Dear Editor,

We are pleased to send you a modified version of the manuscript entitled "Sun-induced Chlorophyll fluorescence and PRI improve remote sensing GPP estimates under varying nutrient availability in a typical Mediterranean savanna ecosystem” which is being revised for possible publication in Biogeoscience.

We are grateful for the relevant feedbacks and comments received and we have addressed the main comments according to the reviewers’ suggestions.

The following pages contain our responses to each of the comments from the Referees with a detailed description of the modifications introduced in the manuscript as a result of remarks from the Referees. As it was suggested, we also included a marked-up manuscript version.

Please note the following distinctions in types:

→ comment from the Reviewer

← response from the authors

We are confident to have fully answered all questions and incorporated all the recommendations in the revised paper, and we hope that the revised manuscript can be accepted for future publication.

Best regards,

Oscar Perez-Priego & co-authors

Referee 1

→ This paper by Perez-Priego et al. evaluates the performance of sun-induced chlorophyll fluorescence and the PRI to estimate GPP variation in response to nutrient availability in a Mediterranean savanna grassland. In addition to being a very well planned and executed study, the authors conduct a detailed analysis comparing the predictive power of SIF and PRI to that of baseline alternative approaches using greenness and other meteorological data. They show that both SIF and PRI correlated well with GPP in response to fertilization, while greenness indices (i.e. NDVI and MTCI) failed to do so. Despite that some sentences are written in a bit complex stile, the work is well presented and the text flows well. Overall, I believe this is a significant and original contribution that adds to the increasing number of studies evidencing the potential of SIF and PRI to improve our capacity to estimate GPP dynamics, in this case, in response to plant nutrient status. I think this study certainly deserves to be published in Biogeosciences after a few issues are addressed.

← We greatly appreciate the Referees' in-depth review and constructive comments.

1) The use of the term GPP is a bit confusing. The authors use the terms GPP2000, maximum daily GPP, daily average GPP, and then "GPP" alone. Accordingly, it is not clear what exactly it is denoted when "GPP" is used alone (instantaneous?, mean?, max?, noon?...). The authors should clarify the terms throughout the paper.

← We thank the Referee for this comment and apologize for the confusion. We agree that GPP is used in a confusing way throughout the manuscript without specifying neither its temporal resolution or the timing on which it was measured (or modeled like in the case of the GPP_{2000}). We have considered the Referee's comment, and have in the abbreviations list the different terms and their description:

- GPP: gross primary productivity or instantaneous gross photosynthetic rate.

-GPP_{noon}: instantaneous gross photosynthetic rate taken at solar noon (between 11:00 and 15:00 pm solar time).

-GPP_{2000}: gross photosynthetic rate estimated at 2000 of PAR derived from the fitting of the light response curve on GPP and PAR (Ruimy et al., 1995)

-GPP_{daily}: mean value of the diurnal time course of gross photosynthetic rate.

These abbreviations have been referred throughout the manuscript as suggested.

2) From the M&M it is understood that chamber measurements were conducted with three rotating chambers and that each measurement lasted for approximately 3 minutes. But there is no information on what was the temporal range of these measurements: Where they conducted from

sunrise until sunset? This seems to be the case otherwise they could not have constructed their light response data in Figure 2. Once this is clarified they could also mention briefly how was maximum daily GPP and daily average GPP calculated. In the same lines, it would be good to mention explicitly how the PLRC curve was obtained (e.g. by pooling together all diurnal measurements for each treatment and sampling date).

← We have better clarified the temporal range of these measurements and how PLRC curves were obtained in lines 327-331 as suggested:

"To assess how GPP is modulated by light among treatments and over the phenological cycle of the herbaceous stratum, we computed the parameters of photosynthetic light response curve (PLRC). Specifically, the Michaelis–Menten function was fitted to GPP and PAR data taken throughout course of the day (from sunrise until sunset) for each field campaign as follows…"

As we mentioned above, a better description regarding the use of GPP has been adopted throughout the manuscript.

3) Apparently, the authors use measured VPD and soil moisture as inputs in the MM model. Although it is stated that soil moisture was measured with a Theta Probe, there is no information as to how VPD was estimated. Perhaps they could add a clarification in Page 11899, lines 16-18: e.g. "Chamber humidity data was used to estimate VPD".

← We have clarified this as suggested (L246-248).

"Vapor pressure deficit (VPD) was computed using Tc and relative humidity, which was derived from water vapor molar fraction measured with the IRGA."

4) It is stated that chamber measurements lasted for 3 minutes, but could the authors provide a bit more of information as to how long it took to reach equilibration before NEE was measured, and similarly, how long it took to reach the steady state for Reco after placing the dark cloth?

← Following both Referee's recommendations, we have further described this in the manuscript (L257-276).

The chamber is open and ventilated during ~1 min after NEE measurement and the opaque blanket covers the chamber just after we place the chamber back on the collar. In addition of this 1 min, we must consider the starting time (~15:25 s) – the period that defines the initial slope of the fit after chamber deployment on which the "undisturbed" flux is estimated. Obviously, this "starting time" time is not fixed since we must also consider the need of both to stabilize the chamber atmosphere following deployment and for transport of sample air from chamber to gas analyzer. This starting time is automatically estimated using a change point detection algorithm

(Killick and Eckley, 2010), and implemented in the bootstrap resampling-based algorithm (Perez-Priego et al., 2015).

5) Page 11911, Lines 22-29. I think the analysis presented in Figure 9 and its implications are very interesting and the authors could expand a bit on it in the discussion. Their analysis in Figure 9 nicely shows the complementarity between NDVI and PRI/SIF. At low GPP levels, NDVI and not Fy760 or PRI respond to GPP, whereas at high GPP levels it is Fy760 and PRI but less NDVI that respond to GPP. Could we build on this complementarity to better track GPP dynamics?

← We have considered the Reviewer's comment and have further discussed the implications of this analysis (L564-569).

"Figure 9 suggests that the relationship between NDVI and sPRI or Fy760 is not unique and NDVI may play an important role in driving GPP in ecosystem characterized by marked seasonal variations. Our results highlight the complementarity between NDVI and Fy760 or sPRI. Particularly, NDVI assisted Fy760 or sPRI in predicting GPP under conditions with low biomass (i.e. low LAI), when confounding factors may affect Fy760 or sPRI."

6) As far as I understood the authors were feeding the MM models with field data (both VPD and soil water content). But, what would happen to MM performance if they would have used modelled/estimated VPD and SWC? Could it be that RSM would have been then far superior than MM? The authors might wish to briefly discuss (or even assess) how uncertainties in VPD and SWC estimates would propagate and affect the performance of MM in a real case scenario where no field data is available. As it stands, the comparison between MM and RSM might favor MM.

← We agree with the Reviewer that this is a relevant and interesting point that requires a further evaluation of both MM and RSM approaches for up-scaling purposes. Such would involve explicitly addressing uncertainties in the different forcing fields of the MM and RSM models, which goes beyond the objectives of this current work and would justify a unique study per se. Therefore, we have acknowledged this point in the discussion section of the manuscript (L601-606):

"From a practical point of view, the forcing variables of RSM approaches may show a better observational coverage. In effect, the satellite-based retrievals of RSM forcing variables could additionally overcome representativeness limitations and potential regional or seasonal biases in meteorological fields (Dee et al., 2011). The uncertainties in forcing variables of MM (i.e. temperature, VPD and soil moisture) could propagate and affects the GPP estimates."

7) Page 11907. Line 5. There is no mention or data on GPP2000 in Fig. 2. (see also Page 11907, Line 16). The authors seem to refer to the differences in GPP2000 and GPP between treatments several times in the results and discussion but that analysis is not explicitly shown. How about

adding GPP (daily mean, or max, or noon) and GPP2000 into the analysis presented in Fig 3 with two additional panels?

← The methods describes widely how GPP2000 was estimated and results from the analysis were explicitly mentioned (L440-452; "GPP2000 was higher in +N and +NP treatments (18.6 and 20.1 μmolCO2m-2s-1, respectively) compared to C and +P treatments (14.9 and 15.4 μmolCO2m-2 s-1, respectively)…". We again agree with the Referee that the use of the terminology and acronyms used to describe the different metrics for the photosynthesis (e.g. GPP, GPP2000 etc) was confusing. Improvements have been made to address this issue. Regarding adding GPP in Figure 3, the results were structured over the manuscript in separated sections, where we try to walk the reader through nutrient-induced changes in separate variables i) photosynthesis (Fig 2) and ii) vegetation optical properties (Fig 3). For this reason, we would rather prefer to keep this structure.

Minor corrections: - Page 11906. Lines 20-22. Is this correct? If I checked it right I am getting 79.7 +-16.5 and 75.9 +-10.5. Are these significantly different?

← The Referee is right and this error has been corrected in the manuscript. Now the sentence states (L430-431): "Regarding variations in the fraction of plant forms, no significant differences were found between treatments."

-Page 11907, Line 15, is it Table 1 instead?

← This typo has been corrected.

-Page 11907, Line 27. I am not sure do I understand what the authors mean with "As for chamber measurements,: : :" Did you measure optical properties both outside and inside the chamber? I could not see mention to that in M&M.

← We apologize for the confusion and we have explicitly added "As for GPP" instead (L461).

-Page 11908. Line 21. Is there a typo in the "p<0.1"? The significance threshold is usually set to p values equal or below 0.05.

← There is not typo here. We obtained p values of 0.0513 and 0.0878 for +NP and +N treatments, respectively, which barely would fail to reach statistical significance. However, if we consider sample size effects and the scatter in Fy760 owing to typical noise in the SIF measurements, we could either set the threshold of <0.1 for significance or to say barely significant. We have added these considerations in the improved version of the manuscript (L480-483).

“However, barely significant differences were found in the relationship between GPPnoon and Fy760 ($p<0.1$, Fig 4b) and significant between GPPnoon and MTCI ($p<0.01$, Fig 4d) between N addition treatments (+N and +NP) and C treatments (C and +P).”

-Page 11909, Line22. Add “under high light” after photosynthetic capacity. A reference to the results where this is shown could be also added.

← We have added the term and included the reference as suggested (L509-510).

-Page 11910, Lines 3-5. Point (2) should be rephrased. Increased photosynthetic capacity does not increase F per se, actually it should decrease it because photosynthesis and fluorescence compete for excitation. I believe the feedback the authors mean from the Cendrero-Mateo et al. paper refers to the simultaneous increase in fluorescence and photosynthesis because of decreased NPQ. Rough suggestion: : : : “and on (2) the increased photosynthetic capacity that results in reduced NPQ activity and consequently increases the fluorescence signal (Cendrero-Mateo et al. 2015).“

← We very much appreciate the expertise that the Reviewer brings to this paper and the sentence has been rewritten as suggested (L515-517).

-Page 11911, Line 25. How was this 37.5% obtained?

← This is a result from the relative variation of modeled GPP (from 25 to 40 mmol CO2 m-2 s-1) at highest NDVI values (see Fig. 9). Nevertheless, consistent with other comments from the Referee regarding Fig. 9, the paragraph has been rewritten, and no numbers are now given (L564-570).

-Page 11913. Line 12 and Page 11903, Line 5. “Meteo-driven models” vs “Meteorology-driven methods”. Better to use only one.

← Corrected.

Referee 2

→ Perez-Priego et al. report on an experiment in a Spanish oak savanna where the herbaceous understory has been to a N and P fertilizer application in a full factorial design. The authors measured the CO2 gas exchange using ecosystem chambers and determined, notably from the same plots, hyperspectral reflectance and several canopy structural attributes (LAI, C/N contents). The objective of the paper is to assess how fertilizer application effects CO2 gas exchange and hyperspectral reflectance and how to best model GPP using spectral vegetation indices with or without additional modifiers driven by meteorological parameters.

I think this is a unique paper as it combines the 'classical' ecological approach of field manipulation with the question of how to improve remote sensing of GPP. The key point here is that by this experimental design the authors are able to produce GPP and spectral vegetation indices which are scale-consistent, in contrast to other attempts of this kind where coarse-scale satellite remote sensing is combined with eddy covariance flux estimates from time-varying flux footprints.

The structure of the paper is OK and it is generally well written, although at times the style could be improved (it is however always clear what the authors intend to say). Methods appear sound and the graphical presentation is flawless.

According to my opinion, the paper can thus be accepted after minor revisions.

← The authors thank the Referee for this positive assessment. Our replies to specific comments are found below.

Detailed comments:

→ (1) p. 11893, l. 3: while I am not a specialist for savanna ecosystems, but would not be 'understory' a suitable and more accessible term for what the authors refer to as 'herbaceous stratum'; if so, please replace throughout the paper

← The authors agree that "understory" is a suited term for savanna ecosystems, however, in this case it can be confusing due to 1) the experiment was restricted to an open grassland area (out of the tree influence, and 2) "understory" is a general term that may include other plant forms (i.e. shrubs), which are absent in this experiment. For these reasons, we would rather prefer to keep the use of "herbaceous stratum".

(2) p. 11894: l. 14-16: in my view LUE models operate solely on the assumption that LUEmax is correct for the respective application; for example, you would not use the LUEmax of a tropical forest for a desert ecosystem; neither should one use the same LUEmax for the same ecosystem if nutrient availability, which is know to affect LUE, is different

← We fully agree with the Referee's comment and we have pointed out this in the paragraph (L105-106).

"ii) potential LUE (or maximum, LUE_m), normally taken from look-up tables and associated with plant functional types (Heinsch et al., 2006)"

(3) p. 11896, l. 12: another suitable reference would be Porcar-Castell et al. (2015) from the EuroSpec SI.

← This reference has been included as suggested.

(4) p. 11896, l. 23: I am a strong believer in hypothesis-driven research; given the 'classical' ecological experimental design, this paper lends itself to formulate a few hypothesis, which would further strengthen the paper.

← We thank the Referee for the suggestion and we have considered to reformulate the last part of the Introduction clarifying the main working objectives (L166-170):

"The main objective of this study was to evaluate whether traditional LUE models driven by meteorological and phenological data (MM) entail a limited assessment of the environmental controls on GPP. More particularly, we evaluated if the effects of varying nutrient availability on GPP estimates as tracked by chlorophyll fluorescence and PRI can be equally explained by meteorology-driven models."

(5) p. 11897, l. 19-24: the abbreviations for the treatments are not used consistently throughout the paper, e.g. sometimes +N or only N is used; make sure that the same abbreviations are used throughout the text, tables and figures.

← In the manuscript the following convention was adopted: "+N" refers to Nitrogen addition treatment (see L195), while only "N" is used to refer to Nitrogen (N) content in plants. Like N, similar distinction for both "+P" and "P" abbreviations were taken. Following this convention, we have been carefully revised the manuscript.

(6) p. 11900, l. 9: does 3min apply to the combined NEE and RECO measurement or individually to both (i.e. a total of 6min for NEE & RECO)? If so, I suppose that the temperature for the RECO measurement will be higher compared to the NEE measurement, which will bias estimated GPP. Is this an issue and can the authors quantify the effect? In this section it may also be worth stating that apparently a quadratic fit was applied to the dry mole fractions and the flux inferred from the first derivative at t=0 (even though this is detailed in Perez-Priego et al. 2015, this is fundamental information required here).

← We thank the Referee for these questions and comments. We clarified and improved the description of the chamber method and flux calculation approaches.

As it has been explained in the manuscript (L256-259) the chamber was open and ventilated during 1 min prior to measurement, so that initial air composition and temperature in the confined environment of the chamber represented natural atmospheric conditions (as much NEE as Reco). Considering ~4 min of delay between NEE and Reco measurements, comparable environments were shared for both measurements and hence no biases in GPP by temperature are expected.

Regarding, flux calculations we have added the following paragraph in the methods section (L266-277):

"Shortly, the flux calculation algorithm reduces flux uncertainties by including the change-point detection method to determine the stabilization time, which defines the initial slope of the regressions, and a bootstrap resampling-based method to improve confidence in regression parameters and to optimize the number of data points used for flux calculation. In addition, a statistical analysis of residuals was performed to automatically detect the best fit among alternative regressions (i.e. quadratic, hyperbolic tangent saturating function, exponential, linear). These analyses were implemented in a self-developed R Package (available upon authors request or at the following link http://r-forge.r-project.org/projects/respchamberproc/). GPP measurements were taken over the course of the day (from sunrise to sunset) for each field campaign. Chamber disturbance effects and correction for systematic and random errors (i.e. leakage, water dilution and gas density correction, and light attenuation by the chamber wall) were applied according to Perez-Priego et al., (2015)."

(7) p. 11902, l. 21: if I understood the methods section correctly, gas exchange and hyperspectral measurements were done sequentially, but not simultaneously (even if the time difference may be small).

← Yes the consideration is correct and we have clarified this point. L339-340 now read: "We evaluated direct relationships between midday GPP values (measurements taken around noon with the chamber) and sequentially measurements of Fy760…. "

(8) p. 11907, l. 20: I think with two months of data the authors should not attempt to assess any long-term effects (years to decades); probably the term 'season should be used here.

← We agree that "long-term" is a poor choice of word; this is now referred to "season" (L453).

(9) Fig. 1: the abbreviation SMANIE appears for the first time here and has not be explained before.

← SMANIE it's the acronym of the project, we agree and so the abbreviation has been explained in the Methods Section at the beginning of the "Experimental site and description" section (L178 "A Small scale MANIpulation Experiment (SMANIE) was set up in a Mediterranean savannah…"

(10) Fig. 2: is it possible to re-scale the figs and move the title of sub-panel (b) into the panel for consistency with the other sub-panels?

← We thank the suggestion but data visualization becomes worse when re-scaling the figures. For this reason we would prefer to keep the figure as it is.

Sun-induced Chlorophyll fluorescence and PRI improve remote sensing GPP estimates under varying nutrient availability in a typical Mediterranean savanna ecosystem

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Abstract

This study investigates the performances of different optical indices to estimate gross primary production (GPP) of herbaceous stratum in a Mediterranean savanna with different Nitrogen (N) and Phosphorous (P) availability. Sun-induced chlorophyll Fluorescence yield computed at 760 nm (Fy760), scaled-photochemical reflectance index (sPRI), MERIS terrestrial-chlorophyll index (MTCI) and Normalized difference vegetation index (NDVI) were computed from near-surface field spectroscopy measurements collected using high spectral resolution spectrometers covering the visible near-infrared regions. GPP was measured using canopy-chambers on the same locations sampled by the spectrometers. We tested whether light-use efficiency (LUE) models driven by remote sensing quantities (RSM) can better track changes in GPP caused by nutrient supplies compared to those driven exclusively by meteorological data (MM). Particularly, we compared the performances of different RSM formulations -relying on the use of Fy760 or sPRI as proxy for LUE and NDVI or MTCI as fraction of absorbed photosynthetically active radiation (*f*APAR) - with those of classical MM.

Results showed higher GPP in the N fertilized experimental plots during the growing period. These differences in GPP disappeared in the drying period when senescence effects masked out potential differences due to plant N content. Consequently, although MTCI was tightly related to the mean of plant N content across treatment (r^2=0.86, $p<0.01$), it was poorly related to GPP (r^2=0.45, p<0.05). On the contrary sPRI and Fy760 correlated well with GPP during the whole measurement period. Results revealed that the relationship between GPP and Fy760 is not unique across treatments but it is affected by N availability. Results from a cross-validation analysis showed that MM (AIC_{cv}=127, ME_{cv}= 0.879) outperformed RSM (AIC_{cv}=140, ME_{cv}= 0.8737) when soil moisture was used to constrain the seasonal dynamic of LUE. However, residual analyses demonstrated that GPP predictions with MM are

inaccurate whenever no climatic variable explicitly reveals nutrient-related changes in the LUE parameter. These results put forward that RSM is a valuable means to diagnose nutrient-induced effects on the photosynthetic activity.

Abbreviations:
a, $\mathbf{a_o}$, and $\mathbf{a_1}$ are model parameters; $\mathbf{b_0}$, $\mathbf{b_1}$, $\mathbf{b_2}$, and $\mathbf{b_3}$ are fitting parameters of RSM; **EFPs**, ecosystem functional properties; ***f*(meteo),** limiting functions relying on meteorologically-driven data; ***f*APAR**, fraction of absorbed photosynthetically active radiation; ***f*PAIg**, fraction of *PAIg* in different plant forms; **Fy760**, sun-induced chlorophyll Fluorescence yield at 760 nm; **GPP**, gross primary productivity; $\mathbf{GPP_{noon}}$: instantaneous gross photosynthetic rate taken at solar noon (between 11:00 and 15:00 pm solar time); GPP_{daily}: mean value of the diurnal time course of gross photosynthetic rate; $\mathbf{GPP_{2000}}$, gross primary productivity estimated at 2000 of PAR; **LUE,** light use-efficiency; $\mathbf{LUE_m}$ potential or maximum LUE; **MM**, meteorologically driven model; **MM-VPD,** simplifier model of the original MOD17 that account for VPD in *f*(meteo); **MM(SWC-VPD)** meteorologically-driven model that account for VPD and soil moisture in *f*(meteo); **MTCI**, MERIS terrestrial-chlorophyll index; **NDVI**, Normalized difference vegetation index; **NEE**, net ecosystem CO_2 exchange; **PAIg**, Green Plant Area Index; **PAR**, Photosynthetically active radiation; ***ph***, physiologically-related parameter of RSM referring to either sPRI or Fy760 as a proxy for LUE; **PLRC**, photosynthetic light response curve; **PRI**, photochemical reflectance index; $\mathbf{R_{eco}}$, daytime ecosystem respiration; **RSM**, remote sensing based models; **SIF**, sun-induced chlorophyll fluorescence; **sPRI**, scaled-photochemical reflectance index; ***st***, structurally-related parameter of RSM referring to either NDVI or MTCI as a proxy for *f*APAR; **SWC**, soil water content; $\mathbf{SWC_{max}}$ parameter of the *f(meteo)* term; **VPD**, vapor pressure deficit; $\mathbf{VPD_{max}}$ and $\mathbf{VPD_{min}}$ are fitting parameters of the *f(meteo)* term; $\boldsymbol{\alpha}$ is a parameter describing the photosynthetic quantum yield; $\boldsymbol{\beta}$ is the parameter that extrapolates to GPP at saturating light condition.

1. Introduction

Human-induced nutrient imbalances are affecting essential processes that lead to important changes in ecosystem structure and functioning (Peñuelas et al., 2013). In spite of the crucial role of nutrients in regulating plant processes, efforts to describe and predict the response of photosynthesis to such changes with remote sensing information have been limited. In the framework of the classical Monteith Light Use Efficiency (LUE) model (Monteith, 1972), estimates of photosynthesis (hereafter gross primary productivity, GPP) are based on three key quantities: i) the fraction of photosynthetically active radiation (*f*APAR) absorbed by the vegetation, ii) potential LUE (or maximum, LUE_m), normally taken from look-up tables and associated with plant functional types (Heinsch et al., 2006) and iii) correction factors related to meteorological conditions that limit LUE_m. Although Nitrogen (N) deficiencies have been recognized one of the main correction factors of LUE_m (Madani et al., 2014), the predictive capability of LUE models is usually circumspect as they operate based on the general assumption that plants are under non-limiting nutrient conditions.

Very little attention has been given to nutrient-induced effects on *f*APAR and LUE in common formulations of LUE models. Light absorption by plant is given by chlorophyll pigments that enable photosynthetic processes. Assuming a correlation between leaf chlorophyll pigments and leaf N content, note that N atoms are basic components of the chlorophylls molecular structure, several studies have demonstrated that leaf N content can be estimated through chlorophyll-related hyperspectral vegetation indices (Baret et al., 2007; Schlemmer et al., 2013). Among these indices, the MERIS Terrestrial Chlorophyll Index (MTCI, Dash and Curran, 2004) has been used as a proxy for *f*APAR (Rossini et al., 2010; Wang et al., 2012). However, leaf N content is functional trait that controls GPP not only because it scales with chlorophylls but also regulates enzyme kinetic processes driving photosynthesis and hence the physiological status of the plant (Huang et al., 2004; Walker et al., 2014). Then, prescribing biome-specific LUE parameters and correcting LUE_m only for climatic and environmental conditions may hamper the accurate prediction of GPP (Yuan et al., 2014). For these reasons, recent literature has called for better physiological descriptors of the dynamic behavior of LUE (Guanter et al., 2014).

The sun-induced chlorophyll fluorescence (SIF) or physiological-related reflectance indices such as the photochemical reflectance index (PRI) provide a new optical means to spatially infer LUE (Damm et al., 2010; Guanter et al., 2014; Rossini et al., 2015) and can provide diagnostic information regarding plant nutrient and water status (Lee et al., 2013; Pérez-Priego et al., 2005; Suárez et al., 2008; Tremblay et al., 2012). From a physiological perspective, the efficiency of green plants to transform absorbed light into chemical energy during photosynthesis can be characterized by two main photo-protective mechanisms: i) non-photochemical quenching that can be detected using the Photochemical Reflectance Index (PRI), originally proposed by (Gamon et al., 1992) to track changes in the de-epoxidation state of the xanthophyll cycle pigments, and ii) Chlorophyll fluorescence, the dissipation of energy that exceeds photosynthetic demand (Krause and Weis, 1984). The PRI has been directly correlated with LUE (Drolet et al., 2008; Gamon et al., 1997; Nichol et al., 2000; Peñuelas et al., 2011; Rahman et al., 2004). However, such relation may vary because of the sensitivity of the PRI to confounding factors like those associated with temporal changes in the relative fraction of chlorophyll:carotenoids pigment composition (Filella et al., 2009; Porcar-Castell et al., 2012), viewing angles and vegetation structure (Garbulsky et al., 2011; Grace et al., 2007; Hall et al., 2008; Hilker et al., 2008).

Alternatively, the estimation of SIF by passive remote sensing systems has been proven feasible in recent years from satellite (Frankenberg et al., 2014; Lee et al., 2013; Parazoo et al., 2014) to the field (Damm et al., 2010; Guanter et al., 2013; Meroni et al., 2011), and opens further possibilities to directly track the dynamics of LUE (Damm et al., 2010; Guanter et al., 2014). Although SIF correlates with LUE, such relations might not be conservative since chlorophyll fluorescence emission varies among species types (Campbell et al., 2008) or with stress conditions such as nutrient deficiencies (Huang et al., 2004; McMurtrey et al., 2003) or drought (Flexas et al., 2002; Pérez-Priego et al., 2005). Likewise with the PRI, the retrieval of SIF from the apparent reflectance signal is not trivial as long as it is affected by the vegetation structure or canopy background components (Zarco-Tejada et al., 2013).

Comparable spatial and temporal resolutions of radiometric and ground-based GPP measurements are essential to accurately optimize LUE model parameters, particularly in heterogeneous ecosystems. Previous studies have related ecosystem-scale eddy covariance fluxes to radiometric measurements taken in single points to constraint LUE models. However, the explanatory power of LUE models might be greatly reduced by the spatial mismatch between radiometric and eddy covariance flux footprints (Gelybó et al., 2013; Porcar-Castell et al., 2015). Similar issues occur in small-scale factorial experiments where comparable measurements on an intermediate scale between leaf-scale cuvette measurements and ecosystem-scale eddy covariance measurements are required. Here, we tried to overcome such limitations by combining ground-based radiometric and CO_2 fluxes measurements with similar extension of the measurement footprint using portable spectrometers and canopy chambers in a nutrient-manipulation experiment.

The main objective of this study was to evaluate whether traditional LUE models driven by meteorological and phenological data (MM) entail a limited assessment of the environmental controls on GPP. More particularly, we evaluated if the effects of varying nutrient availability on GPP estimates as tracked by chlorophyll fluorescence and PRI can be equally explained by meteorology-driven models. To address the main objective we:

a) assess the effect of different nutrient supplies on grassland photosynthesis and optical properties and their relationships during a phenological cycle, including both growing and drying periods,

b) evaluate the performance of different LUE modeling approaches with varying nutrient availability and environmental conditions.

2. Material and Methods

2.1. Site description and experimental design

A Small scale nutrient Manipulation Experiment (SMANIE) was set up in a Mediterranean savannah in Spain (39°56'24.68''N, 5°45'50.27''W; Majadas de Tietar, Caceres,

Fig. 1). The site is characterized by a mean annual temperature of 16°C, mean annual precipitation of ca. 700 mm, falling mostly from November until May, and by a very dry summer. Similar to most Mediterranean grasland, grazing (<0.7 cows ha^{-1}) is the main land use in the site. The site is defined as a typical Mediterranean savanna ecosystem, low density of oak trees (mostly *Quercus Ilex* (L.), ~20 trees ha^{-1}) dominated by a herbaceous stratum. The experiment itself was restricted to an open grassland area which was not influenced by tree canopy. The herbaceous stratum is dominated by species of the three main functional plant forms (grasses, forbs and legumes). The fraction of the three plant forms varied seasonally according to their phenological status (Table 1). Overall, leaf area measurements of the herbaceous stratum characterized the growing season phenology as peaking early in April and achieving senescence by the end of May (Table 1).

The experiment consisted of four randomized blocks of about 20 m x 20 m. Each block was separated into four plots of 9 m x 9 m with a buffer of 2 m in between to avoid boundary effects. In each block, four treatments were applied (see Fig. 1):

(a) control treatment (C) with no fertilization;

(b) Nitrogen addition treatment (+N) with an application of 100 kg N ha^{-1} as potassium nitrate (KNO_3) and ammonium nitrate (NH_4NO_3);

(c) Phosphorous addition treatment (+P) with an application of 50 kg P ha^{-1} as monopotassium phosphate (KH_2PO_4); and

(d) N and P addition treatment (+NP), juxtaposing treatments (b) and (c).

Each fertilizer was dissolved in water and sprayed on foliage early in the growing season (March 21st, 2014). The same amount of water used in the fertilizer solutions (~ 2 L m^{-2}) was sprayed on the C treatment to avoid water imbalances among treatments.

Within each plot, two permanent, non-disturbed parcels (32 in total, see black squares in Fig 1) were dedicated to monitor CO_2 fluxes (net ecosystem CO_2 exchange, NEE; and daytime ecosystem respiration, R_{eco}). While NEE measurements were performed over the course of the day (from early in the morning to late afternoon), spectral measurements were conducted simultaneously with flux measurements only around noon on half of the parcels (16 in total).

Flux and spectral measurements were carried out in four field campaigns:

- Campaign #1: before fertilization (March 20th, 2014),
- Campaign #2: three weeks after fertilization (April 15th, 2014) during the peak of the growing period,
- Campaigns #3 and #4: on May 7th and 27th, 2014, respectively, concurring with the drying period were performed to evaluate joint effects related to physiological senescence processes.

Ancillary measurements were taken in every field campaign as follows: green plant area index (PAI_g) and aboveground biomass were directly measured by harvest in four parcels (0.25m x 0.25m) within each plot in the area surrounding that where spectral and flux measurements were taken. All samples were refrigerated just after collection, and transported for laboratory analyses. Fresh samples were separated into functional groups, the sample was scanned and green plant area was measured using image analysis (WinRHIZO, Regent Instruments Inc., Canada). Afterwards, fresh samples were dried in an oven at 65 °C for 48 hours and weighed to determine dry biomass. To analyze the nutrient content in leaf mass, biomass subsamples were ground in a ball mill (RETSCH MM200, Retsch, Haan, Germany) and total C and N concentrations were determined with an elemental analyzer (Vario EL, Elementar, Hanau, Germany). P concentrations were also measured: 100-mg biomass subsamples were diluted in 3 ml of HNO_3 65%, (Merck, Darmstadt, Germany) and microwave digested at high pressure

(Multiwave, Anton Paar, Graz, Austria; Raessler et al. (2005). Afterwards, elemental analysis was conducted using inductively coupled plasma - optical emission spectrometry (ICP-OES, Optima 3300 DV, Perkin Elmer, Norwalk, USA).

2.2 Flux measurements and Meteorological data

Net CO_2 fluxes were measured with three transparent chambers of a closed dynamic system. The chambers consisted of a cubic (0.6m x0.6m x0.6 m) transparent low-density polyethylene structure connected to an infrared gas analyzer (IRGA LI-840, Lincoln, NE, USA), which measures CO_2 and water vapor mole fractions (W) at 1 Hz. The chambers were equipped with different sensors to acquire environmental and soil variables, all installed at the chamber ceiling: Photosynthetically Active Radiation (*PAR)* was measured with a quantum sensor (Li-190, Li-Cor, Lincoln, NE, USA) placed outside of the chamber to be handled and leveled; air and vegetation temperatures were measured with a thermistor probe (T_a, type 107, Campbell Scientific, Logan, Utah, USA) and an infrared thermometer (T_c, IRTS-P, Apogee, UT, USA); atmospheric pressure (P) was measured inside the chamber using a barometric pressure sensor (CS100, Campbell Scientific, Logan, Utah, USA). The chambers were also equipped with soil temperature and humidity sensors; soil water content was determined with an impedance soil moisture probe (Theta Probe ML2x, Delta-T Devices, Cambridge, UK) at 5 cm depth and soil temperature (type 107, Campbell Scientific, Logan, Utah, USA) at 10 cm depth. Vapor pressure deficit (VPD) was computed using T_c and relative humidity, which was derived from water vapor molar fraction measured with the IRGA.

The chamber operated as a closed dynamic system. A small pump circulates an air flow of 1 L min^{-1} through the sample circuit: air is drawn from inside the chamber - through three porous-hanging tubes spatially distributed through the chamber headspace - to the infrared gas analyzer; this air flow is then returned to the chamber. The hanging tubes allowed spatially distributed sampling, obviating the need to homogenize air during chamber deployment. Nevertheless, one small fan (12V, 0.14A) was fixed at 0.3 m on a floor corner of the chamber and angled 45º upward.

A 0.6x0.6m metal collar was installed in each permanent parcel of each plot. The collar provided a flat surface onto which the bottom of the chamber was placed. The chamber was open and ventilated during 1 min prior to measurement, so that initial air composition and temperature in the confined environment of the chamber represented natural atmospheric conditions (as much NEE as Reco). For the NEE measurement, the transparent chamber was placed on the collar (closed position, lasted 3 minutes as a general rule), and fluxes were calculated from the rate of change of the CO_2 molar fraction (referenced to dry air) within the chamber. Similar procedure was carried out for R_{eco} but using an opaque blanket that covered the entire chamber and kept it dark during the measurements (PAR values around 0). Fluxes were calculated according to Pérez-Priego et al. (2015).

Shortly, the flux calculation algorithm reduces flux uncertainties (i.e. NEE and R_{eco}) by including the change-point detection method to determine the stabilization time, which defines the initial slope of the regressions, and a bootstrap resampling-based method to improve confidence in regression parameters and to optimize the number of data points used for flux calculation. In addition, a statistical analysis of residuals was performed to automatically detect the best fit among alternative regressions (i.e. quadratic, hyperbolic tangent saturating function, exponential, linear). These analyses were implemented in a self-developed R Package (available upon authors request or at the following link http://r-forge.r-project.org/projects/respchamberproc/). NEE and R_{eco} measurements were taken over the course of the day (from sunrise to sunset) for each field campaign. Chamber disturbance effects and correction for systematic and random errors (i.e. leakage, water dilution and gas density correction, and light attenuation by the chamber wall) were applied according to Perez-Priego et al., (2015).

2.3 Field spectral measurements

Midday spectral measurements at canopy level were carried out under clear sky conditions using two portable spectrometers (HR4000, OceanOptics, USA) characterized by different spectral resolutions. Spectrometer 1, characterized by a Full Width at Half Maximum (FWHM) of 0.1 nm and a 700-800 nm spectral range was specifically designed for the estimation of sun-induced chlorophyll fluorescence at the O_2-A band (760 nm). Spectrometer 2 (FWHM = 1 nm, 400 - 1000 nm spectral range) was used for the computation of reflectance and vegetation indices. Spectrometers were housed in a thermally regulated Peltier box, keeping the internal temperature at 25°C in order to reduce dark current drift. The spectrometers were spectrally calibrated with a source of known characteristics (CAL-2000 mercury argon lamp, OceanOptics, USA) while the radiometric calibration was inferred from cross-calibration measurements performed with a calibrated FieldSpec FR Pro spectrometer (ASD, USA). This spectrometer was calibrated by the manufacturer with yearly frequency.

Incident solar irradiance was measured by nadir observations of a leveled calibrated standard reflectance panel (Spectralon; LabSphere, USA). Measurements were acquired using bare fiber optics with an angular field of view of 25°. The average canopy plane was observed from nadir at a distance of 110 cm (43 cm diameter field of view) allowing for collecting measurements of 50% of the surface area covered by the chamber measurements. The manual rotation of a mast mounted horizontally on the tripod allowed sequential observation of the vegetated target and the white reference calibrated panel. More in detail, every acquisition session consisted in the consecutive collection of the following spectra: instrument dark current, radiance of the white reference panel, canopy radiance and radiance of the white reference panel. The radiance of the reference panel at the time of the canopy measurement was then estimated by linear interpolation.

For every acquisition, 3 and 10 scans (for Spectrometers 1 and 2, respectively) were averaged and stored as a single file. Five measurements were collected for each plot. Spectral data were acquired with dedicated software (Meroni and Colombo, 2009) and processed with a specifically developed IDL (ITTVIS IDL 7.1.1) application. This application allowed the basic processing steps of raw data necessary for the computation of the hemispherical conical reflectance factor described by Meroni et al. (2011).

The following indices were selected as suitable to investigate long term nutrient-mediated effects on photosynthesis. The NDVI (Rouse et al., 1974) was selected because it correlates well with plant area and among traditional spectral vegetation indices is used worldwide by classical LUE models as a surrogate for *f*APAR (Di Bella et al., 2004). The MTCI (Dash and Curran, 2004) was selected because it was specifically designed for canopy chlorophyll content estimation, and recently used as proxy for *f*APAR as well as NDVI. In this study we used the PRI and SIF as surrogates for LUE. A scaled PRI (sPRI) calculated as (PRI+1)/2 was used. SIF was estimated by exploiting the spectral fitting method described in Meroni et al. (2010), assuming linear variation of the reflectance and fluorescence in the O_2-A absorption band region. The spectral interval used for SIF estimation was set to 759.00 - 767.76 nm for a total of 439 spectral channels used. For methodological distinction among existing approaches, hereafter SIF is referred to as F760. Because F760 is affected by PAR we use the apparent chlorophyll fluorescence yield (Fy760; Rossini et al., 2010) computed as the ratio between F760 and the incident radiance in a nearby spectral region. A summary of the formulation to compute the vegetation indices and their corresponding target and proxy in the LUE model approach are presented in Table 2.

2.4 Relationship between GPP and remote sensing data

Ecosystem-level GPP was computed as the difference between NEE and daytime R_{eco} taken consecutively with the chambers. To assess how GPP is modulated by light among treatments and over the phenological cycle of the herbaceous stratum, we computed the parameters of photosynthetic light response curve (PLRC). Specifically, the Michaelis–Menten function was fitted to GPP and PAR data taken throughout the course of the day (from sunrise until sunset) for each field campaign and treatment as follows:

$$GPP_i = \frac{\alpha \times \beta \times PAR_i}{\beta + PAR_i \times \alpha}, \qquad [1]$$

where α is a parameter describing the photosynthetic quantum yield (μmol CO_2 μmol photons^{-1}), and β is the parameter that extrapolates to GPP at saturating light condition (μmol CO_2 m^{-2} s^{-1}). According to Ruimy et al. (1994), we used the optimized parameters of the PLRC as defined in Eq. (1) to estimate the GPP at 2000 μmol quantum m^{-2} s^{-1} of PAR (hereafter referred to GPP_{2000}).

We evaluated direct relationships between those GPP measurements taken around noon (between 11:00 and 15:00 pm solar time) with the chamber (GPP_{noon}) and sequentially measurements of Fy760 and spectral indices (NDVI, sPRI, MTCI). In addition, to avoid confounding factors in the relationship between Fy760 and sPRI and photosynthesis, we also used GPP_{2000} as a maximum photosynthetic capacity descriptor.

2.5 Monteith's light-use efficiency modelling approaches

Following Monteith's LUE framework (Eq. 2) two alternative modeling approaches were used:

$$GPP = LUE \times fAPAR \times PAR, \qquad [2]$$

i. ***Meteo-driven methods (MM);*** based on the MOD17 formulation, fAPAR is approached through the relationship with NDVI and includes limiting functions f(meteo), which are based on climatic driving parameters to limit maximum LUE (LUE_{max}). Alternatively, Eq. (2) was reformulated as follows:

$$GPP = LUE_{max} \times f(meteo) \times (a_0 \times NDVI + a_1) \times PAR, \qquad [3]$$

where LUE_{max}, a_o, and a_1 are model parameters. Three different f(meteo) functions were tried;

a) **MM-VPD,** this method is a simplification of the original MOD17, in which *f*(meteo) includes two linear ramp functions of both maximum and minimum vapour pressure deficit (VPD) and minimum temperature (T). Since minimum temperature was not limiting at the site, we fixed the *f*(meteo) parameters as suggested by Heinsch et al. (2006) but constraining only a function based on VPD as follows:

$$f(meteo) = \left[1 - \left(\frac{VPD - VPD_{min}}{VPD_{max} - VPD_{min}}\right)\right], \qquad [4]$$

then, VPD_{max} and VPD_{min} are defined as the three parameters of the *f(meteo)* term.

b) **MM-SWC**, where *f*(meteo) includes a soil water content (SWC) function (Migliavacca et al., 2011) as the limiting factor of LUE_{max}:

$$f(meteo) = \frac{1}{1 + exp^{(SWC_{max} - a \times SWC)}}, \qquad [5]$$

here, SWC_{max} and *a* are defined as the parameters of the *f(meteo)* term.

c) **MM (SWC-VPD)**, where *f*(meteo) includes both soil water content and VPD functions as limiting factors:

$$f(meteo) = \left[1 - \left(\frac{VPD - VPD_{min}}{VPD_{max} - VPD_{min}}\right)\right] \times \left[\frac{1}{1 + exp^{(SWC_{max} - a \times SWC)}}\right], \qquad [6]$$

here, VPD_{max}, VPD_{min}, SWC_{max} and *a* are defined as the parameters of the *f(meteo)* term.

ii. ***RS-based method (RSM);*** based on a solution of Eq.(1) as follows:

$$GPP = LUE \times fPAR \times PAR = (a_0 \times Ph + a_1) \times (a_2 \times St + a_3) \times PAR$$

$$= (b_0 \times Ph + b_1 \times St + b_2 \times Ph \times St + b_3) \times PAR\ , \qquad [7]$$

where four alternative model formulations were obtained from the combination of the sPRI or Fy760 as the physiological related proxy (*Ph*) for LUE, and NDVI or MTCI as structural-related (*St*) proxy for *f*APAR. In Eq. 7, b_0, b_1, b_2, and b_3 are fitting parameters (Rossini et al., 2010).

2.5 Statistical analysis and model performance

All model formulations were optimized using GPP_{noon} and spectral measurements taken at midday. Since the means of spectral measurements per treatment could have unequal variance, a Welch's t-test was performed to evaluate significant differences between the mean values of the different vegetation indices for each treatment and over the four field campaigns. In addition, an analysis of covariance (ANCOVA) was used to test whether or not there was a significant interaction by the treatment effect between GPP_{noon} and Fy760 and different spectral indices. Like vegetation indices, a t-test was performed to the daily average of GPP taken over the course of the day (GPP_{daily}).

2.5.1 Cross-validation analyses and model evaluation

Different model formulations were evaluated in leave-one-out (loo) cross-validation: from the whole dataset composed by *n* observations, one data point at a time was removed. The model was fitted against the $n-1$ remaining data points (training set) while the excluded data (validation set) were used for model evaluation. The cross-validation process was then repeated *n* times, with each of the *n* observations used exactly once as the validation set. For each validation set of the cross-validated model, statistics were calculated.

Model accuracy was evaluated by means of different statistics according to Janssen and Heuberger (1995): root mean square error (RMSE), relative root mean square error (rRMSE) determination coefficient (r^2) and model efficiency (ME). The model performances in loo cross-validation were also calculated and reported as $RMSE_{cv}$, $rRMSE_{cv}$, r^2cv and ME_{cv}.

The Akaike Information Criterion (AIC_{cv}) was used to evaluate the trade-off between model complexity (i.e. number of parameters) and explanatory power (i.e. goodness-of-fit) of the different model formulations proposed. The AIC_{cv} is a method based on information theory that is useful for statistical and empirical model selection purposes (Akaike, 1998). Following Anderson et al. (2000), in this analysis we used the following definition of AIC_{cv}:

$$AIC_{cv} = 2(\rho + 1) + n\left[ln\left(\frac{RSS_{cv}}{n}\right)\right] \quad [8]$$

where *n* is the number of samples (i.e. observations), *p* is the number of model parameters and RSS_{cv} is the residual sum of squares divided by n.

The LUE model formulations proposed in Section 2.4 can be ranked according to AIC_{cv}, where the model with lowest AIC_{cv} is considered the best among the different model formulations.

All model parameters (MM, and RSM) were estimated by using a Gauss-Newton nonlinear least square optimization method (Bates and Watts, 2008), and standard errors of parameters were estimated by bootstrapping (number of sampling, n = 500; Efron and Tibshirani (1994)), both implemented in the R standard package (R version 3.0.2, R Development Core Team, 2011).

3. Results

3.1 Effects of fertilization on plant nutrient contents and GPP

Fertilization caused strong variations in leaf N and P content among treatments, plant forms and across field campaigns (Table 2); while total N content in plants ranged slightly between 13.8±1.2 and 15.4±1.7 mg g^{-1} for the C and +P treatments over the whole experiment, the largest increases in total N content were found in the peak of the growing season (#2, March 20th, 2014), when +NP and +N treatments reached values of up to 23.7±2.0 and 23.5±4.1 mg g^{-1}, respectively. Although slightly lower, the differences in total N content between C and +P, and +NP and +N remained high over the drying period. Total P content was higher in +NP and +P treatments after fertilization, as compared to +N and C treatments. Consequently, the N:P ratio at the first campaign after fertilization (#2) achieved values of up to 14.2, 6.6, 6, and 3.7, in +N, C, +NP, and +P treatments, respectively. Similar differences in N:P between treatments were also observed during the drying period (#3 and #4, Table 2). On the other hand, PAI_g ranged from 0.4 m^2 m^{-2} in campaign #4 to up to 2.5 m^2 m^{-2} in campaign #2. No differences were found in PAI_g among treatments since grazing apparently offset any potential difference in the green aboveground production. Regarding variations in the fraction of plant forms, no significant differences were found between treatments.

Fertilization caused significant differences in the GPP_{daily} ($p<0.05$) between N-addition treatments (mean values of 19.62±4.15 and 18.19±5.67 μmolCO_2 m^{-2} s^{-1} for +N and +NP, respectively) and C and +P treatments (14.31±5.39 and 14.40±4.09 μmolCO_2 m^{-2} s^{-1}, respectively) in the peak of the growing season (campaign #2); a relative difference of 37% in GPP_{daily} values was found between +N and +NP and C treatments. During the drying period, however, GPP was substantially down regulated (campaigns #3 and #4) and no significant differences were found in GPP_{daily}, regardless of differences in plant N content observed among treatments. The potential photosynthetic capacity GPP_{2000} (Fig 2) derived from PLRC was similar in the four treatments in the pretreatment period (campaign #1, Fig 2a). GPP_{2000} varied throughout the season and peaked in the campaign #2 (April 15th) in all treatments. At this time PLRC of the +N and +NP treatments diverged clearly from no N addition treatments (C and +P, Fig 2b). GPP_{2000} was higher in +N and +NP treatments (18.6 and 20.1μmol CO_2 m^{-2} s^{-1}, respectively) compared to C and +P treatments (14.9 and 15.4 μmol CO_2 m^{-2} s^{-1}, respectively). After campaign #2, when the soil layer at 5 cm depth dried out appreciably (volumetric water content achieved values of 3% vol., data not shown), vegetation progressively senesced and GPP_{2000} in turn was down-regulated and converged to similar values in all treatments, regardless the higher N content observed in +N and +NP treatments as compared with C and +P treatments (Table 1). During the drying season, GPP_{2000} decreased in all treatments ranging between 5.6 and 8 μmolCO_2 m^{-2} s^{-1} and no differences among treatments was observed (Fig 2 c and d). These results indicate that the senescence of the herbaceous stratum, which is regulated by water availability, strongly modulated the photosynthetic capacity of the vegetation over the season.

3.2 – Effects of fertilization on remote sensing data

Optical properties of the analyzed plots were similar during campaign #1, before the nutrient application. A pronounced seasonal time course was observed for both *Ph* (sPRI and Fy760) and structural indices (*St*; NDVI and MTCI) with maximum values during the second campaign. It is interesting to note that while for *St* indices the maximum values were reached in +N plots, +NP plots showed maximum *Ph* values. Vegetation indices and Fy760 then decreased in the drying period (Figure 3). As for GPP, differences between treatments were more evident during campaign #2 when C plots showed statistically lower values for all the indices considered, while only MTCI was able to detect significant differences between N fertilized plots (+N and +NP). Furthermore significant differences in Fy760 and MTCI between C and the other three treatments were found ($p<0.05$) in the drying period (campaign #4,). NDVI varied significantly with changes in PAI_g with values of 0.4 in the campaign #4 up to 0.8 in the campaign #2 ($p<0.001$, $r^2=0.79$).

3.3 Relationship between remote sensing data and GPP

While *Ph* indices (Fy760 and sPRI) varied linearly with GPP_{noon} in all treatments ($p<0.001$, $r^2=0.66$ for Fy760 and $p<0.001$, $r^2=0.79$ for sPRI, respectively, Fig 4 a and b,), different patterns were observed for *St*: NDVI and GPP were best fitted by an exponential regression ($p<0.001$, $r^2=0.77$ Fig 4 c), while a weak linear relationship between MTCI and GPP_{noon} ($p<0.05$, $r^2=0.45$, Fig 4 d) was found. Although a weak relation between MTCI and GPP_{noon} was found, MTCI was strongly correlated with plant N content (y=14.17x-2.49, $p<0.001$, $r^2=0.86$). Note that these results are computed excluding data taken in the pre-treatment campaign (#1) and differences in the relationship between remote sensing data and GPP_{noon} among treatments can be only attributed to nutrient-induced effects. The ANCOVA test did not show significant differences neither in slope nor intercept of the relationship between GPP_{noon} and sPRI, and NDVI across treatments. However, barely significant differences were found in the relationship between GPP_{noon} and Fy760 ($p<0.1$, Fig 4b) and significant between GPP_{noon} and MTCI ($p<0.01$, Fig 4d) between N addition treatments (+N and +NP) and C treatments (C and +P).

Similar to GPP_{noon}, GPP_{2000} was also significantly related to mean midday sPRI ($r^2=0.76$, $p<0.001$, Fig. 5a) and Fy760 ($r^2=0.76$, $p<0.001$, Fig. 5b). As expected, an exponential regression fitted best for NDVI, while a poor relationship with MTCI was found (data not shown).

3.4 Modeling GPP

Based on the AIC_{cv} criterion, MM (VPD- SWC) outperformed MM-VPD, MM-SWC and RSM models. Although MM (VPD-SWC) showed high accuracy in the predictions (ME_{cv}=0.879, r^2_{cv}=0.881), this model had a tendency to underestimate observation at high GPP_{noon} values (see comparison between model predictions and observations, Figures 6a-6c). Note that the highest biases in modeled GPP_{noon} values among MM models belong to +N and +NP treatments in field campaign #2. Since the four treatments experienced the same environmental conditions (i.e. comparable values of SWC, VPD, air temperature), this bias can be attributed to the higher N content (+N and +NP treatments) as compared to C and +P treatments. Remarkably, residuals of the MM (VPD-SWC) taken from periods with moist soil (SWC>15) were significantly correlated with sPRI and Fy760 ($p<0.05$, Fig. 7 a and b, respectively). However, no biases between residuals and predictions were observed in RSM over the span of values and treatments (Fig. 8). Results from the evaluation of model performance indicated that RSM performs best when NDVI rather than MTCI, is used as *St* in the Eq.7 and, hence, as a proxy for *f*APAR (Table 3). Our results indicated that RSM performs best when either *Ph* (sPRI or Fy760) is combined with NDVI as *St*.

4. Discussion

4.1 Effects of nutrients on GPP and remote sensing data and their relationships

Nutrient fertilization, particularly N inputs, induced physiological changes manifested as an increase in photosynthetic capacity under high light conditions (Fig. 2; Hirose and Werger (1994). As we expected, plant N content showed to be a trait of photosynthesis that influences a variety of aspects of photosynthetic physiology (Ciompi et al., 1996; Sugiharto et al., 1990).

These physiological changes were reflected on the optical properties, particularly on fluorescence and sPRI. The increase in fluorescence with N fertilization inputs was recently explained as the combined effect that a higher N content has on 1) chlorophyll content, which magnifies APAR and enhances fluorescence signal, and on 2) the increased photosynthetic capacity that results in reduced NPQ activity and consequently increases the fluorescence signal (Cendrero-Mateo et al., 2015).The relationships between GPP_{noon} and Fy760 is not unique and may vary from optimal to non-optimal environmental conditions (i.e. nutrient deficiencies, water stress), when other regulatory mechanisms might reduce the degree of coupling between fluorescence and photosynthesis (Cendrero-Mateo et al., 2015; Porcar-Castell et al., 2012). Although Fy760 was positively correlated with GPP_{noon}, barely significant differences in the slope of this relationship were observed between treatments (Fig. 4 b). Further studies are needed to fully explore the relationship between Fy760 and GPP_{noon} under different stress conditions and over different ecosystems. However, if confirmed, the effect of nutrient availability on the relationship between Fy760 and GPP_{noon} could have important implications in GPP modeling. This result suggests that the inclusion of a correction factor related to leaves N:P stoichiometry should be considered when modeling GPP assuming a linear relationship with fluorescence at plant functional type level (Guanter et al., 2014; Joiner et al., 2013).

In this study we also explored the capability of remote sensing to describe ecosystem functional properties defined as those quantities that summarize and integrate ecosystem processes and responses to environmental conditions and can be retrieved from ecosystem level fluxes (e.g. GPP_{2000}) and structural measurements (Reichstein et al., 2014). GPP at light saturation (i.e. GPP_{2000}) is one example of an ecosystem functional property, shown here to be quite correlated to sPRI and Fy760 (Fig. 5). This result suggests that sPRI and Fy760 open also new opportunities for remote sensing products to describe the spatiotemporal variability of essential descriptors of ecosystem functioning (Musavi et al., 2015). Inferring GPP_{2000} using remote-sensing has important implication both for monitoring global carbon cycle and for benchmarking terrestrial biosphere models.

MTCI was tightly related with N content (r^2=0.86, p<0.001), independent of other structural variables (i.e. PAI_g), and can be used as a good indicator of N availability. Although MTCI has been proven to be very sensitive to variations in chlorophyll contents (Dash and Curran, 2004) and hence linkable with light absorption processes, it was weakly correlated with GPP, particularly in plots added with N (+N and +NP; r^2=0.27, p<0.01, Fig 4 d). A quite wide range of GPP_{noon} values were found at high values of MTCI – high GPP_{noon} values corresponding to the growing season and low ones to the drying period – which can be explained by two simultaneous mechanisms.

First, despite the high plant N content, physiological mechanisms including stomatal control or reduced carboxylation efficiency down-regulate GPP (Huang et al., 2004) and ultimately might break the relationship between GPP_{noon} and MTCI. Second, MTCI tracks changes in N content regardless changes in canopy structure occuring during the dry season when grass achieved senescence (i.e. green to dry biomass ratio, PAI_g). More studies aimed at the separation of the combined effects of N and changes in green/dry biomass fractions on *f*APAR are essential. On the other hand, although NDVI followed the seasonal dynamic of PAIg, it saturated at high GPP_{noon} values indicating the low ability of this index to detect spatial variations induced by N fertilization.

Although optical measurements were taken at high spatial resolution (<0.36 m^2), the separation of confounding factors affecting sPRI or Fy760 is essential to elucidate the mechanistic association between sPRI or Fy760 and GPP. Like sPRI, the retrieval of Fy760 from the apparent reflectance signal can be also affected by vegetation structure or canopy background components (Zarco-Tejada et al., 2013). After optimization and selection of the best model parameters using NDVI and sPRI (or Fy760) as driver, we analyzed the response of simulated GPP to variations in NDVI and sPRI (or Fy760, Fig 9). Results indicate that at high GPP levels, Fy760 and sPRI but less NDVI shaped GPP. However, at low GPP levels, either Fy760 or sPRI responded to GPP on a small scale (Fig 9b). Figure 9 suggests that the relationship between NDVI and sPRI or Fy760 is not unique and NDVI may play an important role in driving GPP in ecosystem characterized by marked seasonal variations. Our results highlight the complementarity between NDVI and Fy760 or sPRI. Particularly, NDVI assisted Fy760 or sPRI in predicting GPP under conditions with low biomass (i.e. low LAI), when confounding factors may affect Fy760 or sPRI. In semi-arid ecosystems, the lack of sensitivity of sPRI or Fy760 to changes in GPP during dry conditions have been explained by the soil background effect on the reflectance signal (Barton and North, 2001; Mänd et al., 2010; Zarco-Tejada et al., 2013). Accordingly, Rahman et al., (2004) pointed out that conditions where sPRI performs best are in dense canopies with low portion of bare soil.

4.2 Performances of different LUE modeling approaches.

Here we aim at answering the question how can we better simulate GPP using LUE modeling with varying nutrient availability and environmental conditions by drawing comparisons between the two model philosophies; RSM against MM approaches. There are an increasing number of studies focused on the development of LUE models driven by remotely sensed information to better explain spatio-temporal variations of GPP (Gitelson et al., 2014; Rossini et al., 2012; Rossini et al., 2014). However, nutrient availability (and in particular N) greatly influence the spatial variability of LUE even within the same plant-functional type (e.g. grasslands) and further studies are essential. The slightly better performance in cross validation of the MM (VPD-SWC) against all model configurations, including RSM, supports the importance of a joint use of SWC and VPD as key parameters to constraint LUE in arid and semi-arid ecosystems (Prince and Goward, 1995). However, residual analyses demonstrated that MM (VPD-SWC) was unable to track N-induced differences in GPP during the growing period, when both parameters are not limiting (Fig. 7). By contrast, accurate estimates of GPP were obtained with RSM both over the drying and the growing periods. These results also indicate the importance of physiological descriptors to constrain LUE, which prevails over structural factors controlling *f*APAR (i.e. green biomass) under given environmental conditions and encourage the use of hyperspectral remote sensing for diagnostic upscaling of GPP.

With sPRI or Fy760 as a proxy for LUE, RSM is presented as a valuable means to diagnose N-induced effects on physiology. Our results show the limits of MM in predicting the spatial and temporal variability of GPP when LUE is not controlled by meteorological drivers alone (VPD, temperature, soil moisture)**.** Accordingly, GPP is eventually biased whenever neither climatic nor structural state variables explicitly reveal spatial changes in the LUE parameter associated with plant nutrient availability; residuals showed a clear tendency to underestimate the highest modeled GPP values, significantly correlated to Fy760 and sPRI (Fig.7). From a practical point of view, the forcing variables of RSM approaches may show a better observational coverage. In effect, the satellite-based retrievals of RSM forcing variables could additionally overcome representativeness limitations and potential regional or seasonal biases in meteorological fields (Dee et al., 2011). The uncertainties in forcing variables of MM (i.e. temperature, VPD and soil moisture) could propagate and affects the GPP estimates.

5. Concluding remarks

1. Fy760 and sPRI correlated well with GPP: both increased with N content and decreased with senescence.
2. MTCI can be used as a good descriptor of N content in plants but the relationship with GPP breaks down under drought conditions.
3. Meteo-driven models were able to describe temporal variations in GPP, and soil moisture can be a key parameter to better track the seasonal dynamics of LUE in arid environments. However, meteo-driven models were unable to describe N-induced effects on GPP. Important implication can be derived from these results and uncertainties in the prediction of global GPP still remain when meteo-driven models do not account for plant nutrient availability.
4. sPRI or Fy760 provide valuable means to diagnose nutrient-induced effects on the photosynthetic activity and, therefore, should be included in diagnostic GPP models.

Author contribution

OPP, MM, and MRo conceived the analyses, wrote the introduction, results and discussion, and led the preparation and revision of the manuscript; FF, TJ made hyperspectral measurements, computed spectral indices and fluorescence, and wrote part of the methods section; JH, MS and OPP made chamber measurements, soil and vegetation lab analysis and wrote part of the methods section; JH organized the dataset; OK provided technical assistance in the design and construction of the chambers and data acquisition system and wrote part of the methods section; GM and AC designed the fertilization protocol, organized sampling, provided technical assistance for the managing of the experiment and contributed to data interpretation; TW and OPP developed the R package for flux calculations, computed GPP and flux uncertainties and contributed to statistical analyses and interpretation. NC and MRe contributed to analyses and interpretation and to draft the manuscript. All authors discussed the results and contributed to the manuscript.

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Figure Captions

Fig 1. Overview of the experimental site (SMANIE): the experimental blocks are drawn on an image acquired with the hyperspectral AHS (Sensytech Inc., Beverly, MA, USA) sensor during April 2014.

Fig 2. Photosynthetic light response curves derived for each growing period: (a) pretreatment and (b) post-treatment and drying periods (c and d). Treatments are presented in different colors. Lines represent the Michaelis–Menten function fitting gross photosynthesis (GPP, $\mu molCO_2m^{-2}s^{-1}$) and photosynthetic active radiation (PAR, $\mu molm^{-2}s^{-1}$).

Fig 3. Seasonal time course of mean midday physiologically-driven vegetation indices; (a) scale photochemical reflectance index, sPRI (b) apparent fluorescence yield (Fy760), and structure-driven vegetation indices, (c) NDVI, and (d) MTCI among C, +N, +NP and +P treatments in a Mediterranean grassland in Spain. Bars indicate standard deviation, N = 4. Different letters denote significant difference between treatments (Weilch t test, $P < 0.05$).

Fig 4. Relationship between GPP_{noon} and remote sensing data: (a) scaled photochemical reflectance index (sPRI), (b) apparent fluorescence yield, (c) normalized difference vegetation index (NDVI), and (d) MTCI. Square symbols represent measurements taken in the pre-treatment (#1) and circles after fertilization (#2–#4). Data were obtained at midday and lines represent results from the regressions for each treatment excluding measurements in the pre-treatment.

Fig 5. Relationship between GPP2000 and average values of sPRI and (b) apparent fluorescence yield (Fy760). Lines represent results the best linear regressions fitting the data.

Fig 6. Comparison between measured GPP and GPP modeled with the best performing LUE model for each kind of formulation: MM (VPD, panel a), MM (SWC, panel b), MM (including VPD and SWC, panel c), RSM (sPRI-NDVI panel d), and RSM (Fy760-NDVI, panel e). Results from the cross-validation analysis are presented in Table 3.

Fig 7. Correlation between residuals of the MM (VPD-SWC) model and (a) scaled photochemical reflectance index (sPRI) and (b) chlorophyll fluorescence yield (Fy760) taken from periods with high soil water content (SWC>15%, red circles). No correlation was observed when SWC<15% ($p>0.5$, black circles).

Fig 8. Plot between residuals of both the Meteo-driven model (MM-VPD) and Remote Sensing-based method (RSM) and modeled GPP values. Both lines represent the local polynomial regression fitting of the residuals against predicted values.

Fig 9. Contour plot indicating how variation in photosynthesis (GPP, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) are explained by variations in the LUE and fPAR parameters of the RSM. While (a) sPRI and (b) Fy760 are indistinctly used as a proxy of LUE, the NDVI is taken as *f*PAR.

Table Captions

Table 1. Ancillary data resulting from the analysis. Green Plant Area Index (PAIg), fraction of PAI in different plant forms (fPAI), and C, N, and P plant content. The N:P ratio also is shown. Data correspond to the mean value and standard deviation (SD) of the subsamples taken in each plot and treatment.

Table 2. Spectral vegetation indices computed in this study. Vegetation indices are classified into two major classes based on their suitability in inferring fAPAR (structural related indices) and LUE (physiologically-related indices) parameters. R denotes the reflectance at the specified wavelength (nm). NDVI: normalized difference vegetation index; MTCI: MERIS terrestrial chlorophyll index; NDI: normalized difference index; sPRI: scaled Photochemical Reflectance Index; Fy760: apparent fluorescence yield at 760 nm.

Table 3. Results from the model evaluation one leave out cross-validation analysis across LUE model configurations and vegetation indices. Based on AICcv, the best performance among formulation test for each method is highlighted text bold.

Table 4. Abbreviations.

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