

Supporting information

Article title: Nitrogen and Phosphorus effect on Sun-Induced Fluorescence and Gross Primary Productivity in Mediterranean grassland

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Supplementary Figures

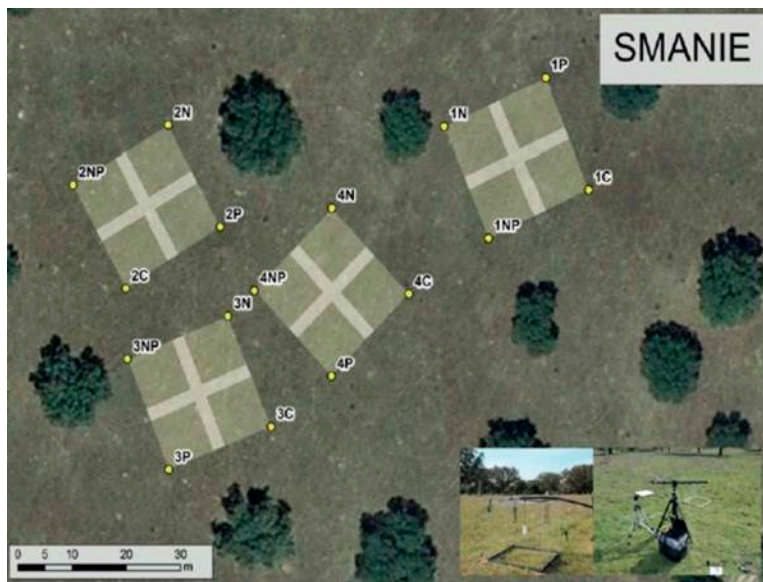


Figure s1. Aerial photograph of the experimental site (SMANIE) modified from Migliavacca et al., 2017. The position of the four blocks is indicated in transparent green and the treatment type is indicated at the corners of the blocks. The bands within each block indicate the buffer of 2 m used to separate the different treatments. In the lower right corner the transparent chambers used to measure the CO₂ and water fluxes are shown in the left figure and the manual system used to collect the spectral measurements is shown in the right figure.

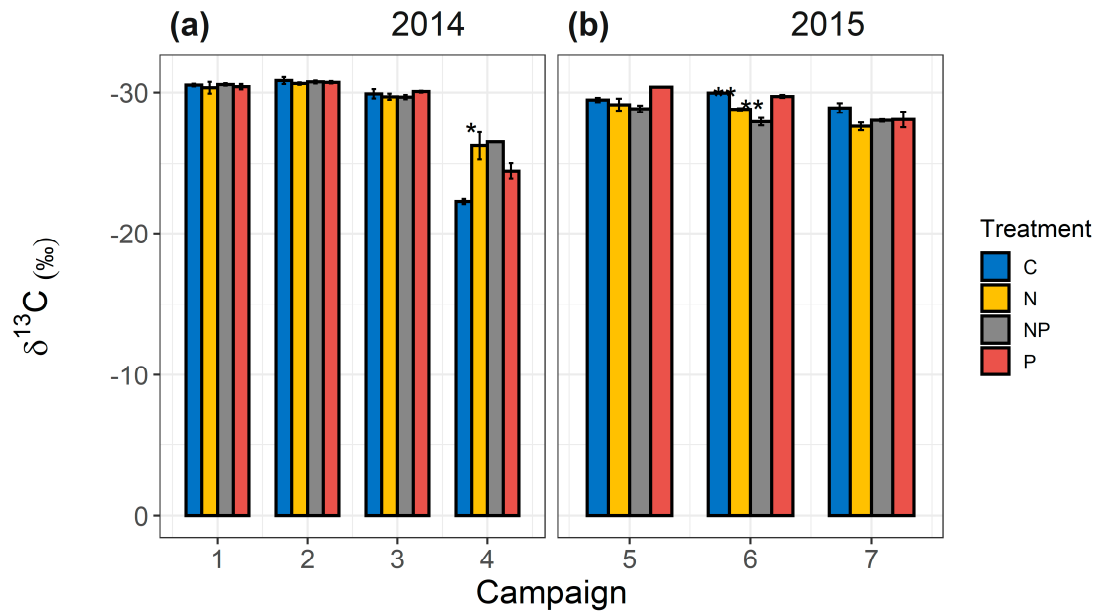


Figure S2. Group differences among treatment of carbon isotopic signature ($\delta^{13}\text{C}$) in 2014 (a) and 2015 (b). Group differences in (a), (b) were analyzed with ANOVA and Tukey post-hoc test. "*" refers to a significant difference from the control (C) treatment with p value < 0.05 and "***" refers to a significant difference from the C treatment with p value < 0.01.

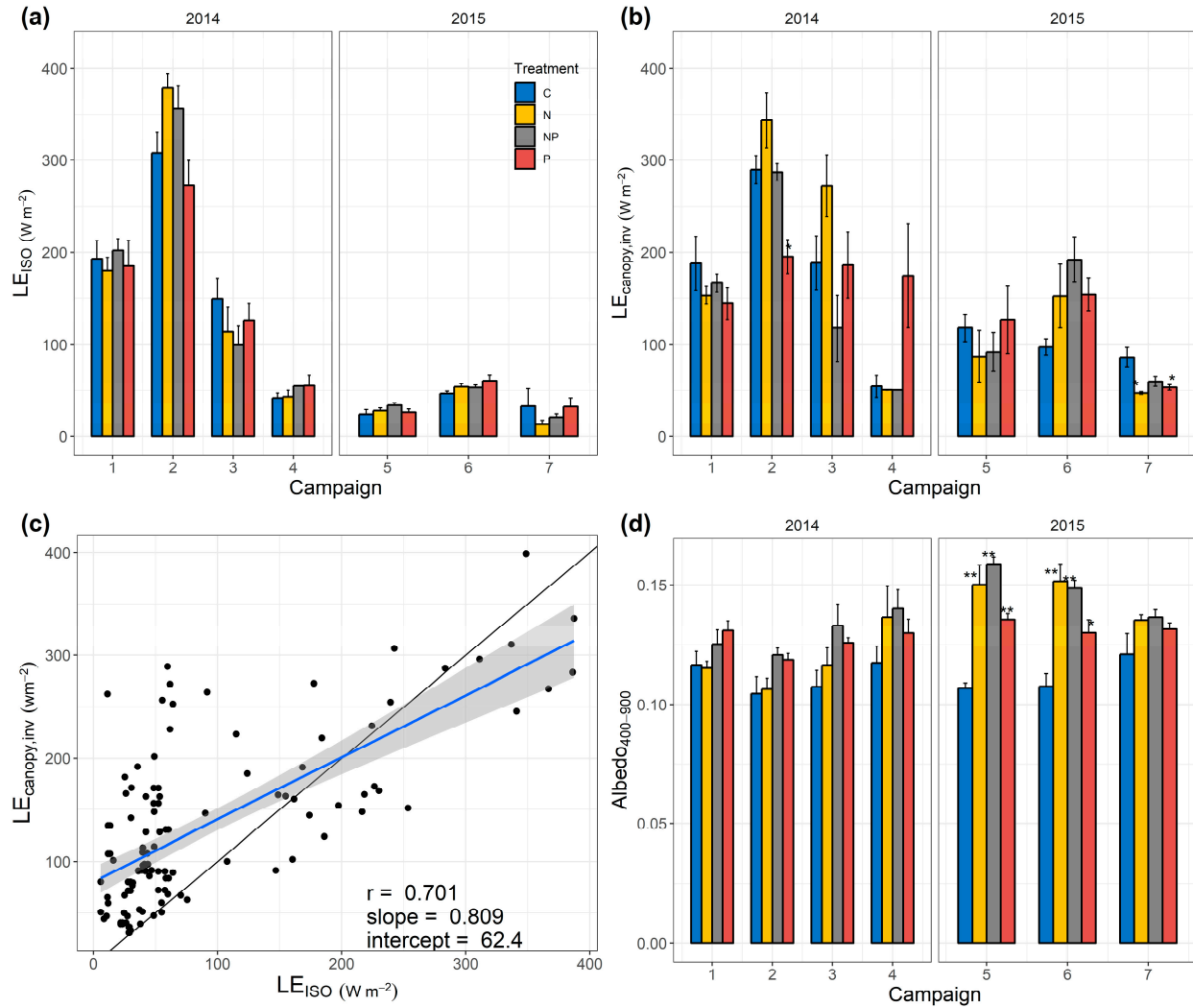


Figure S3. Group differences among treatment of transpiration (LE_{ISO}) (a) latent heat flux of the canopy estimated through SCOPE inversion ($LE_{canopy,inv}$) (b) integrated albedo between 400-900 nm ($Albedo_{400-900}$) (d) and relation among the two transpiration estimates (c) (note that the LE_{ISO} has been converted to W/m^2). Group differences in (a), (b) and (d) were analyzed with ANOVA and Tukey post-hoc test. “**” refers to a significant difference from the control (C) treatment with p value < 0.05 and “***” refers to a significant difference from the C treatment with p value < 0.01. Dashed lines represent the 1:1 line. Blue lines represent the regression from the total least square regression.

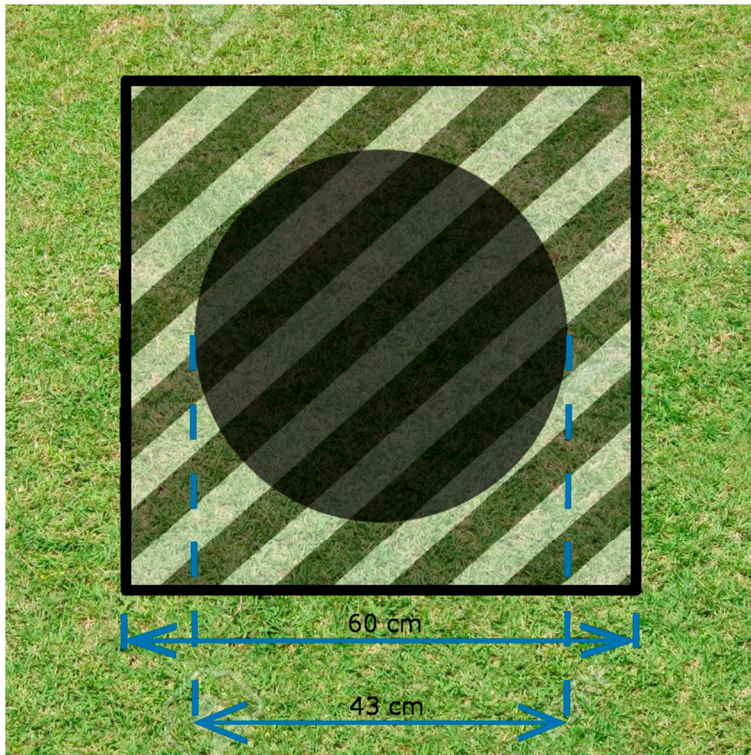


Figure S4. Schematic of the radiometric footprint (black circle) and the gas exchange footprint (black diagonal stripes) with the experimental set-up. When performing radiometric measurements, a frame painted with black mat color was put on the edges of the collar to prevent reflections from the metallic collar.

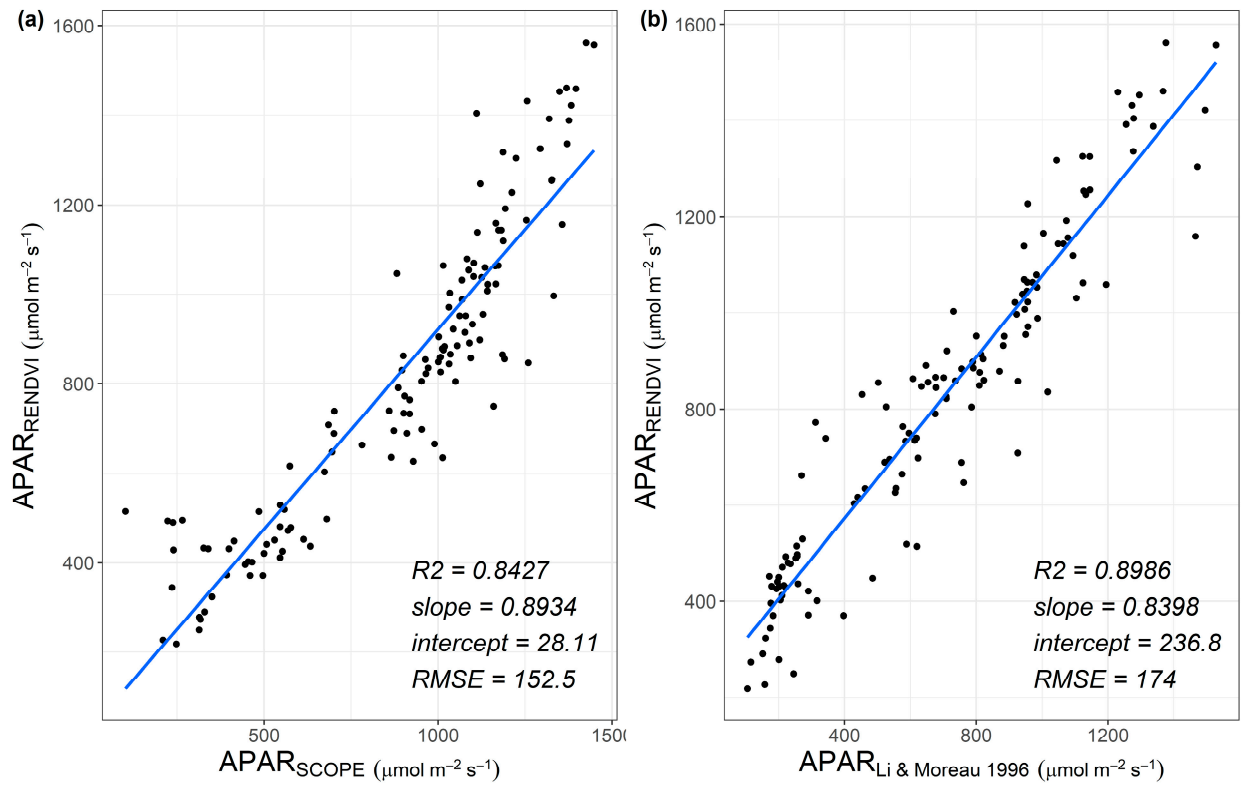


Figure S5. Scatterplot of modeled (from forward runs of SCOPE) vs absorbed photosynthetic active radiation (APAR) calculate from the RENDVI index (a), and APAR calculated with method of Li & Moreau 1996 vs $APAR_{RENDVI}$. Coefficient of determination (R^2), slope and intercept and Root Mean Square Error (RMSE) of the ordinary least square regression are reported in the bottom right. Blue lines represent the regression from the ordinary least square regression.

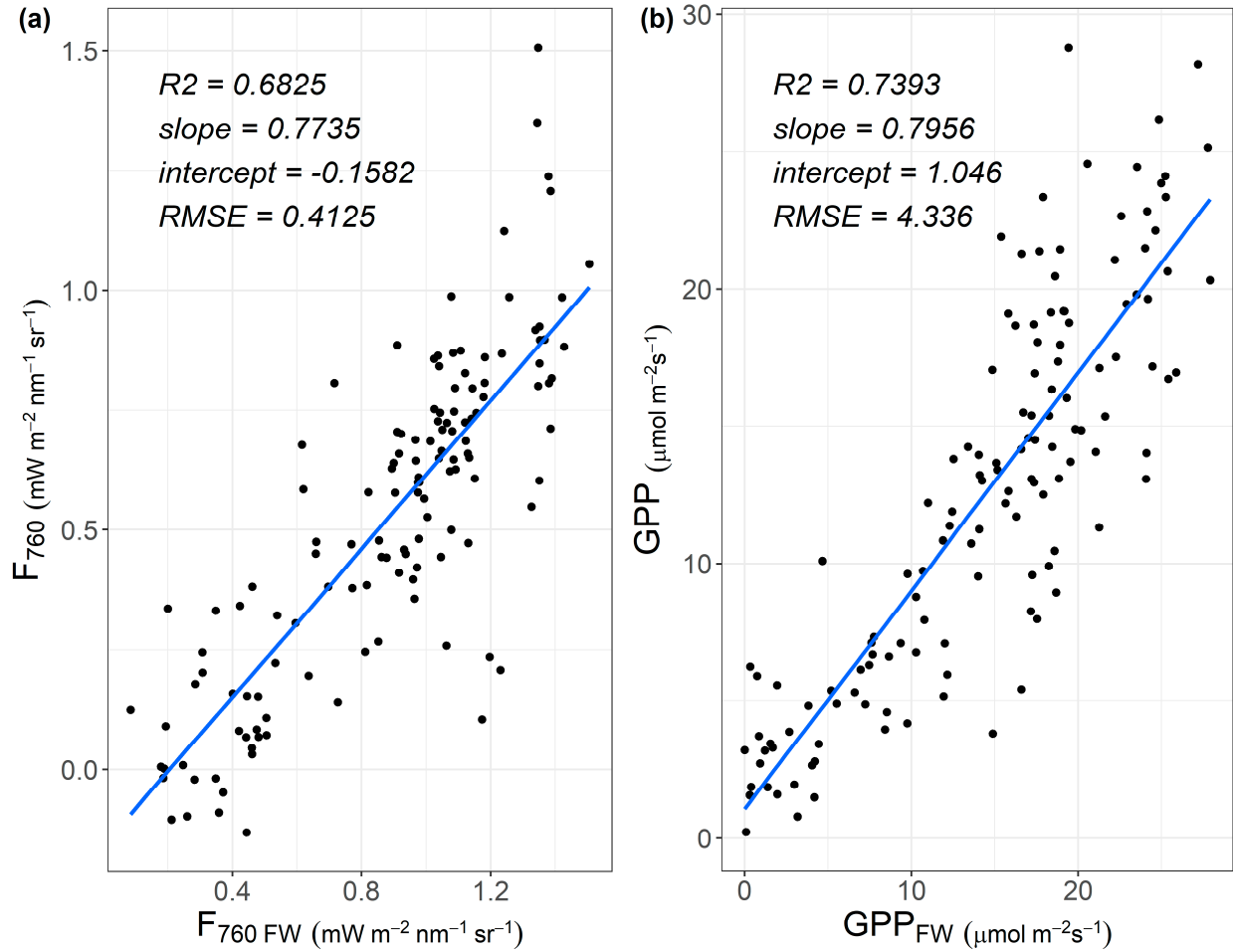


Figure S6. Scatterplot of modeled (forward SCOPE runs) vs observed fluorescence at 760 nm (F_{760}) (a) and scatterplot of modelled (from forward SCOPE runs) vs observed gross primary production (GPP) (b). Coefficient of determination (R^2), slope and intercept and Root Mean Square Error (RMSE) of the ordinary least square regression are reported in the top left. Blue lines represent the regression from the ordinary least square regression.

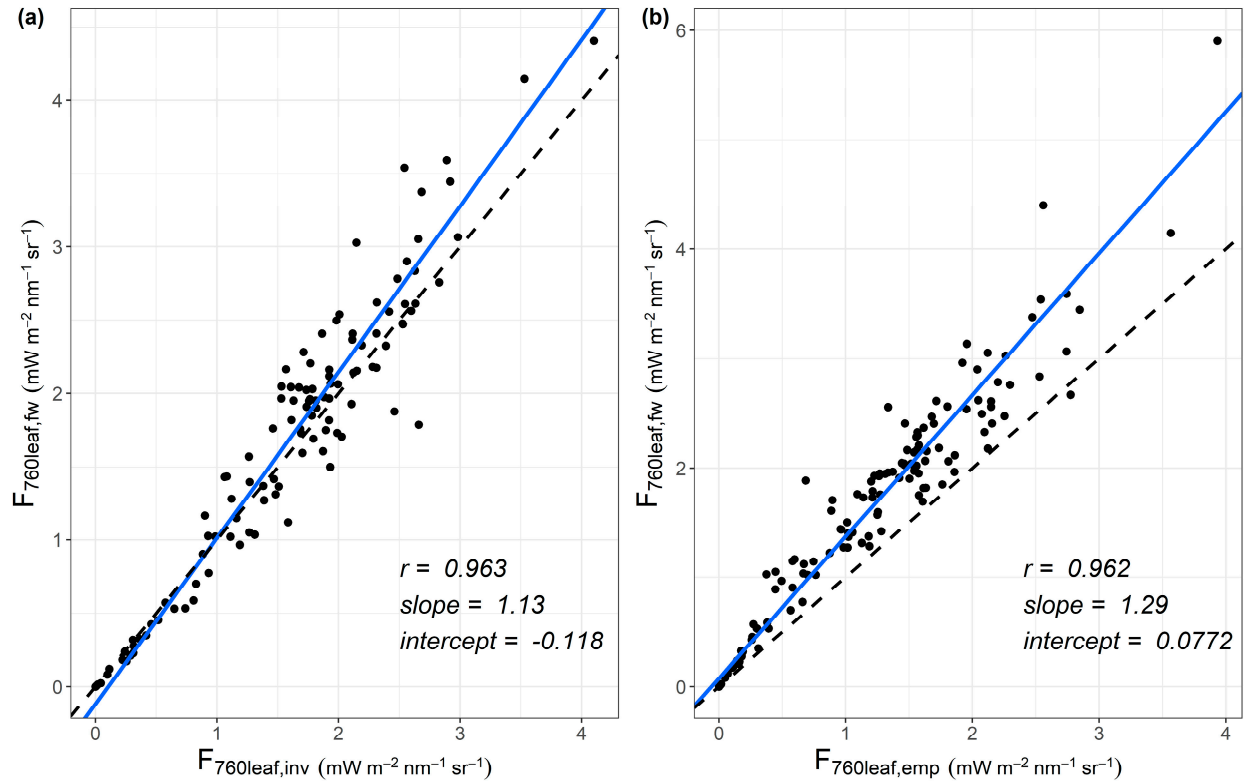


Figure S7. Scatterplot of the relationship between Fluorescence emitted by all leaves at 760 nm calculated by forward runs of SCOPE ($F_{760\text{leaf},\text{fw}}$) and Fluorescence emitted by all leaves at 760 nm calculated with the empirical approach ($F_{760\text{leaf},\text{emp}}$) (a) and relationship between $F_{760\text{leaf},\text{fw}}$ and fluorescence emitted by all leaves at 760 nm calculated from SCOPE inversion ($F_{760\text{leaf},\text{inv}}$) (b). Pearson correlation (r), slope and intercept from the Total Least Square regression is provided in the bottom right. Dashed lines represent the 1:1 line. Blue lines represent the regression from the total least square regression.

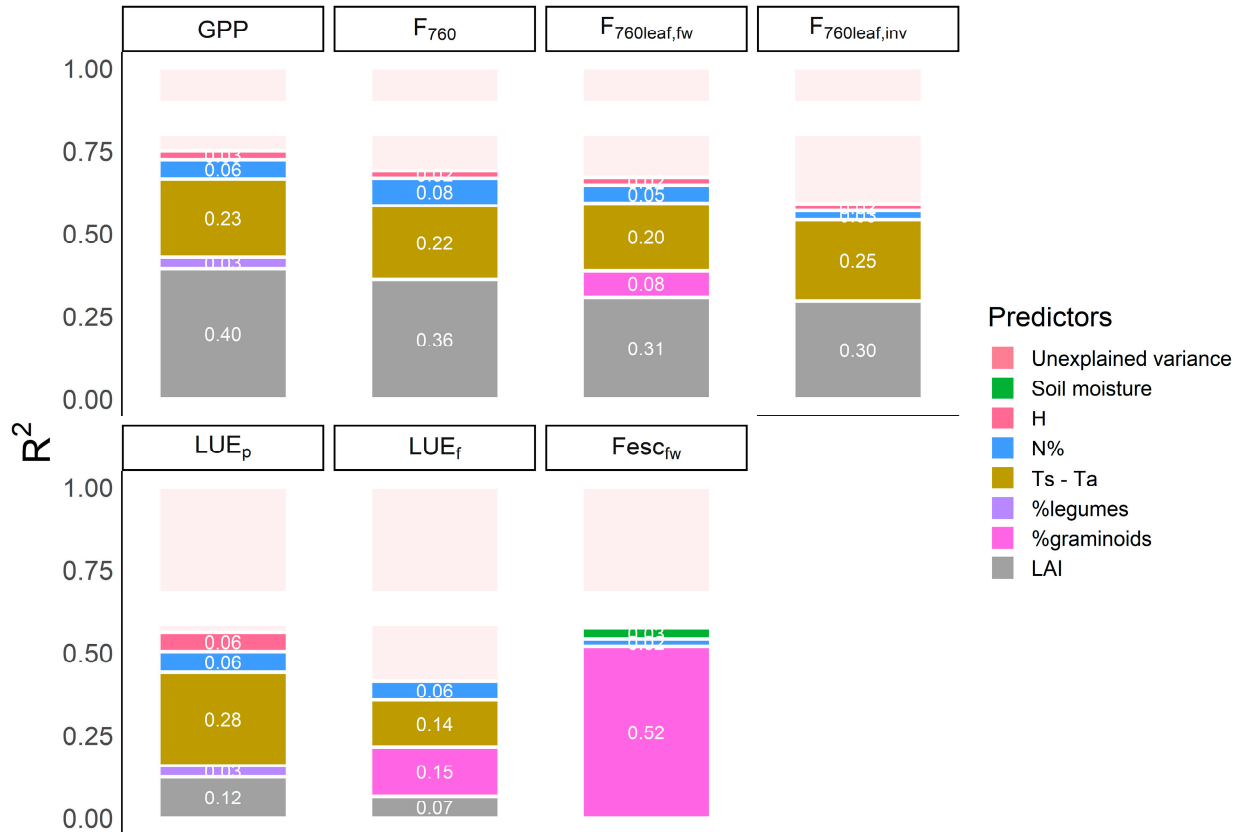


Figure S8. Relative importance analysis with LMG (Lindeman, Merenda and Gold) method of gross primary production (GPP), observed fluorescence at 760 nm (F_{760}), Fluorescence emitted at 760 nm calculated by forward runs of SCOPE ($F_{760\text{leaf, fw}}$), Light use efficiency of photosynthesis (LUE_p), light use efficiency of fluorescence emission at 760 nm (LUE_f), escape probability of F_{760} calculated from forward runs of SCOPE (F_{escfw}). Predictors included in the analysis are: soil moisture, Shannon biodiversity index (H), canopy nitrogen content (N%), surface-air temperature (Ts - Ta), relative abundance of legumes (%legumes), relative abundance of graminoids (%graminoids) and leaf are index (LAI).

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Leaf.Nitrogen ~ Treatment.N + Treatment.NP + Treatment.P
%graminoids ~ Treatment.N + Treatment.NP + Treatment.P
APAR ~ Leaf.Nitrogen + %graminoids
F.emitted760 ~ Leaf.Nitrogen + APAR
F760 ~ F.emitted760 + %graminoids
GPP ~ Leaf.Nitrogen + APAR
Surf.temp ~ Leaf.Nitrogen + %graminoids + APAR

GPP ~~ F.emitted760
GPP ~~ Surf.temp
F.emitted760 ~~ Surf.temp

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Figure S9. Set of equations that represent the model structure for the path analysis written as R markdown. Variables are the left are dependent variables and variables to the right of “~” are predictors. “~” denotes direct relationships, whereas “~~” denote covariance.

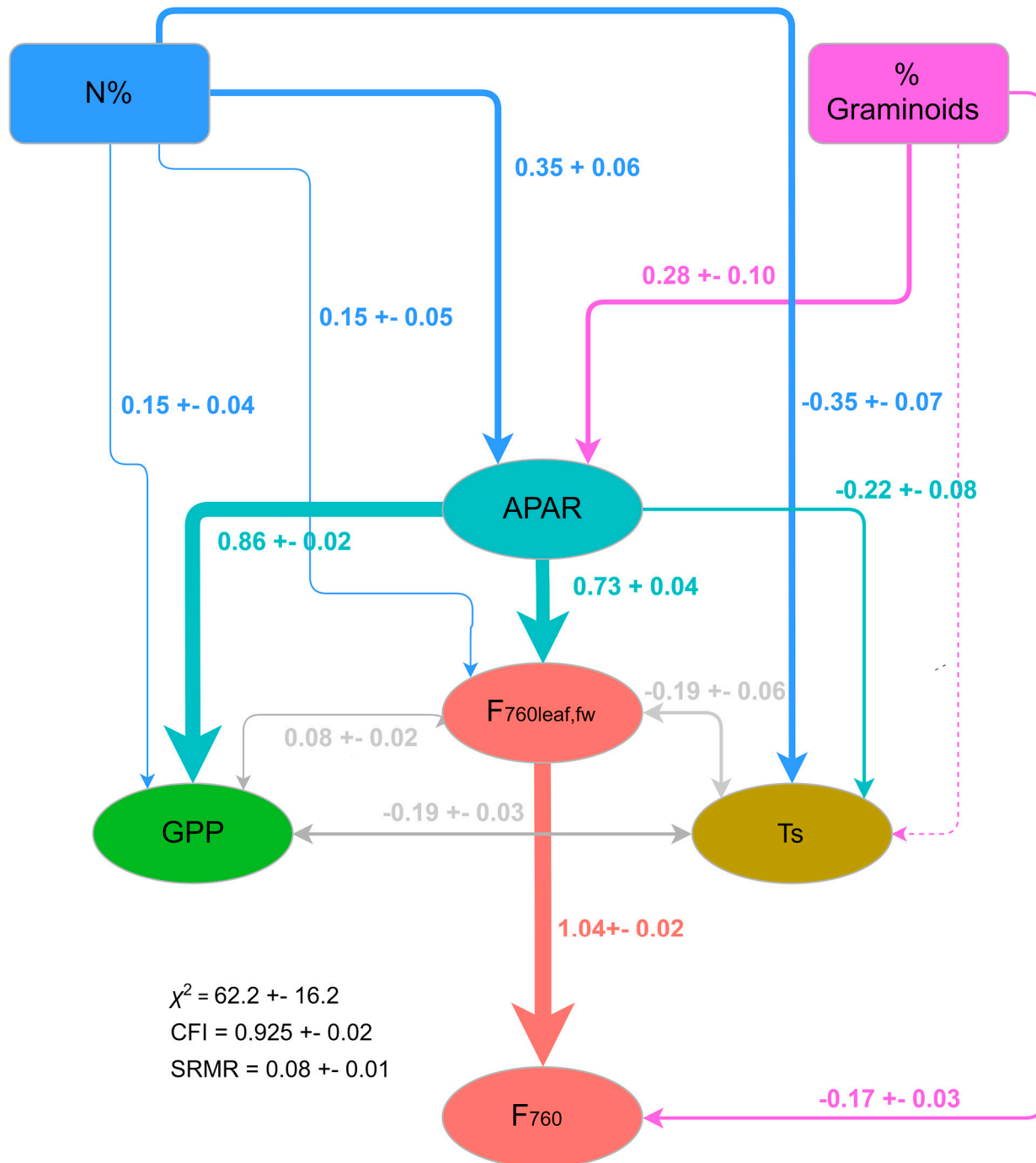


Figure S10. Path analysis without the Nitrogen (N) treatment. Path analysis displays the role of canopy nitrogen content (N%) and relative graminoids abundance (%graminoids) on the energy partitioning at the leaf and canopy level. Photosynthetic active radiation (PAR); Absorbed by vegetation photosynthetic active radiation (APAR), Fluorescence emission by all leaves at 760 nm calculated by forward runs of SCOPE ($F_{760\text{leaf},\text{fw}}$); gross primary production (GPP), Surface temperature (T_s) and observed fluorescence at 760 nm (F_{760}). The strength of the relationship among variables is expressed by the standardized coefficient (β) of the path analysis. Each standardized coefficient has a standard error obtain from bootstrapping ($n=100$ times). The width of the arrows is proportional to their standardized coefficient (β). Colored lines

(both solid or dotted) represent direct relationships between variables, whereas gray double-headed arrows represent the covariance among variables. Solid and dotted lines indicate significant ($p < 0.05$) and non-significant relationships, respectively. The width of the arrows is proportional to their standardized coefficient (β). The different colors are introduced to increase readability of the standardized path coefficients. The fit by the overall model is measured by means of Chi-squared (χ^2), comparative fit index (CFI) and standardized root mean square of residual (SRMR).

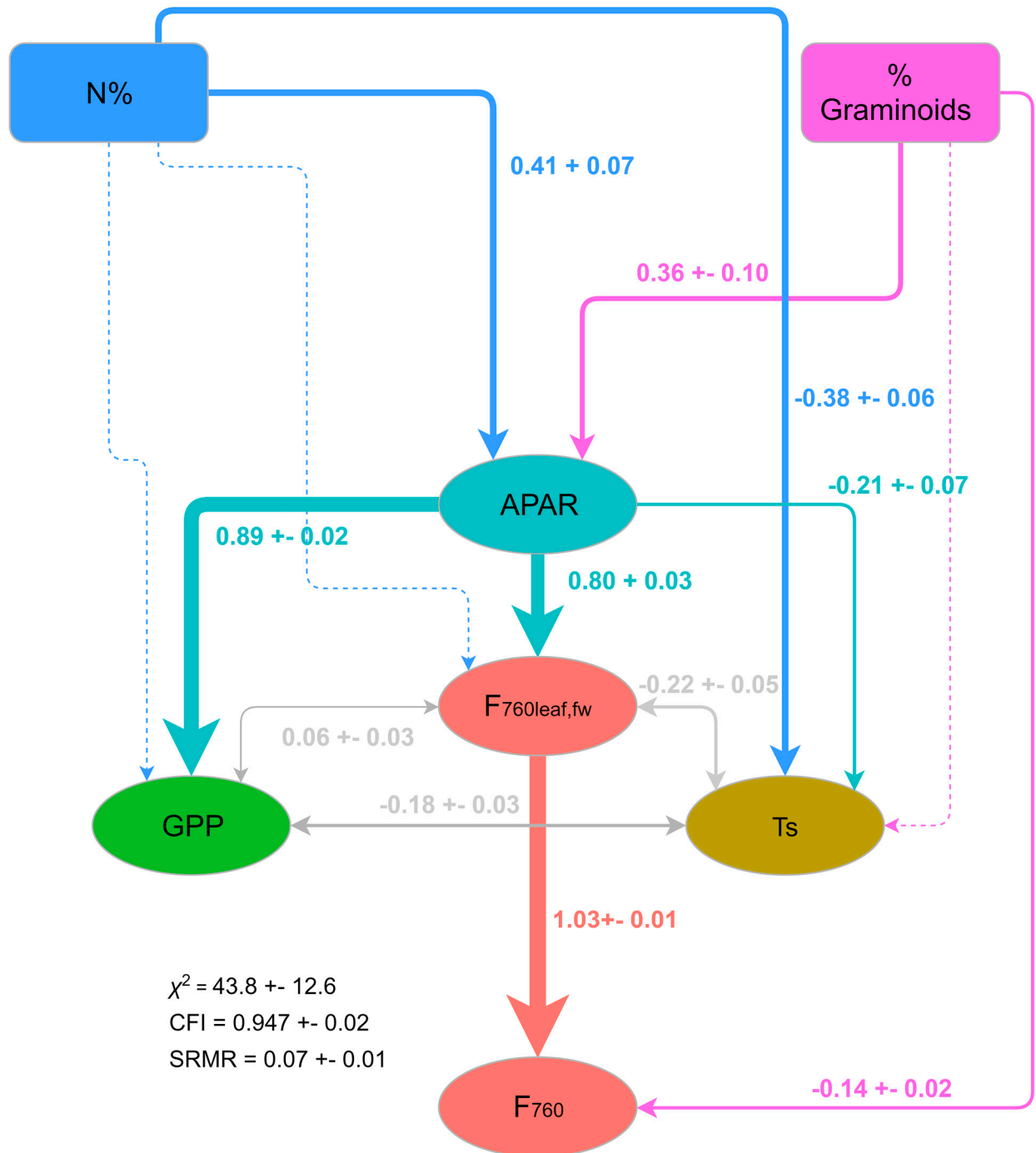


Figure S11. Path analysis without the Nitrogen and Phosphorus (NP) treatment. Path analysis displays the role of canopy nitrogen content (N%) and relative graminoids abundance (%graminoids) on the energy partitioning at the leaf and canopy level. Photosynthetic active radiation (PAR); Absorbed by vegetation photosynthetic active radiation (APAR), Fluorescence emission by all leaves at 760 nm calculated by forward runs of SCOPE ($F_{760\text{leaf, fw}}$); gross primary production (GPP), Surface temperature (Ts) and observed fluorescence at 760 nm (F_{760}). The strength of the relationship among variables is expressed by the standardized coefficient (β) of the path analysis. Each standardized coefficient has a standard error obtain from bootstrapping (n=100 times). The width of the arrows is proportional to their standardized coefficient (β). Colored lines (both solid or dotted) represent direct relationships between variables, whereas gray double-headed arrows represent the covariance among variables. Solid and dotted lines indicate significant ($p < 0.05$) and non-significant relationships, respectively. The width of the arrows is proportional to their standardized coefficient (β). The different colors are introduced to increase readability of the standardized path coefficients. The fit by the overall model is measured by means of Chi-squared (χ^2), comparative fit index (CFI) and standardized root mean square of residual (SRMR).

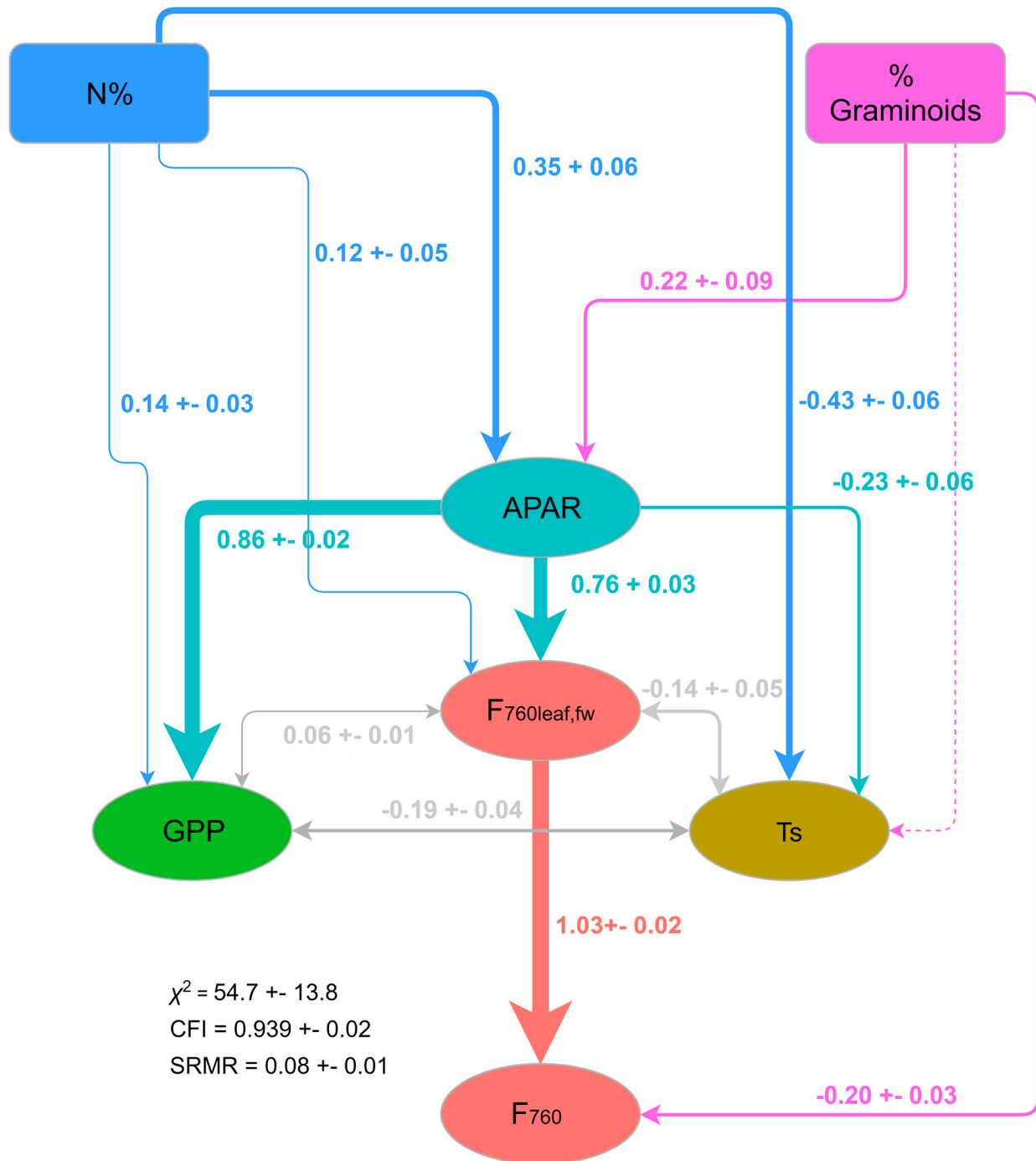


Figure S12. Path analysis without the Phosphorus (P) treatment. Path analysis displays the role of canopy nitrogen content (N%) and relative graminoids abundance (%graminoids) on the energy partitioning at the leaf and canopy level. Photosynthetic active radiation (PAR); Absorbed by vegetation photosynthetic active radiation (APAR), Fluorescence emission by all leaves at 760 nm calculated by forward runs of SCOPE ($F_{760leaf,fw}$); gross primary production (GPP), Surface temperature (T_s) and observed fluorescence at 760 nm (F_{760}). The strength of the relationship among variables is expressed by the standardized coefficient (β) of the path analysis. Each standardized coefficient has a standard error obtain from bootstrapping (n=100 times). The width of the arrows is proportional to their standardized coefficient (β). Colored lines

(both solid or dotted) represent direct relationships between variables, whereas gray double-headed arrows represent the covariance among variables. Solid and dotted lines indicate significant ($p < 0.05$) and non-significant relationships, respectively. The width of the arrows is proportional to their standardized coefficient (β). The different colors are introduced to increase readability of the standardized path coefficients. The fit by the overall model is measured by means of Chi-squared (χ^2), comparative fit index (CFI) and standardized root mean square of residual (SRMR).

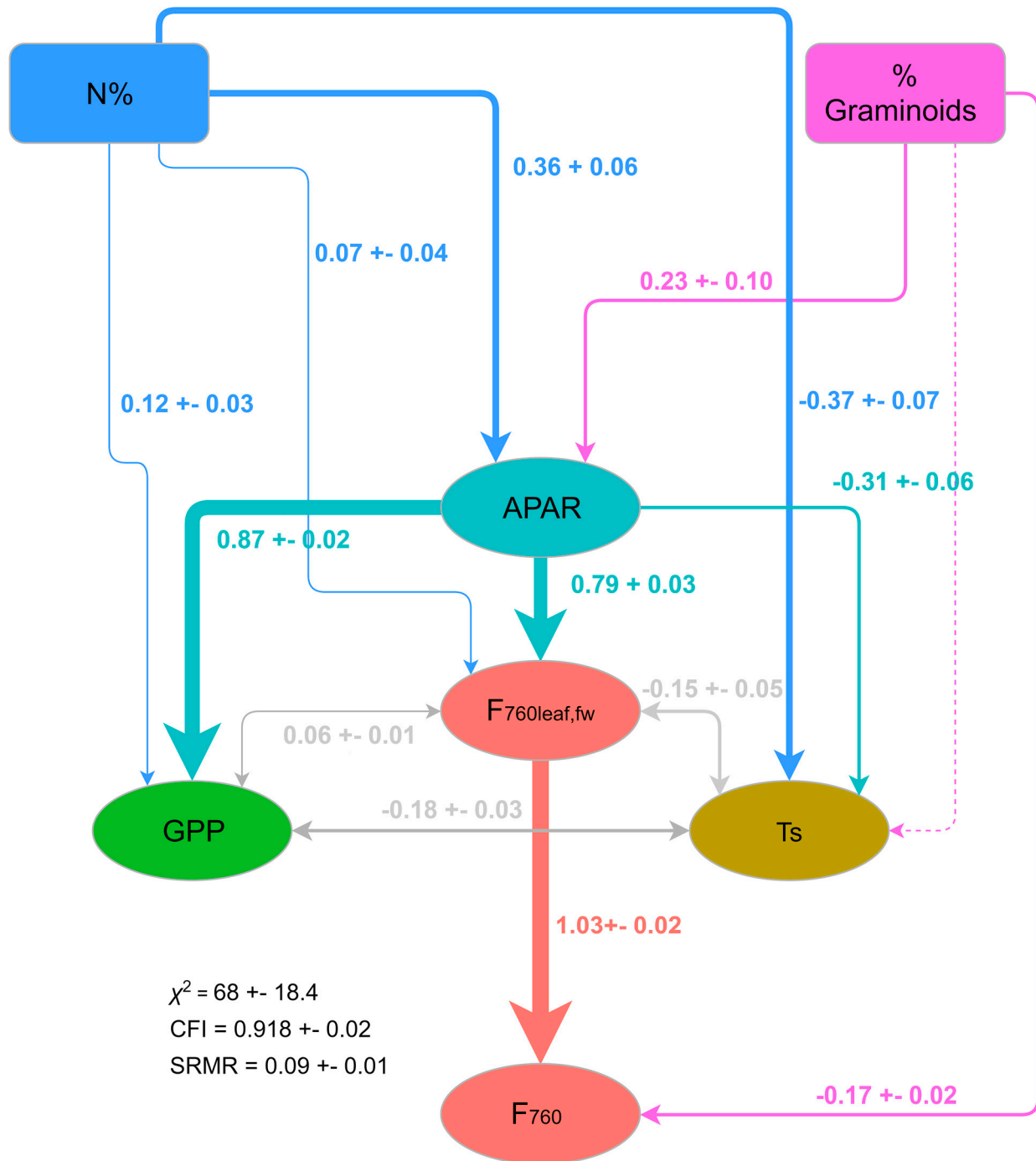


Figure S13. Path analysis without the Control treatment(C). Path analysis displays the role of canopy nitrogen content (N%) and relative graminoids abundance (%graminoids) on the energy partitioning at the leaf and canopy level. Photosynthetic active radiation (PAR); Absorbed by vegetation photosynthetic active radiation (APAR), Fluorescence emission by all leaves at 760 nm calculated by forward runs of SCOPE ($F_{760\text{leaf},fw}$); gross primary production (GPP), Surface temperature (T_s) and observed fluorescence at 760 nm (F_{760}). The strength of the relationship among variables is expressed by the standardized coefficient (β) of the path analysis. Each standardized coefficient has a standard error obtain from bootstrapping (n=100 times). The width of the arrows is proportional to their standardized coefficient (β). Colored lines

(both solid or dotted) represent direct relationships between variables, whereas gray double-headed arrows represent the covariance among variables. Solid and dotted lines indicate significant ($p < 0.05$) and non-significant relationships, respectively. The width of the arrows is proportional to their standardized coefficient (β). The different colors are introduced to increase readability of the standardized path coefficients. The fit by the overall model is measured by means of Chi-squared (χ^2), comparative fit index (CFI) and standardized root mean square of residual (SRMR).

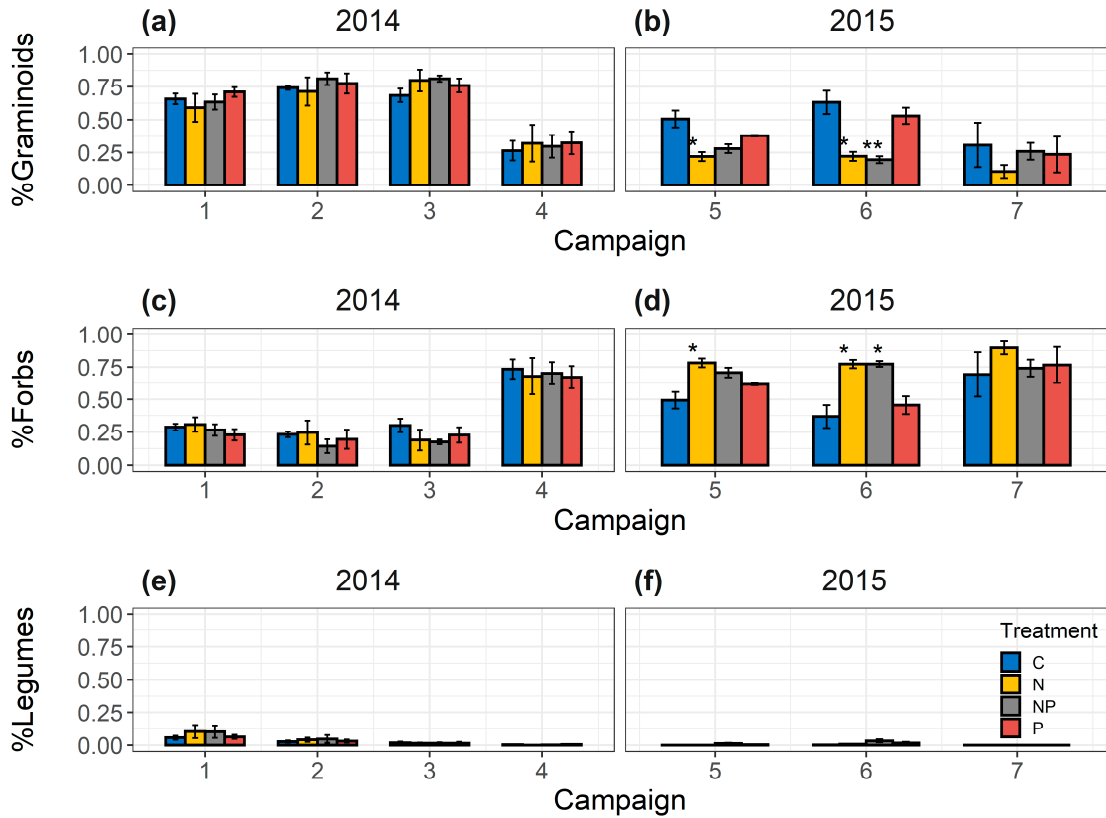


Figure S14. Bar graph representing differences among treatments of graminoids relative abundance (%graminoids) in 2014 (a) and 2015 (b), forbs relative abundance (%Forbs) in 2014 (c) and 2015 (d) and legumes relative abundance (%Legumes). Data are divided among campaigns. Bar graphs represent means and error bars represent 1 standard error. Group differences were analyzed with ANOVA with the Welch correction and individual differences among groups were evaluated with the Games-Howell post hoc test. "*" refers to a significant difference from the control (C) treatment with p value < 0.05 and "***" refers to a significant difference from the C treatment with p value < 0.01 .

Table 1. Evaluation of the relationship between Gross Primary Production (GPP) and Fluorescence at 760 nm (F_{760}) and between GPP and Fluorescence at emission level at 760 nm ($F_{760leaf, fw}$) among different treatments.

Year	Treatment	Variable	Intercept P value	Interaction P value	Intercept value	Slope value
2014	C	$F_{760leaf, fw}$	0.03		4.09	5.30
2014	C	F_{760}	0.04		4.48	17.11
2014	N	$F_{760leaf, fw}$	0.38	0.95	2.40	5.39
2014	N	F_{760}	0.39	0.8	2.44	18.42
2014	NP	$F_{760leaf, fw}$	0.92	0.89	0.23	5.11
2014	NP	F_{760}	0.77	0.69	-0.79	19.19
2014	P	$F_{760leaf, fw}$	0.07	0.86	3.14	5.04
2014	P	F_{760}	0.13	0.96	2.60	17.36
2015	C	$F_{760leaf, fw}$	< 0.01		4.68	4.57
2015	C	F_{760}	< 0.01		4.76	14.93
2015	N	$F_{760leaf, fw}$	0.57	0.02	0.89	8.46
2015	N	F_{760}	0.59	0.09	0.84	23.29
2015	NP	$F_{760leaf, fw}$	0.03	0.09	2.66	7.21
2015	NP	F_{760}	0.02	0.32	2.65	19.50
2015	P	$F_{760leaf, fw}$	0.06	0.57	3.94	5.51
2015	P	F_{760}	0.07	0.64	3.74	17.32

Gross Primary Production (GPP) is the dependent variable. Fluorescence emitted by all leaves at 760 nm calculated from forward SCOPE runs ($F_{760leaf, fw}$) and observed Fluorescence at 760 nm (F_{760}) are the independent variables. The column intercept reports the p values of the intercept from the multiple linear model and the column Interaction reports the p values of the interaction between Independent variable and Treatment factor in relation to the Control (C) treatment from the ANCOVA. Bold p values refer to p values < 0.05. The values of intercept and slope from the linear model $GPP \sim F_{760}$ among treatments and the linear model $GPP \sim F_{760leaf, fw}$ among treatments are reported. N refers to Nitrogen addition treatment, P is the phosphorus addition treatment and NP is the nitrogen and phosphorus addition treatment.

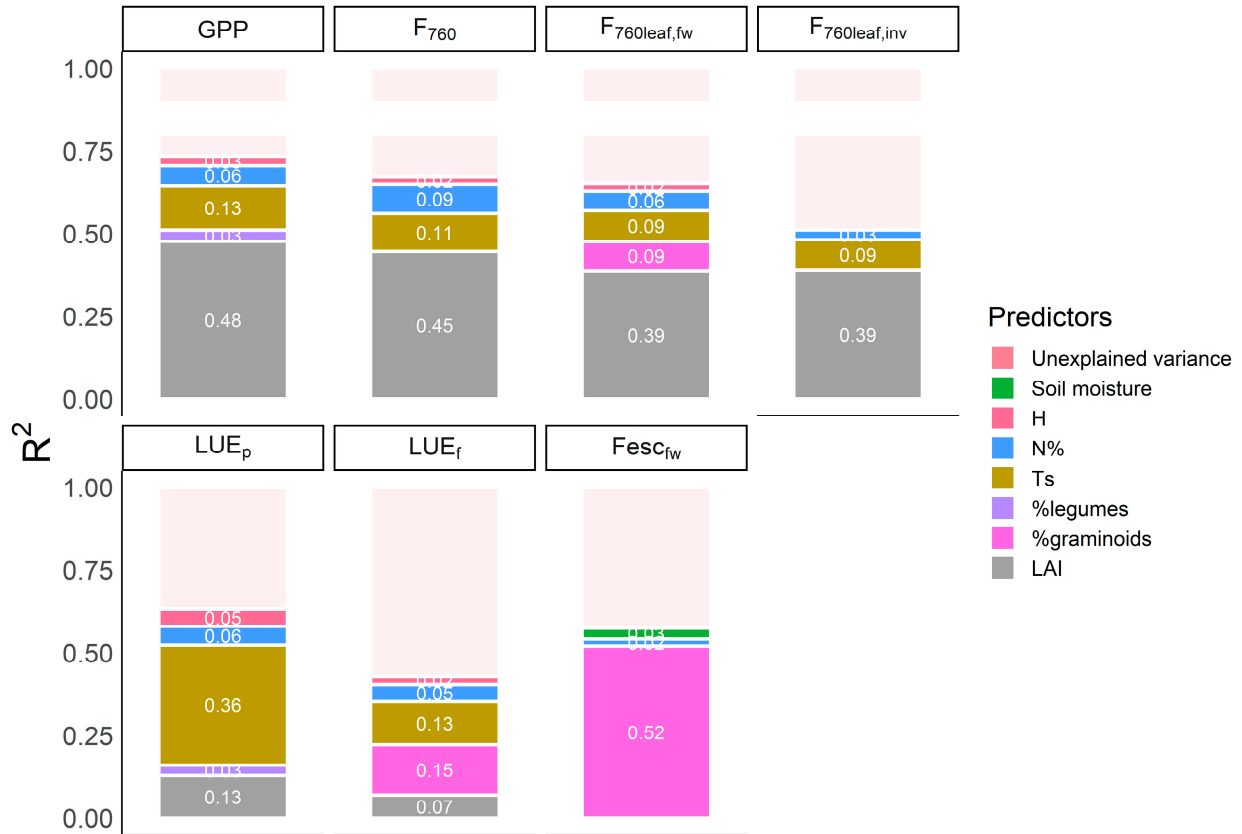


Figure S15. Relative importance analysis with LMG (Lindeman, Merenda and Gold) method of Gross Primary Production (GPP), observed fluorescence at 760 nm (F_{760}), Fluorescence emitted at 760 nm calculated by forward runs of SCOPE ($F_{760\text{leaf},fw}$), Light use efficiency of photosynthesis (LUE_p), light use efficiency of fluorescence emission at 760 nm (LUE_f), escape probability of F_{760} calculated from forward runs of SCOPE ($Fesc_{fw}$). Predictors included in the analysis are: soil moisture, Shannon biodiversity index (H), canopy nitrogen content (N%), surface temperature (Ts), relative abundance of legumes (%legumes), relative abundance of graminoids (%graminoids) and leaf area index (LAI).

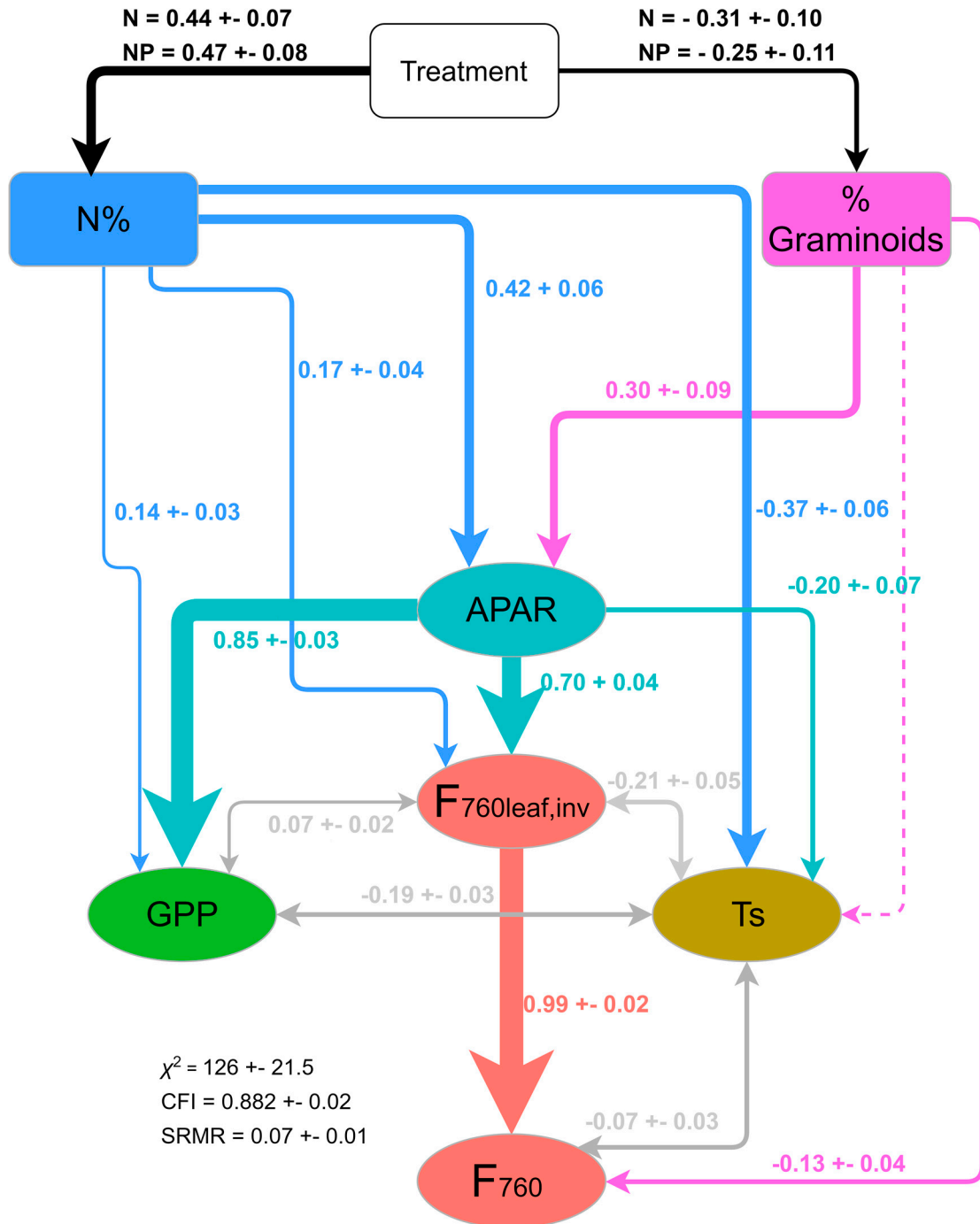


Figure S16. Path analysis with Fluorescence emission at 760 nm calculated from SCOPE inversion $F_{760leaf,inv}$. Path analysis displays the role of canopy nitrogen content (N%) and relative graminoids abundance (%graminoids) on the energy partitioning at the leaf and canopy level. Photosynthetic active radiation (PAR); Absorbed by vegetation photosynthetic active radiation (APAR), Fluorescence emission by all leaves at 760 nm calculated by forward runs of SCOPE ($F_{760leaf,inv}$); gross primary production (GPP), Surface temperature (Ts) and observed fluorescence at 760 nm (F_{760}). The strength of the relationship among variables is expressed by the standardized coefficient (β) of the path analysis. Each standardized coefficient has a standard error obtain from bootstrapping ($n=100$ times). The width of the arrows is

proportional to their standardized coefficient (β). Colored lines (both solid or dotted) represent direct relationships between variables, whereas gray double-headed arrows represent the covariance among variables. Solid and dotted lines indicate significant ($p < 0.05$) and non-significant relationships, respectively. The width of the arrows is proportional to their standardized coefficient (β). The different colors are introduced to increase readability of the standardized path coefficients. The fit by the overall model is measured by means of Chi-squared (χ^2), comparative fit index (CFI) and standardized root mean square of residual (SRMR).

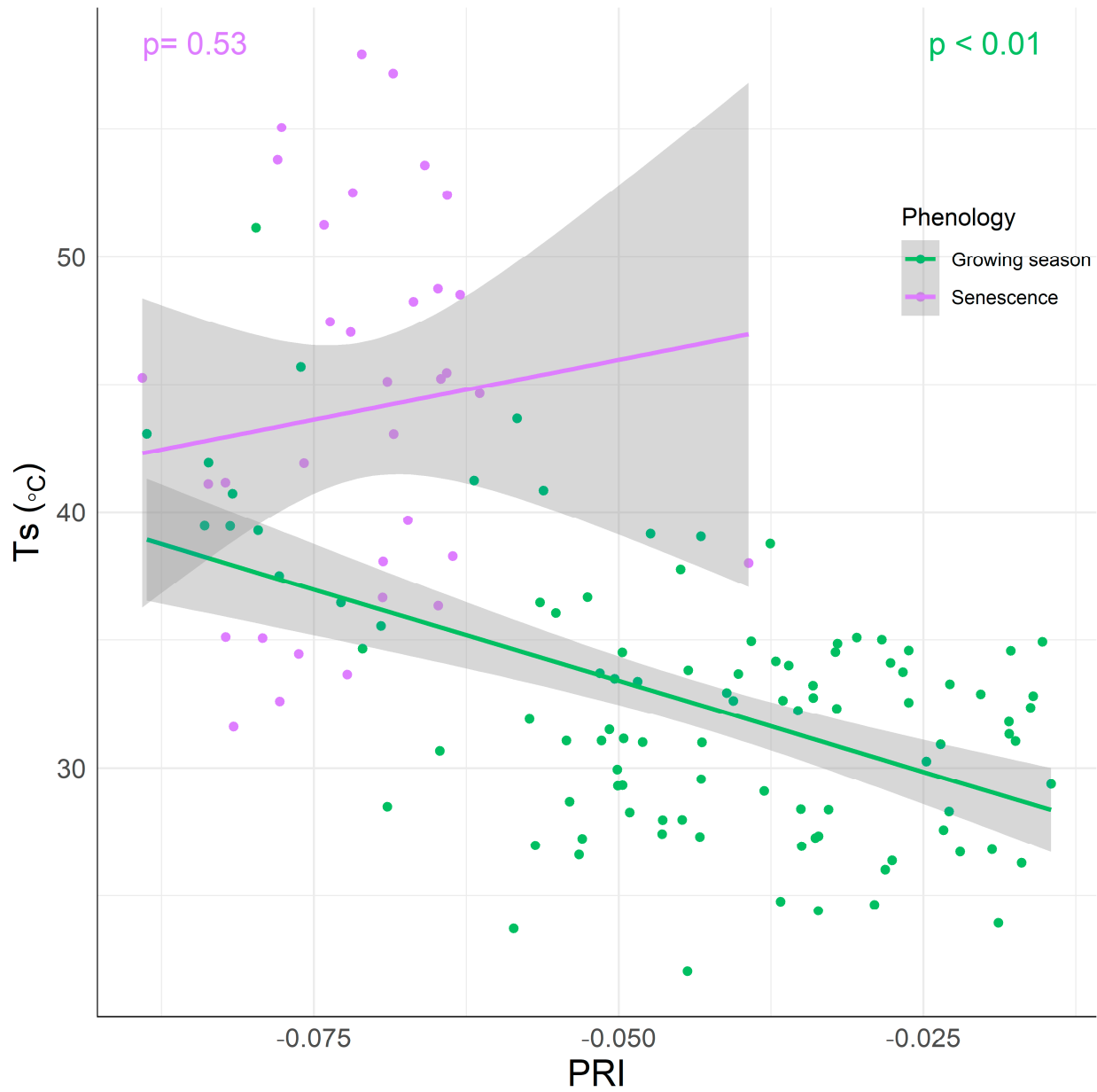


Figure S17. Scatterplot of Surface temperature (T_s) vs Photochemical Reflectance Index (PRI). “Growing season” includes the campaigns 1,2,3,5,6 The p values of the ordinary least square regressions are reported in the top. Colored lines represent the regression from the ordinary least square regression.

