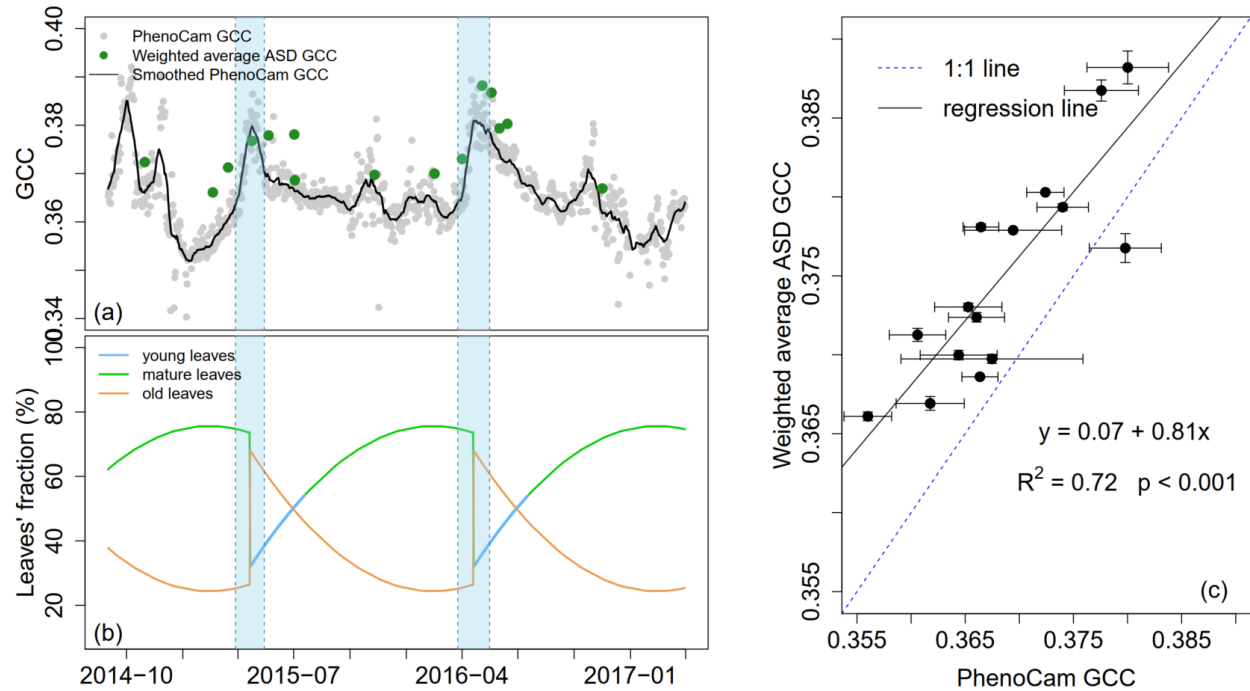
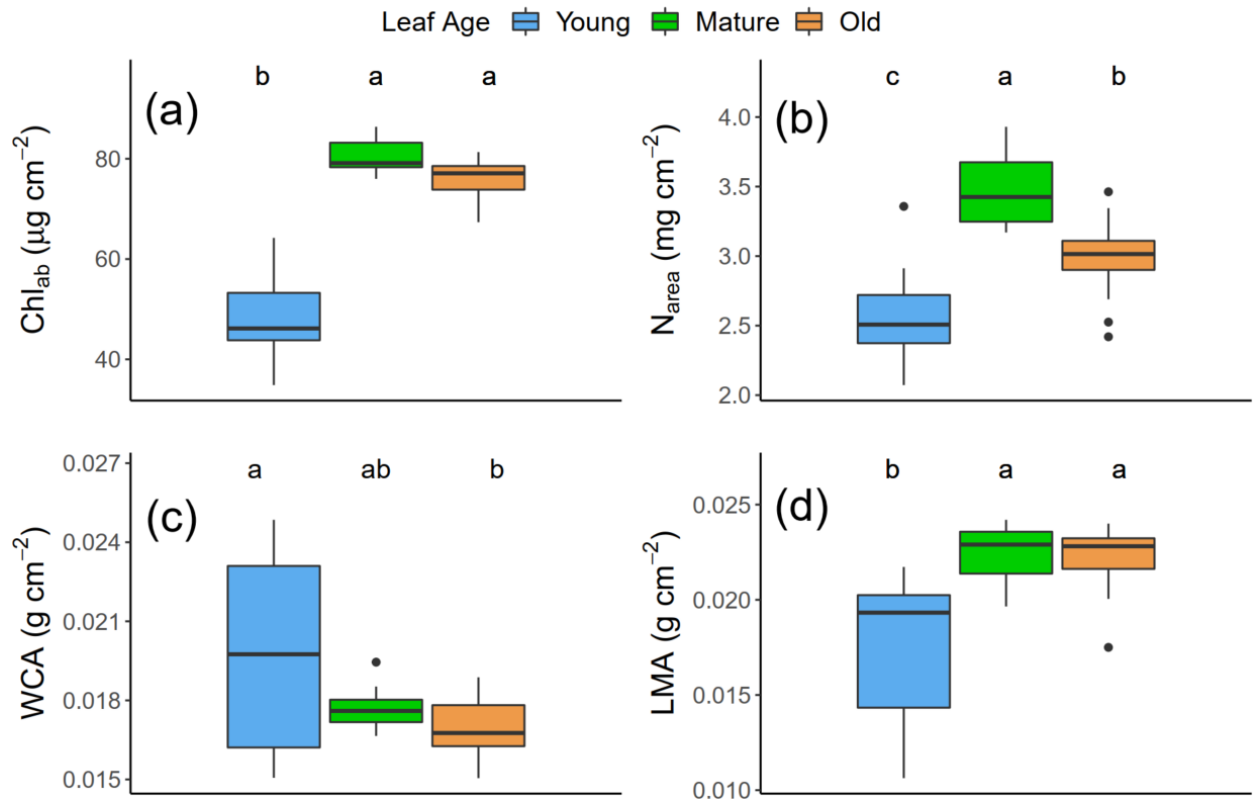


Figure 3. Comparison of age-weighted average ASD green chromatic coordinate (GCC_{ASD}) with PhenoCam GCC. (a) Comparison of time series of PhenoCam GCC and age-weighted average GCC_{ASD} . The weighted averaged GCC_{ASD} was calculated according to the estimated fraction of new, mature, and old leaves from (b). (b) Estimated fraction of different-age leaves. Leaf age fraction was estimated according to field observations (see details in Note S3). The drop of current leaves during leaf flushing was caused by the current leaves being assigned into the old leaves category. The blue shaded area indicates the leaf flushing period visually determined by PhenoCam images. (c) Scatterplot of PhenoCam GCC and age-weighted average GCC_{ASD} . The error bars in (c) stand for the standard deviation.

3.1.2 Relationship between GCC_{ASD} and leaf traits among different leaf age classes

Leaf traits such as Chl_{ab} , N_{area} , and LMA were significantly lower in young than in mature and old leaves, while WCA was larger in young leaves than in mature and old leaves (Figure 4). In contrast, all these traits were similar or only slightly different between mature and old leaves. This difference in leaf traits (Figure S11) coincide with the distinct optical properties (i.e. green reflectance (Figure S12)) and GCC_{ASD} (Figure 2) in young leaves.

374 The linear relationship between leaf traits and GCC_{ASD} differed among different age classes and
 375 traits (Figure 5). We found significant negative linear relationships between GCC_{ASD} and Chl_{ab} or
 376 N_{area} in young leaves, while no significant linear relationships were observed for WCA and LMA.
 377 Generally, there were no significant relationships between GCC_{ASD} and leaf traits in mature and
 378 old leaves, except for the positive linear relationship for GCC_{ASD} and WCA in old leaves (Figure
 379 5c). These leaves also offered lower variability than young leaves in both axes compared (spectral-
 380 trait).



382 Figure 4. Comparison of biological traits (a-d) among different leaf-age classes. Biological traits are
 383 chlorophyll content per leaf area (including chlorophyll a, and b: Chl_{ab}), nitrogen content per leaf area (N_{area}),
 384 water content per area (WCA), leaf mass per area (LMA) observed among different age classes. The bold
 385 lines within the boxes are the medians, and the boxes are the interquartile ranges (Q25, Q75). The black
 386 points outside the boxes represent outliers. Tukey's honest significant difference test (Tukey's HSD)

evaluated biological traits differences among the three age groups, and letters a, b, and c indicate the significant differences ($p < 0.05$).

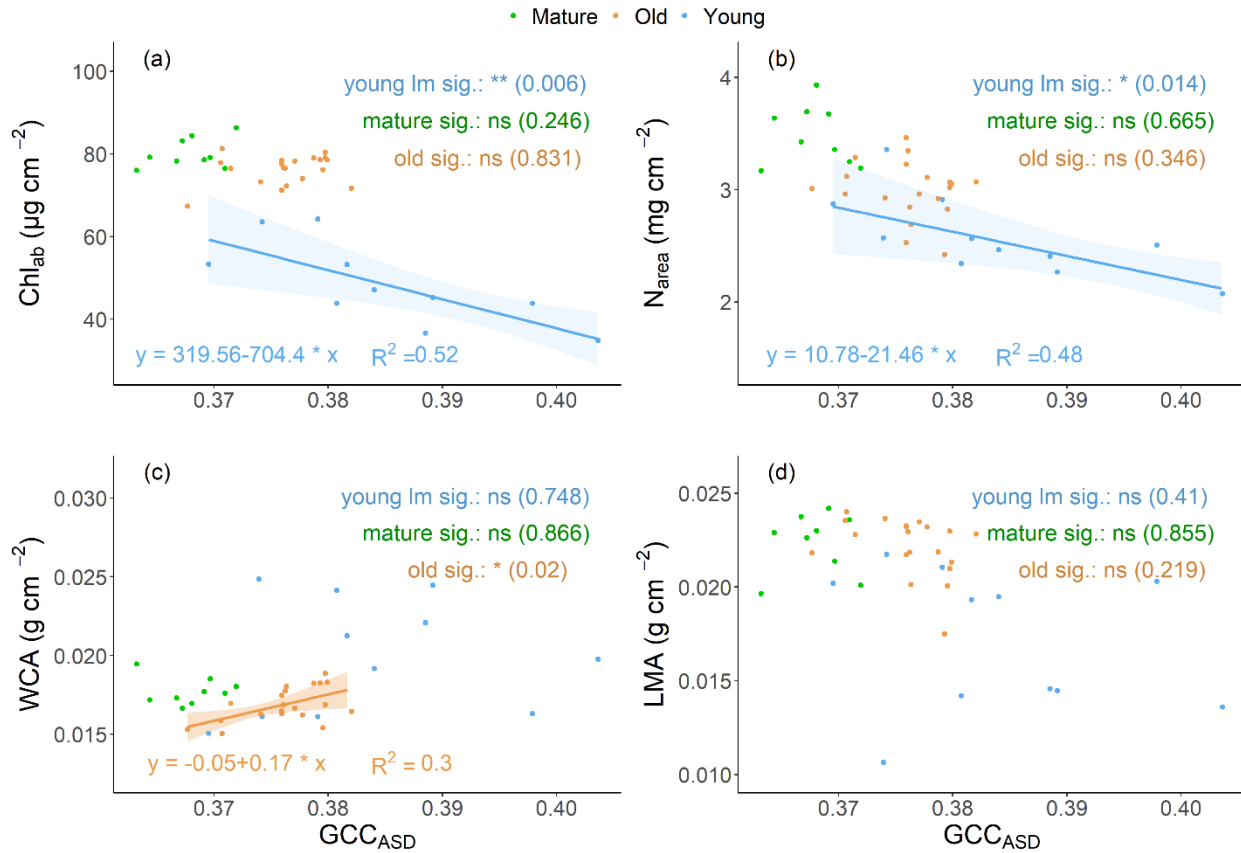
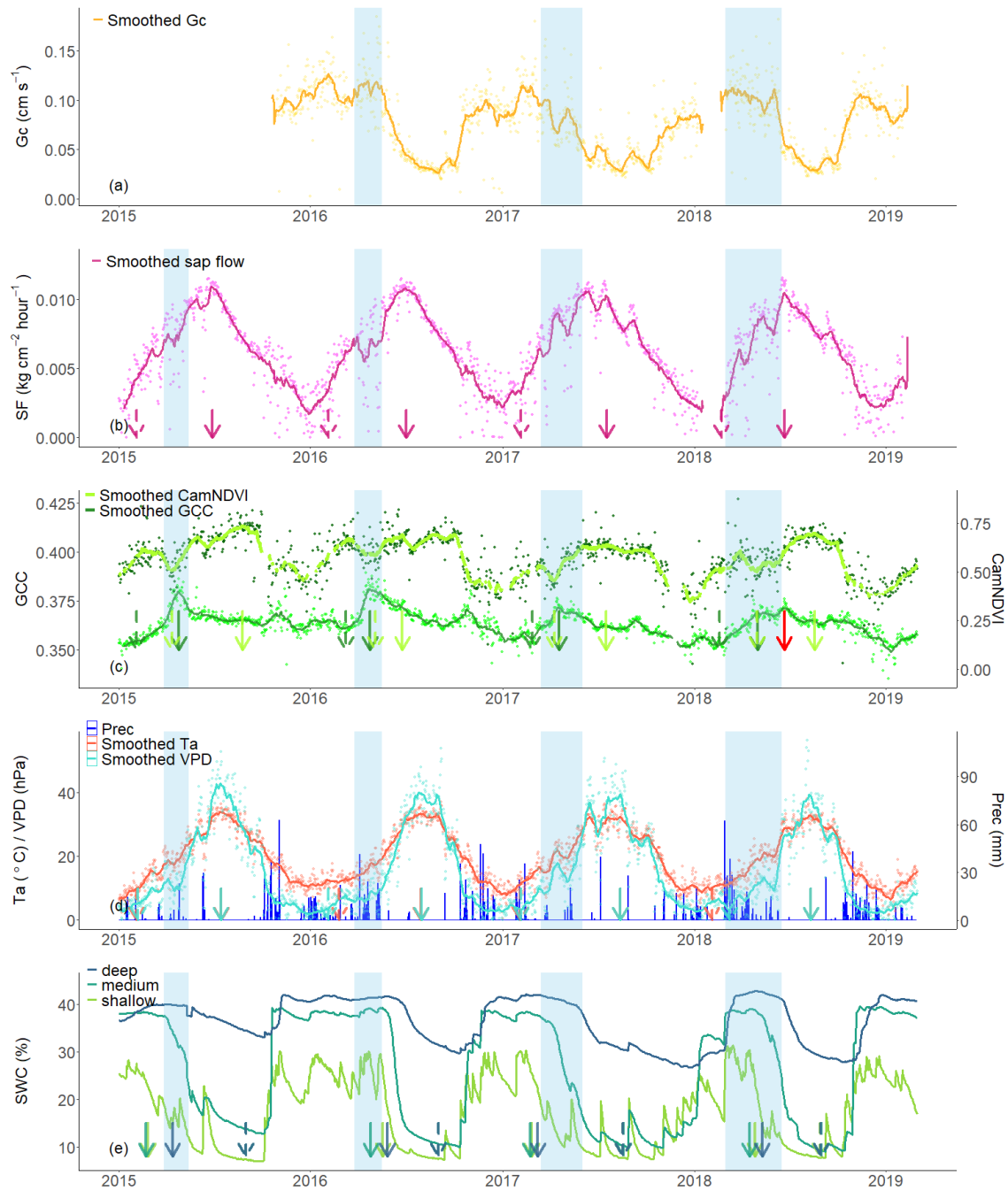


Figure 5. Relationship between GCC_{ASD} and biological traits for young, mature, and old leaves. Biological traits are chlorophyll content per leaf area (including chlorophyll a, and b: Chl_{ab}), nitrogen content per leaf area (N_{area}), water content per leaf area (WCA), and leaf mass per area (LMA). Young, mature, and old leaves are defined as the leaves younger than 3 months, younger than 1 year but older than 3 months, and older than 1 year, respectively. The shaded area around each regression line represents 95% confidence interval. The linear regression lines and their equations and coefficient of determination (R²) are displayed at the bottom of each panel if the regression is statistically significant. The significances (with p-value in the brackets) of linear regression for different age classes are displayed in the upper right in each panel. The p-value is reported as follows: *.01 ≤ p < .05, **.001 ≤ p < .01, and ns when p > .05.

3.2 Relationship between trees' water fluxes and their explanatory variables

3.2.1 Temporal variations

Figure 6 shows the temporal variation of trees' greenness and water fluxes for the different periods (Figure 6). Both GCC and SF started to increase at the beginning of the year, but they differed largely in the peak timing. GCC peaked in the middle of the flushing period in spring ($\text{DOY } 127 \pm 30 \text{ days}$), whereas SF peaked in summer ($\text{DOY } 188 \pm 10 \text{ days}$), which was earlier than the period of highest T_a , VPD, and CamNDVI (Figure 6). Compared to GCC, CamNDVI decreased at the beginning of the leaf flushing and then increased until autumn. SWC decreased rapidly before or during the leaf flushing, with the larger changes in the shallow and medium compared to deep soil depth. SWC reached the lowest value in late summer unless some rainfall occurred late in the season (Figure 6).



411

412

413

414

415

416

Figure 6. Time series of daily (a) canopy conductance (G_c), (b) SF (sap flux density), (c) PhenoCam green chromatic coordinate (GCC) and normalized difference vegetation index (CamNDVI), (d) meteorological factors (precipitation (Prec), air temperature (Ta), and vapor pressure deficit (VPD)), and (e) soil water content (SWC) of three different depths during 2015-2019 for evergreen oak trees. G_c time series starts in autumn 2015 because canopy surface temperature (T_s) used to calculate G_c was available only since that

time. Apart from Prec and SWC, all the variables are smoothed with a 20-day average moving window. The arrows represent the start (dashed) and the peak (solid) of the time series in each growing period (in panel b-f; the details of extraction method can be found in Note S5) between February and August each year. The blue shaded area indicates the leaf flushing period visually determined by PhenoCam images. The red arrow in panel (d) shows the second leaf flush after a caterpillar attack.

3.2.2 Relationships between water fluxes and their explanatory variables

The results of the multi-model GAM analysis indicated that the Gc model that included all the drivers (M4, “All VIs”) performed best according to the AIC values and explained 87% of the variance for variation of Gc (Table 2). It was followed by M2 (“GCC”). The GAM smoothing functions (Figure 7) showed that Ta and GCC were the most important drivers (larger partial effects than other drivers) for estimating variation of Gc. Specifically, GCC positively correlated with its partial effects on Gc. In contrast, it seems that CamNDVI had a varied relationship with its partial effects on Gc (decreasing and then slight increasing with the increase of CamNDVI value), with less partial contribution than GCC (Figure 7).

Likewise, M4 (“All VIs”) was the best model for estimating SF and explained 96% of the variance for variation of SF (Table 2). The partial effects plots indicated that the variation of SF was most sensitive to VPD changes, especially with a very strong positive relationship at low VPD values (Figure 8). The other drivers had a similar partial contribution to the variation of SF.

Table 2. Statistics of best Generalized Additive Model (GAM) for canopy conductance (Gc) and sap flux density (SF)^{1, 2}. Abbreviations: N, number of non-NA observations; R_{adj}^2 , adjusted coefficient of

	Best GAM	N	R_{adj}^2	ΔAIC_{M1-M4}	ΔAIC_{M2-M4}	ΔAIC_{M3-M4}
Gc	Gc ~ Intercept***+					
	s(SWDR)*** +					
	s(Ta)*** +					
	s(Ws)*** +	953	0.87	218.0	62.3	137.2
	s(SWC _{shallow})*** +					
	s(SWC _{deep})*** +					
	s(GCC)*** +					
	s(CamNDVI)***					
SF	SF ~ Intercept*** +					
	s(SWDR)*** +					
	s(VPD)***+					
	s(Ws)*** +	953	0.96	260.0	50.8	183.8
	s(SWC _{shallow})*** +					
	s(SWC _{deep})*** +					
	s(GCC)***+					
	s(CamNDVI)***					

determination; ΔAIC , difference of Akaike Information Criteria (AIC) values between different GAM models.

¹ s (variables) stands for smooth function for each predictor. The symbols “***” indicate the predictor’s partial effect significance levels of 0.001.

² The AIC differences (ΔAIC) between M1 and M4, M2 and M4, M3 and M4 were shown in the last three columns (M1-M4 are the four GAM models in Table1). ΔAIC compares the full model performance with the performance of the models only driven by climate, by climate and GCC, or by climate and CamNDVI, respectively.

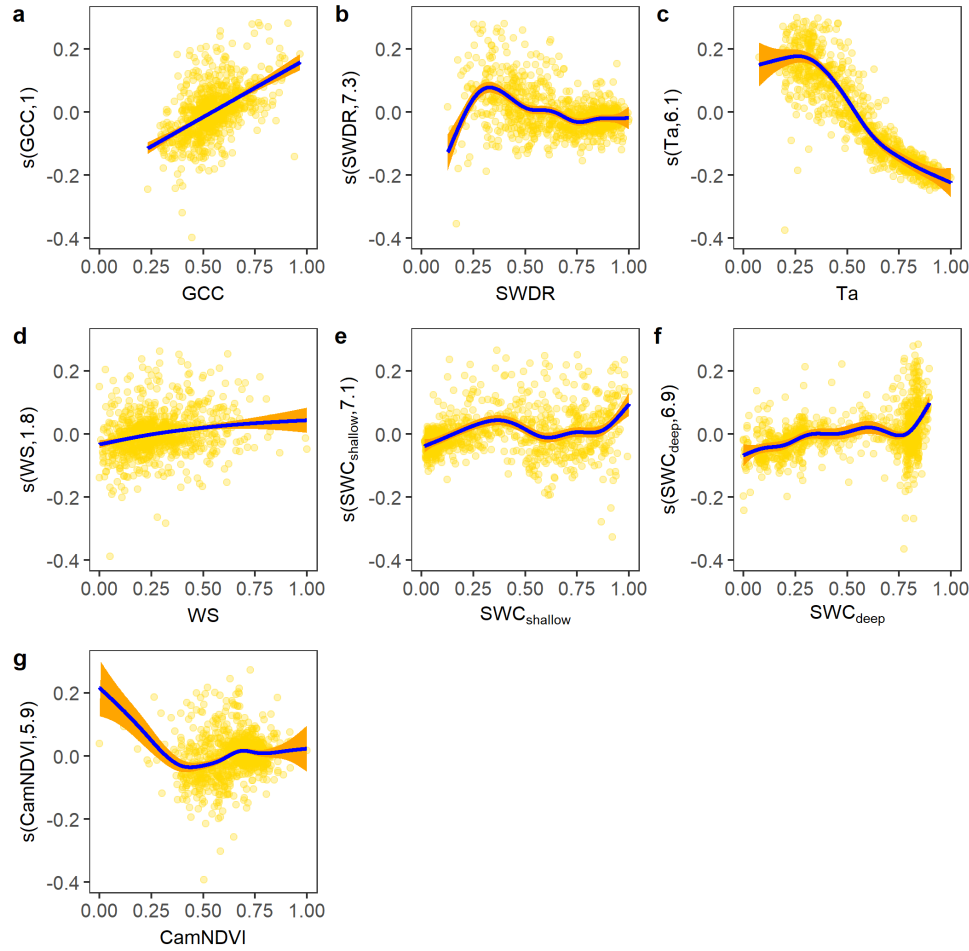
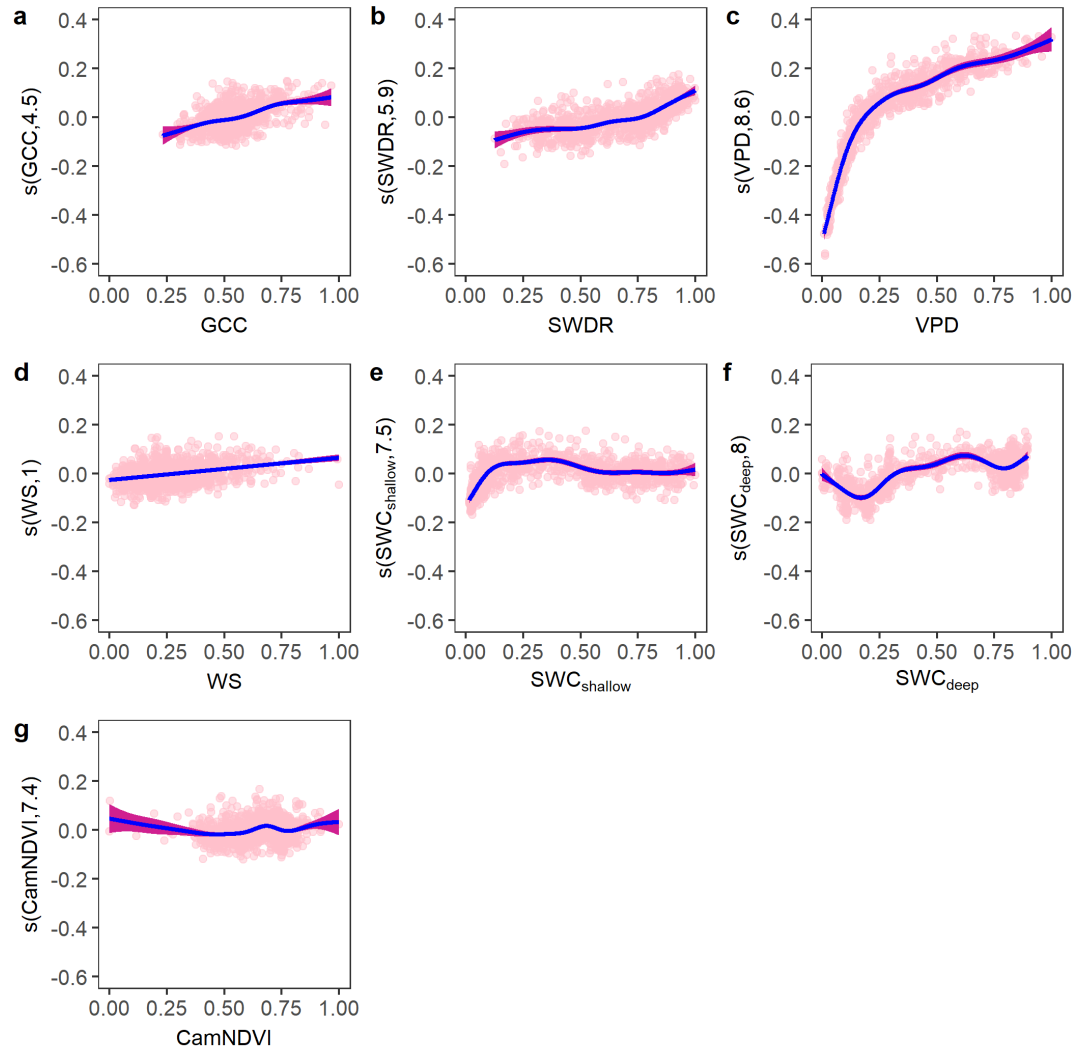


Figure 7. Partial effect plots for the drivers of canopy conductance (G_c) from the GAM analysis. The data points in each panel are the partial residuals. The y axis stands for the partial effects of each driver to the variation of G_c . The blue lines are GAM fitting lines. The orange shaded area represents the 95% confidence interval. The values in brackets in the y-axis labels are the effective degrees of freedom (edf) directly related to the complexity of the smooth function. The explanatory variables of G_c and their abbreviations are: PhenoCam green chromatic coordinate (GCC) and normalized difference vegetation index (CamNDVI), shortwave downwelling radiation (SWDR), air temperature (T_a), wind speed (WS), vapor pressure deficit (VPD), soil water content at shallow and deep soil depth (SWC_{shallow} , SWC_{deep}).



454

455

456

457

458

459

460

461

462

Figure 8. Partial effect plots for the drivers of sap flux density (SF) from the GAM analysis. The points in each panel are the partial residuals. The y axis stands for the partial effects of each driver to the variation of Gc. The blue lines are GAM fitting lines. The shaded area represents the 95% confidence interval. The values in brackets in the y-axis labels are the effective degrees of freedom (edf), directly related to the complexity of the smooth function for each variable. The explanatory variables of SF and their abbreviations are: PhenoCam green chromatic coordinate (GCC) and normalized difference vegetation index (CamNDVI), shortwave downwelling radiation (SWDR), air temperature (Ta), wind speed (WS), vapor pressure deficit (VPD), soil water content at shallow and deep soil depth (SWC_{shallow}, SWC_{deep}).

4. Discussion

4.1 New leaves flushing explains the rapid increase of canopy greenness in spring

Combining leaf and canopy-scale measurements, we proved that the annual variability of the evergreen Holm oak optical properties is dominated by the sprout and development of young leaves. Mature and old leaves' biophysical and optical properties are relatively similar and steady in time (Pacheco-Labrador *et al.*, 2017). Contrarily, young leaves feature distinct properties after sprouting. Such difference reduces as the new leaves mature, at the same time that the mature leaves become more and more abundant in the crown (Figure 3b). The combination of different properties and abundances in time leads to the rapid increase of PhenoCam GCC during the leaf flushing period.

Young leaves of Holm oak trees in our study ecosystem present significantly lower Chl_{ab} , N_{area} , LMA, and higher WCA than mature and old leaves (Figure 4). This variation is consistent with previous studies in Mediterranean (Niinemets *et al.*, 2004; Mediavilla *et al.*, 2011) and tropical evergreen tree species (Chavana-Bryant *et al.*, 2017; Wu *et al.*, 2017a). According to Figure 5 and sound radiative transfer theory (Féret *et al.*, 2017), the lower Chl_{ab} (correlated with N_{area}) is the main responsible for the lower VIS absorption and the higher GCC_{ASD} values in young leaves. These leaves darken (Figure S9), and their GCC_{ASD} rapidly decreases with maturation (Figure S12) as leaves accumulate more pigments, among other biochemical and anatomical changes (Sims & Gamon, 2003; Kokaly *et al.*, 2009; Serbin *et al.*, 2014; Chavana-Bryant *et al.*, 2017). In addition, as young leaves appear, a similar effect is observed in the canopy, both by the PhenoCam as well as more advanced optical sensors (Figure S13, Note S6). Leaf reflectance in the NIR and the SWIR regions also vary with the leaf age due to the development of complex internal cellular structure, epicuticular waxes or cuticles, and variation of leaf water and dry matter content in the leaves

(Reicosky & Hanover, 1978; Mulroy, 1979; Kokaly *et al.*, 2009; Asner *et al.*, 2015). Since LMA and WCA mainly related to the radiation absorption in the SWIR (Jacquemoud *et al.*, 1996), we observed the significant changes observed in WCA and LMA (Figure 4) had little effect on GCC_{ASD} (Figure 5). The variation in LMA and WCA can be characterized with high-resolution spectra measurements becoming more available (Wu *et al.*, 2017a; Meireles *et al.*, 2020).

We represented the canopy-scale GCC variation using the weighted leaf-level GCC_{ASD} by considering the distinct differences in spectral reflectance between young and mature/old leaves (Figure 2 and Figure 3). PhenoCam GCC and the weighted average of GCC_{ASD} run in parallel and were strongly correlated (Figure 3). These results align with previous studies, which found that leaf demography contributes significantly to the observed variation of canopy reflectance (Roberts *et al.*, 1998; Wu *et al.*, 2017a) and satellite greenness (Wu *et al.*, 2018). We are aware that the uncertainties in leaf age fractions propagate to the comparison between weighted average GCC_{ASD} and PhenoCam GCC. The direct up-scaling of GCC_{ASD} (weighted average) omits spectral contributions from branches, shadows, and different leaf angles and multiple-scattering. However, the structural properties of the Holm oaks in the site are known to be relatively stable (Melendo-Vega *et al.*, 2018). Thus, the variability of the upscaled leaf GCC should still covary with PhenoCam GCC despite a bias. This covariance proves the control of young leaves on canopy greenness variability during the phenological cycle. Wu *et al.* (2017b) have illustrated estimation of photosynthesis for tropical evergreen canopies can be improved by considering the effect of different leaf-age leaves' fraction and properties into the ecological modelling. Hence, proper consideration of leaf demography could improve the representation of greenness and functioning, providing insights into the functioning and properties of the different leaf cohorts and the resources

management strategies of the trees in response to the environment (Albert *et al.*, 2018; Gast *et al.*, 2020).

4.2 Can greenness explain the canopy conductance variation of evergreen broadleaved trees?

Variation of Gc in evergreen broadleaved trees can be explained by both of the changes in greenness and environmental drivers such as Ta (Figure 6 and Figure 7). Gc decreased with VPD due to stomatal closure, preventing excessive water loss under high evaporative demand (Way *et al.*, 2015; Slot & Winter, 2017). This is a recurrent strategy of iso-hydric vegetation such as the Mediterranean Holm oak in our study ecosystem (Quero *et al.*, 2011). In contrast, the GCC positively correlated to the variation of Gc (Figure 7). This positive correlation could be attributed to the leaf ontogeny information conveyed in GCC. Previous studies have found that young leaves exhibit higher canopy conductance than mature leaves (Mediavilla & Escudero, 2003; Whitehead *et al.*, 2011). As we showed, canopy GCC conveys age-related physiological information from different leaf-age classes (Figure 4). Therefore, it is plausible to use GCC as one of the explanatory variables to explain Gc's variation in evergreen broadleaved trees.

VPD is the main driver of SF for evergreen broadleaved trees, while greenness marginally explains the variation of SF (Figure 6 and Figure 8). Consistent with previous studies (Gates, 1968; O'Grady *et al.*, 1999; Vicente-Serrano *et al.*, 2020), the water transport of trees is accelerated by the rise of evaporative demand especially when the water is not limited during the period of the spring in our study sites (Figure 6). With continuous high temperature and low precipitation from mid-to-late summer (Perez-Priego *et al.*, 2017; Luo *et al.*, 2018), water from deep soil accessed by deep roots from trees also becomes limiting (Figure 6; Moreno *et al.* (2005)). Consequently, the driving force from VPD to the SF becomes flat, which is also confirmed from former studies in other semi-arid ecosystems (Skelton *et al.*, 2013; Zha *et al.*, 2017; El-Madany *et al.*, 2020). We only found

marginal contribution from VIs to the variation of SF (Figure 8). The development of observation networks such as PhenoCam (Richardson *et al.*, 2018) and global sap flow networks (Poyatos *et al.*, 2020) would enable us to further explore and validate the relationships we observed between greenness and water fluxes for evergreen broadleaved trees.

We demonstrated that linking evergreen broadleaved trees' greenness with their water flux dynamics in semi-arid tree-grass ecosystems is feasible. We showed that PhenoCam data might help to interpret the variation of Gc, SF, and potentially the changes in stem growth (peak of GCC shows the coordination with the start of trunk perimeter demonstrated in Figure S14). Further research can seek additional connections of greenness with canopy development by focusing on the specific important periods (e.g. rapid greenness increases), which might help us better understand the variation of functioning. For instance, compared to the rapid increase of GCC during the leaf flushing period, we observed the first decreasing and then increasing CamNDVI (Figure 6). This is most likely resulting from the changes in LAI induced by litterfall according to a study conducted on the same species in the Mediterranean ecosystem (Soudani *et al.*, 2012). Including more field LAI and litterfall measurements in this period, we could better disentangle the contribution from different leaf-age (young, mature, and old) leaves to the changes in trees' water fluxes through using leaf demography-ontogeny models (Wu *et al.*, 2016; Wu *et al.*, 2017b). The linkage between PhenoCam greenness and leaf measurements as well as plants' functioning (water fluxes and stem growth) highlights the opportunities to biologically interpretation of near-surface remote sensing greenness in different biomes and better representation of the functioning with the continuous growth of PhenoCam network.

5. Conclusion

In this work, we analyzed the relationships between evergreen trees' greenness (at the leaf- and canopy-scales) and their leaf traits, canopy conductance, and sap flow. We showed that: 1) young leaves flushing with distinct leaf spectral properties lead to a rapid increase of PhenoCam canopy GCC in spring; 2) variation of PhenoCam GCC is related to the leaf traits properties in different leaf-age classes, and considering the leaf fraction of different leaf-age leaves and their spectral properties can well explain the variation of canopy GCC; 3) PhenoCam GCC is an important explanatory factor of variation in the canopy conductance for evergreen broadleaved trees. VPD mainly drives while greenness marginally explains the variation of sap flow. In summary, we demonstrated that variation of PhenoCam GCC is biologically linked to leaf ontogeny and trait variation among different leaf-age classes. It can be used to identify the leaf flushing and explain the variation of trees' functioning. This highlights the opportunities to biologically interpret the near-surface greenness in different biomes and improve predicting or representation of plant functioning with the continuous growth of PhenoCam and functioning-related global observation networks.

Acknowledgments

The authors acknowledge the Alexander von Humboldt Foundation for supporting this research with the Max Planck Prize to Markus Reichstein. Yunpeng Luo and Mirco Migliavacca gratefully acknowledge the financial support from the China Scholarship Council. ADR acknowledges support for the PhenoCam network from the National Science Foundation (DEB- 1702697). Javier Pacheco-Labrador and Mirco Migliavacca acknowledge the German Aerospace Center (DLR) project OBEF-Accross2 "The Potential of Earth Observations to Capture Patterns of Biodiversity" (Contract No. 50EE1912). The research also received funding from the European Union's Horizon

2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 721995 and Ministerio de Economía y Competitividad through FLUXPEC CGL2012-34383 and SynerTGE CGL2015-G9095-R (MINECO/FEDER, UE) projects. We are grateful to all the colleagues who contributed to the acquisition and processing of the field data.

References

- Ahlström A, Raupach MR, Schurgers G, Smith B, Arneth A, Jung M, Reichstein M, Canadell JG, Friedlingstein P, Jain AK, et al. 2015. The dominant role of semi-arid ecosystems in the trend and variability of the land CO₂ sink. *Science* **348**(6237): 895-899.
- Albert LP, Wu J, Prohaska N, de Camargo PB, Huxman TE, Tribuzy ES, Ivanov VY, Oliveira RS, Garcia S, Smith MN, et al. 2018. Age-dependent leaf physiology and consequences for crown-scale carbon uptake during the dry season in an Amazon evergreen forest. *New Phytologist* **219**(3): 870-884.
- Asner GP, Martin RE, Anderson CB, Knapp DE. 2015. Quantifying forest canopy traits: Imaging spectroscopy versus field survey. *Remote Sensing of Environment* **158**: 15-27.
- Barbeta A, Peñuelas J. 2016. Sequence of plant responses to droughts of different timescales: lessons from holm oak (*Quercus ilex*) forests. *Plant Ecology & Diversity* **9**(4): 321-338.
- Bogdanovich E, Perez-Priego O, El-Madany TS, Guderle M, Pacheco-Labrador J, Levick SR, Moreno G, Carrara A, Pilar Martín M, Migliavacca M. 2021. Using terrestrial laser scanning for characterizing tree structural parameters and their changes under different management in a Mediterranean open woodland. *Forest Ecology and Management* **486**: 118945.
- Brown TB, Hultine KR, Steltzer H, Denny EG, Denslow MW, Granados J, Henderson S, Moore D, Nagai S, SanClements M, et al. 2016. Using phenocams to monitor our changing Earth: toward a global phenocam network. *Frontiers in Ecology and the Environment* **14**(2): 84-93.
- Burgess SSO, Adams MA, Turner NC, Beverly CR, Ong CK, Khan AAH, Bleby TM. 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants†. *Tree Physiology* **21**(9): 589-598.
- Chavana-Bryant C, Malhi Y, Wu J, Asner GP, Anastasiou A, Enquist BJ, Cosio Caravasi EG, Doughty CE, Saleska SR, Martin RE, et al. 2017. Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements. *New Phytologist* **214**(3): 1049-1063.
- El-Madany TS, Carrara A, Martín MP, Moreno G, Kolle O, Pacheco-Labrador J, Weber U, Wutzler T, Reichstein M, Migliavacca M. 2020. Drought and heatwave impacts on semi-arid ecosystems' carbon fluxes along a precipitation gradient. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**(1810): 20190519.
- El-Madany TS, Reichstein M, Carrara A, Martín MP, Moreno G, Gonzalez-Cascon R, Peñuelas J, Ellsworth DS, Burchard-Levine V, Hammer TW, et al. 2021. How Nitrogen and Phosphorus Availability Change Water Use Efficiency in a Mediterranean Savanna Ecosystem. *Journal of Geophysical Research: Biogeosciences* **126**(5): e2020JG006005.
- El-Madany TS, Reichstein M, Perez-Priego O, Carrara A, Moreno G, Pilar Martín M, Pacheco-Labrador J, Wohlfahrt G, Nieto H, Weber U, et al. 2018. Drivers of spatio-temporal variability of carbon dioxide and energy fluxes in a Mediterranean savanna ecosystem. *Agricultural and Forest Meteorology* **262**: 258-278.

- Féret JB, Gitelson AA, Noble SD, Jacquemoud S. 2017. PROSPECT-D: Towards modeling leaf optical properties through a complete lifecycle. *Remote Sensing of Environment* **193**: 204-215.
- Filippa G, Cremonese E, Migliavacca M, Galvagno M, Forkel M, Wingate L, Tomelleri E, Di Cella UM, Richardson AD. 2016. Phenopix: AR package for image-based vegetation phenology. *Agricultural and Forest Meteorology* **220**: 141-150.
- Filippa G, Cremonese E, Migliavacca M, Galvagno M, Sonnentag O, Humphreys E, Hufkens K, Ryu Y, Verfaillie J, Morra di Cella U, et al. 2018. NDVI derived from near-infrared-enabled digital cameras: Applicability across different plant functional types. *Agricultural and Forest Meteorology* **249**: 275-285.
- Friedl MA, McIver DK, Hodges JCF, Zhang XY, Muchoney D, Strahler AH, Woodcock CE, Gopal S, Schneider A, Cooper A, et al. 2002. Global land cover mapping from MODIS: algorithms and early results. *Remote Sensing of Environment* **83**(1-2): 287-302.
- Gast A, Römermann C, Bucher S. 2020. Special issue in honour of Prof. Reto J. Strasser—Seasonal variation and trade-off between frost resistance and photosynthetic performance in woody species. *Photosynthetica* **58**(SPECIAL ISSUE): 331-340.
- Gates DM. 1968. Transpiration and leaf temperature. *Annual Review of Plant Physiology* **19**(1): 211-238.
- Gonzalez-Cascon R, Jiménez-Fenoy L, Verdú-Fillola I, Martín MP. 2017. Aqueous-acetone extraction improves the drawbacks of using dimethylsulfoxide as solvent for photometric pigment quantification in Quercus ilex leaves. *Forest Systems (Online)* **26**(2): 5.
- González-Cascón R, Pacheco-Labrador J, Martín M. 2016. Evolution of spectral behavior and chemical composition in the tree canopy of a dehesa ecosystem. *Revista de Teledetección*(46): 31-43.
- Hanan N, Hill M. 2012. Savannas in a Changing Earth System: The NASA Terrestrial Ecology Tree-Grass Project. White Paper for the NASA Terrestrial Ecology Program. *Earth Science Division, Washington, USA*.
- Hastie TJ, Tibshirani RJ. 1990. Generalized additive models, volume 43 of. *Monographs on statistics and applied probability* **15**.
- Hengl T, Walsh MG, Sanderman J, Wheeler I, Harrison SP, Prentice IC. 2018. Global mapping of potential natural vegetation: an assessment of machine learning algorithms for estimating land potential. *PeerJ* **6**: e5457-e5457.
- Huete A, Didan K, Miura T, Rodriguez EP, Gao X, Ferreira LG. 2002. Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment* **83**(1): 195-213.
- Jacquemoud S, Ustin SL, Verdebout J, Schmuck G, Andreoli G, Hosgood B. 1996. Estimating leaf biochemistry using the PROSPECT leaf optical properties model. *Remote Sensing of Environment* **56**(3): 194-202.
- Keenan TF, Darby B, Felts E, Sonnentag O, Friedl MA, Hufkens K, O'Keef J, Klosterman S, Munger JW, Toome M, et al. 2014. Tracking forest phenology and seasonal physiology using digital repeat photography: a critical assessment. *Ecol Appl* **24**(6): 1478-1489.
- Knauer J, El-Madany TS, Zaehle S, Migliavacca M. 2018. Bigleaf—An R package for the calculation of physical and physiological ecosystem properties from eddy covariance data. *PLOS ONE* **13**(8): e0201114.
- Kokaly RF, Asner GP, Ollinger SV, Martin ME, Wessman CA. 2009. Characterizing canopy biochemistry from imaging spectroscopy and its application to ecosystem studies. *Remote Sensing of Environment* **113**: S78-S91.
- Liu Y, Hill MJ, Zhang X, Wang Z, Richardson AD, Hufkens K, Filippa G, Baldocchi DD, Ma S, Verfaillie J, et al. 2017. Using data from Landsat, MODIS, VIIRS and PhenoCams to monitor the phenology of California oak/grass savanna and open grassland across spatial scales. *Agricultural and Forest Meteorology* **237-238**: 311-325.

- Lopes AP, Nelson BW, Wu J, Graca P, Tavares JV, Prohaska N, Martins GA, Saleska SR. 2016.** Leaf flush drives dry season green-up of the Central Amazon. *Remote Sensing of Environment* **182**: 90-98.
- Luo Y, El-Madany T, Ma X, Nair R, Jung M, Weber U, Filippa G, Bucher SF, Moreno G, Cremonese E, et al. 2020.** Nutrients and water availability constrain the seasonality of vegetation activity in a Mediterranean ecosystem. *Global Change Biology* **26**(8): 4379-4400.
- Luo Y, El-Madany TS, Filippa G, Ma X, Ahrens B, Carrara A, Gonzalez-Cascon R, Cremonese E, Galvagno M, Hammer TW, et al. 2018.** Using Near-Infrared-Enabled Digital Repeat Photography to Track Structural and Physiological Phenology in Mediterranean Tree–Grass Ecosystems. *Remote Sensing* **10**(8): 1293.
- Mediavilla S, Escudero A. 2003.** Stomatal responses to drought at a Mediterranean site: a comparative study of co-occurring woody species differing in leaf longevity. *Tree Physiology* **23**(14): 987-996.
- Mediavilla S, González-Zurdo P, García-Ciudad A, Escudero A. 2011.** Morphological and chemical leaf composition of Mediterranean evergreen tree species according to leaf age. *Trees* **25**(4): 669-677.
- Meireles JE, Cavender-Bares J, Townsend PA, Ustin S, Gamon JA, Schweiger AK, Schaepman ME, Asner GP, Martin RE, Singh A, et al. 2020.** Leaf reflectance spectra capture the evolutionary history of seed plants. *New Phytologist* **228**(2): 485-493.
- Melendo-Vega JR, Martín MP, Pacheco-Labrador J, González-Cascón R, Moreno G, Pérez F, Migliavacca M, García M, North P, Riaño D. 2018.** Improving the Performance of 3-D Radiative Transfer Model FLIGHT to Simulate Optical Properties of a Tree-Grass Ecosystem. *Remote Sensing* **10**(12): 2061.
- Migliavacca M, Galvagno M, Cremonese E, Rossini M, Meroni M, Sonnentag O, Cogliati S, Manca G, Diotri F, Busetto L, et al. 2011.** Using digital repeat photography and eddy covariance data to model grassland phenology and photosynthetic CO₂ uptake. *Agricultural and Forest Meteorology* **151**(10): 1325-1337.
- Moore CE, Brown T, Keenan TF, Duursma RA, van Dijk A, Beringer J, Culvenor D, Evans B, Huete A, Hutley LB, et al. 2016.** Reviews and syntheses: Australian vegetation phenology: new insights from satellite remote sensing and digital repeat photography. *Biogeosciences* **13**(17): 5085-5102.
- Moreno G, Obrador JJ, Cubera E, Dupraz C. 2005.** Fine Root Distribution in Dehesas of Central-Western Spain. *Plant and Soil* **277**(1): 153-162.
- Moser WK, Coble AP, Hallik L, Richardson AD, Pisek J, Adamson K, Graham RT, Moser CF. 2020.** Advances in understanding canopy development in forest trees [Chapter 3]. In: *Stanturf, John A., ed. Achieving sustainable management of boreal and temperate forests. Cambridge, UK: Burleigh and Dodds Science Publishing. p. 59-98.: 59-98.*
- Mulroy TW. 1979.** Spectral properties of heavily glaucous and non-glaucous leaves of a succulent rosette-plant. *Oecologia* **38**(3): 349-357.
- Myneni RB, Keeling CD, Tucker CJ, Asrar G, Nemani RR. 1997.** Increased plant growth in the northern high latitudes from 1981 to 1991. *Nature* **386**(6626): 698-702.
- Nasahara KN, Nagai S. 2015.** Review: Development of an in situ observation network for terrestrial ecological remote sensing: the Phenological Eyes Network (PEN). *Ecological Research* **30**(2): 211-223.
- Niinemets Ü, Tenhunen JD, Beyschlag W. 2004.** Spatial and age-dependent modifications of photosynthetic capacity in four Mediterranean oak species. *Functional Plant Biology* **31**(12): 1179-1193.
- O'Grady AP, Eamus D, Hutley LB. 1999.** Transpiration increases during the dry season: patterns of tree water use in eucalypt open-forests of northern Australia. *Tree Physiology* **19**(9): 591-597.

- Pacheco-Labrador J, El-Madany TS, Martín MP, Migliavacca M, Rossini M, Carrara A, Zarco-Tejada PJ. 2017.** Spatio-temporal relationships between optical information and carbon fluxes in a mediterranean tree-grass ecosystem. *Remote Sensing* **9**(6): 608.
- Pacheco-Labrador J, González-Cascón R, Martín MP, Riaño D. 2014.** Understanding the optical responses of leaf nitrogen in Mediterranean Holm oak (*Quercus ilex*) using field spectroscopy. *International Journal of Applied Earth Observation and Geoinformation* **26**: 105-118.
- Perez-Priego O, El-Madany TS, Migliavacca M, Kowalski AS, Jung M, Carrara A, Kolle O, Martín MP, Pacheco-Labrador J, Moreno G, et al. 2017.** Evaluation of eddy covariance latent heat fluxes with independent lysimeter and sapflow estimates in a Mediterranean savannah ecosystem. *Agricultural and Forest Meteorology* **236**: 87-99.
- Petach AR, Toomey M, Aubrecht DM, Richardson AD. 2014.** Monitoring vegetation phenology using an infrared-enabled security camera. *Agricultural and Forest Meteorology* **195-196**: 143-151.
- Phillips N, Oren R. 1998.** A comparison of daily representations of canopy conductance based on two conditional time-averaging methods and the dependence of daily conductance on environmental factors. *Ann. For. Sci.* **55**(1-2): 217-235.
- Poulter B, Frank D, Ciais P, Myneni RB, Andela N, Bi J, Broquet G, Canadell JG, Chevallier F, Liu YY, et al. 2014.** Contribution of semi-arid ecosystems to interannual variability of the global carbon cycle. *Nature* **509**(7502): 600-603.
- Poyatos R, Granda V, Flo V, Adams MA, Adorján B, Aguadé D, Aïdar MPM, Allen S, Alvarado-Barrientos MS, Anderson-Teixeira KJ, et al. 2020.** Global transpiration data from sap flow measurements: the SAPFLUXNET database. *Earth Syst. Sci. Data Discuss.* **2020**: 1-57.
- Quero JL, Sterck FJ, Martínez-Vilalta J, Villar R. 2011.** Water-use strategies of six co-existing Mediterranean woody species during a summer drought. *Oecologia* **166**(1): 45-57.
- Reicosky DA, Hanover JW. 1978.** Physiological Effects of Surface Waxes: I. Light Reflectance for Glaucous and Nonglaucous *Picea pungens*. *Plant physiology* **62**(1): 101-104.
- Richardson AD, Braswell BH, Hollinger DY, Jenkins JP, Ollinger SV. 2009.** Near-surface remote sensing of spatial and temporal variation in canopy phenology. *Ecological Applications* **19**(6): 1417-1428.
- Richardson AD, Hufkens K, Milliman T, Aubrecht DM, Chen M, Gray JM, Johnston MR, Keenan TF, Klosterman ST, Kosmala M, et al. 2018.** Tracking vegetation phenology across diverse North American biomes using PhenoCam imagery. *Scientific Data* **5**(1): 180028.
- Richardson AD, Jenkins JP, Braswell BH, Hollinger DY, Ollinger SV, Smith M-L. 2007.** Use of digital webcam images to track spring green-up in a deciduous broadleaf forest. *Oecologia* **152**(2): 323-334.
- Roberts DA, Nelson BW, Adams JB, Palmer F. 1998.** Spectral changes with leaf aging in Amazon caatinga. *Trees-Structure and Function* **12**(6): 315-325.
- Rolo V, Moreno G. 2012.** Interspecific competition induces asymmetrical rooting profile adjustments in shrub-encroached open oak woodlands. *Trees* **26**(3): 997-1006.
- Serbin SP, Singh A, McNeil BE, Kingdon CC, Townsend PA. 2014.** Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications* **24**(7): 1651-1669.
- Seyednasrollah B, Young AM, Hufkens K, Milliman T, Friedl MA, Froking S, Richardson AD. 2019.** Tracking vegetation phenology across diverse biomes using Version 2.0 of the PhenoCam Dataset. *Scientific Data* **6**(1): 222.
- Sims DA, Gamon JA. 2003.** Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: a comparison of indices based on liquid water and chlorophyll absorption features. *Remote Sensing of Environment* **84**(4): 526-537.

- Skelton RP, West AG, Dawson TE, Leonard JM. 2013.** External heat-pulse method allows comparative sapflow measurements in diverse functional types in a Mediterranean-type shrubland in South Africa. *Funct Plant Biol* **40**(10): 1076-1087.
- Slot M, Winter K. 2017.** In situ temperature relationships of biochemical and stomatal controls of photosynthesis in four lowland tropical tree species. *Plant, Cell & Environment* **40**(12): 3055-3068.
- Soudani K, Hmimina G, Delpierre N, Pontailier JY, Aubinet M, Bonal D, Caquet B, de Grandcourt A, Burban B, Flechard C, et al. 2012.** Ground-based Network of NDVI measurements for tracking temporal dynamics of canopy structure and vegetation phenology in different biomes. *Remote Sensing of Environment* **123**: 234-245.
- Toomey M, Friedl MA, Frolking S, Hufkens K, Klosterman S, Sonnentag O, Baldocchi DD, Bernacchi CJ, Biraud SC, Bohrer G, et al. 2015.** Greenness indices from digital cameras predict the timing and seasonal dynamics of canopy-scale photosynthesis. *Ecological Applications* **25**(1): 99-115.
- Tucker CJ. 1979.** Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing of Environment* **8**(2): 127-150.
- Vicente-Serrano SM, McVicar TR, Miralles DG, Yang Y, Tomas-Burguera M. 2020.** Unraveling the influence of atmospheric evaporative demand on drought and its response to climate change. *WIREs Climate Change* **11**(2): e632.
- Way DA, Oren R, Kroner Y. 2015.** The space-time continuum: the effects of elevated CO₂ and temperature on trees and the importance of scaling. *Plant Cell Environ* **38**(6): 991-1007.
- Whitehead D, Barbour MM, Griffin KL, Turnbull MH, Tissue DT. 2011.** Effects of leaf age and tree size on stomatal and mesophyll limitations to photosynthesis in mountain beech (*Nothofagus solandrii* var. *cliffortioides*). *Tree Physiology* **31**(9): 985-996.
- Wingate L, Ogee J, Cremonese E, Filippa G, Mizunuma T, Migliavacca M, Moisy C, Wilkinson M, Moureaux C, Wohlfahrt G, et al. 2015.** Interpreting canopy development and physiology using a European phenology camera network at flux sites. *Biogeosciences* **12**(20): 5995-6015.
- Wood SN. 2017.** *Generalized additive models: an introduction with R*: CRC press.
- Wu C, Niu Z, Tang Q, Huang W, Rivard B, Feng J. 2009.** Remote estimation of gross primary production in wheat using chlorophyll-related vegetation indices. *Agricultural and Forest Meteorology* **149**(6): 1015-1021.
- Wu J, Albert LP, Lopes AP, Restrepo-Coupe N, Hayek M, Wiedemann KT, Guan K, Stark SC, Christoffersen B, Prohaska N, et al. 2016.** Leaf development and demography explain photosynthetic seasonality in Amazon evergreen forests. *Science* **351**(6276): 972-976.
- Wu J, Chavana-Bryant C, Prohaska N, Serbin SP, Guan K, Albert LP, Yang X, van Leeuwen WJD, Garnello AJ, Martins G, et al. 2017a.** Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. *New Phytologist* **214**(3): 1033-1048.
- Wu J, Kobayashi H, Stark SC, Meng R, Guan K, Tran NN, Gao S, Yang W, Restrepo-Coupe N, Miura T, et al. 2018.** Biological processes dominate seasonality of remotely sensed canopy greenness in an Amazon evergreen forest. *New Phytologist* **217**(4): 1507-1520.
- Wu J, Serbin SP, Xu X, Albert LP, Chen M, Meng R, Saleska SR, Rogers A. 2017b.** The phenology of leaf quality and its within-canopy variation is essential for accurate modeling of photosynthesis in tropical evergreen forests. *Global Change Biology* **23**(11): 4814-4827.
- Yan D, Scott RL, Moore DJP, Biederman JA, Smith WK. 2019.** Understanding the relationship between vegetation greenness and productivity across dryland ecosystems through the integration of PhenoCam, satellite, and eddy covariance data. *Remote Sensing of Environment* **223**: 50-62.
- Yang H, Yang X, Heskel M, Sun S, Tang J. 2017.** Seasonal variations of leaf and canopy properties tracked by ground-based NDVI imagery in a temperate forest. *Scientific Reports* **7**(1): 1267.

805 **Yang X, Tang J, Mustard JF. 2014.** Beyond leaf color: Comparing camera-based phenological metrics
806 with leaf biochemical, biophysical, and spectral properties throughout the growing season of a
807 temperate deciduous forest. *Journal of Geophysical Research: Biogeosciences* **119**(3): 181-191.
808 **Zha T, Qian D, Jia X, Bai Y, Tian Y, Bourque CPA, Ma J, Feng W, Wu B, Peltola H. 2017.** Soil moisture
809 control of sap-flow response to biophysical factors in a desert-shrub species, *Artemisia ordosica*.
810 *Biogeosciences* **14**(19): 4533-4544.
811 **Zhang X, Friedl MA, Schaaf CB, Strahler AH, Hodges JCF, Gao F, Reed BC, Huete A. 2003.** Monitoring
812 vegetation phenology using MODIS. *Remote Sensing of Environment* **84**(3): 471-475.
813 **Zhao JB, Zhang YP, Tan ZH, Song QH, Liang NS, Yu L, Zhao JF. 2012.** Using digital cameras for
814 comparative phenological monitoring in an evergreen broad-leaved forest and a seasonal rain
815 forest. *Ecological Informatics* **10**: 65-72.

816