Nitrogen Availability and Summer Drought, but not N:P Imbalance Drive Carbon Use Efficiency of a Mediterranean Tree-Grass Ecosystem

5 Richard Nair[1,2], Yunpeng Luo[3], Tarek El-Madany[2], Victor Rolo[4], Javier Pacheco-Labrador[5], Silvia Caldararu[1], Kendalynn A Morris[5], Marion Schrumpf[2,6,] Arnaud Carrara[7], Gerardo Moreno[4], Markus Reichstein[2], Mirco Migliavacca[2,8]

1 Discipline of Botany, School of Natural Sciences, Trinity College Dublin, Dublin Ireland
2 Department of Biogeochemical Integration, Max Planck Institute for Biogeochemistry, 07745 Jena, Germany
10 3 Swiss Federal Institute for Forest, Snow and Landscape Research WSL, 8903 Birmensdorf, Switzerland
4 Forest Research Group, INDEHESA, University of Extremadura, Plasencia, Cáceres, Spain
5 Joint Global Change Research Institute, Pacific Northwest National Laboratory, College Park, Maryland, USA
6 Department of Biogeochemical Processes, Max Planck Institute for Biogeochemistry, 07745 Jena, Germany
7 Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), 46980 Valencia, Spain
15 8 European Commission Joint Research Centre, Via Enrico Fermi 2749, Ispra (VA), Italy

Correspondence to: Richard Nair (richard.nair@tcd.ie)

Abstract.

All ecosystems are simultaneously a source and a sink of atmospheric carbon (C). A change in their balance of net and gross
20 ecosystem carbon uptake, ecosystem-scale carbon use efficiency (CUE_{ECO}), is a change in their ability to buffer climate change.

Anthropogenic nitrogen (N) deposition is increasing N availability, potentially shifting terrestrial ecosystem stoichiometry towards phosphorus (P) limitation. Depending on how gross primary production (GPP, plants alone) and ecosystem respiration (R_{ECO}, plants and heterotrophs) are limited by N, P, or associated changes in other biogeochemical cycles,
25 CUE_{ECO} may change. Seasonally, CUE_{ECO} also varies as GPP is more coupled to the growing season than respiration.

We worked in a Mediterranean tree-grass ecosystem (locally called 'dehesa') characterized by mild, wet winters and summer droughts. We examined CUE_{ECO} from eddy covariance fluxes over six years under control, +N and +NP fertilized treatments on three timescales: annual, seasonal (determined by vegetation phenophase) and two-weekly aggregations. Finer aggregation allowed consideration of responses to specific vegetation and meteorological conditions. We predicted that
30 CUE_{ECO} should be increased by wetter conditions, and by NP fertilization.

Milder and wetter years with proportionally longer growing seasons increased CUE_{ECO}, as did N fertilization, regardless of whether P was added. Using a generalized additive model, whole ecosystem vegetation status and water deficit indicators, which both varied with treatment, were the main determinants of biweekly differences in CUE_{ECO}. The direction of water effects depended on the timescale considered and occurred alongside treatment-dependent water depletion.

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1 Introduction

Understanding links between ecosystem carbon (C) stocks and atmospheric carbon dioxide (CO_2) is important because of atmospheric CO_2 concentrations are a driver of climate change and climate change also changes these links (Cao & Woodward, 1998). Ecosystems sequester C via photosynthesis (i.e. Gross Primary Production, GPP) and release it via

40   ecosystem respiration (R_{ECO}). However, ecosystems are made up of many individuals and processes responding to environmental conditions, integrating biogeochemical cycling of C, N, P, and water through life history, adaptation, acclimation and competition (Leibold et al., 2017). Thus biogeochemical cycles are coupled and changes in one can affect others (Gentine et al., 2019; Zaehle, 2013). Hence developing environmental resource imbalances, such as progressive N limitation due to CO_2 fertilization (Coskun et al., 2016; Craine et al., 2018; Y. Luo et al., 2004) and N:P imbalance due to N

45   deposition (Du et al., 2020; Peñuelas et al., 2012, 2013, 2020) affect GPP and R_{ECO} in different ways (El-Madany et al., 2021). The difference between GPP and R_{ECO} is Net Ecosystem Production (NEP). The ratio of NEP/GPP is ecosystem scale carbon use efficiency (CUE_{ECO}), so it incorporates both GPP and R_{ECO} (e.g. Fernández-Martínez et al., 2014; Sinsabaugh et al., 2017). CUE_{ECO} is neither stable in time (e.g. von Buttlar et al., 2018) nor between ecosystem types (DeLucia et al., 2007; Zhang et al., 2009). Other definitions of CUE (Manzoni et al., 2018) only consider vegetation (i.e. net primary

50   productivity over GPP) or microbial growth (biomass change over C uptake). As CUE_{ECO} represents whole ecosystems, it is directly scalable to global concerns (Bradford & Crowther, 2013).

Carbon use efficiency concepts normalize for productivity variations and can compare dissimilar communities in space (Chen & Yu, 2019; DeLucia et al., 2007; Liu et al., 2019) or time (Gang et al., 2019). Although CUE_{ECO} is less variable than

55   its component fluxes (Manzoni et al., 2018), climate and soil nutrients affect CUE_{ECO} (DeLucia et al., 2007; Gang et al., 2022; Zhang et al., 2014). Hence understanding CUE_{ECO} informs predictive capability for both maintaining C uptake capacity (1/3 of human emissions) and C stores (3 x atmospheric pool) in the terrestrial biosphere (Friedlingstein et al., 2022). But predictive understanding of variation in CUE_{ECO} are still lacking, just as similar issues exist for subcomponents vegetation (Gang et al., 2022) and microbial CUE (Qiao et al., 2019).

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Control of ecosystem function is complex because ecosystems consist of independent individuals and their interactions. Co-limitation between nutrients, water and carbon fixation rate is common, especially on sub-annual timescales where seasonal droughts or winter periods restrict growth. Generally, primary productivity is limited by water availability and nutrient availability, and decomposers by C supply (Soong et al., 2020). Hence CUE_{ECO} could respond to nutrient availability, or not,

65   depending on the link between productivity and turnover. Further, both ecosystem (Migliavacca et al., 2015) and soil respiration (Bahn et al., 2009) respond to short-term GPP, so GPP could directly drive CUE_{ECO}. Soils also contain large C stocks, and turnover is linked to productivity (Stoner et al., 2021). But microbes are the main CO_2 source from these soil stocks (Bond-Lamberty et al., 2018), so responses could be via alterations in C supply or nutrient mining responses under

stoichiometric shifts (Hicks et al., 2021; Janssens et al., 2010). Microbial and plant activity also depend on water availability,
but vegetation often senesces, or dies in droughts, so further lags may also occur as vegetation responses are slower than
microbes (Pereira et al., 2007).

Phenology determines sub-annual CUE_{ECO} as GPP completely depends on active green vegetation (the 'growing season') but
respiration can occur from turnover of dead biomass without this constraint. Short-term endogenous (e.g. bud burst, Jeong et
al., 2012) or exogenous (e.g. rain pulses, Xu et al., 2004) events also affect photosynthesis, respiration and resource
assignment. Hence instability is expected. Indeed, patterns of resource distribution between processes (Lapointe, 2001;
Maseyk et al., 2008), organs (W. Luo et al., 2013), tissue stoichiometry (Meunier et al., 2017), allometry (Weiner, 2004)
and community structure vary sub-annually, determining GPP and R_{ECO} independently (Butterfield & Malmström, 2009;
Caldararu et al., 2014; Keenan et al., 2014; Y. Luo et al., 2020). Phenology itself also responds to climate change (Piao et al.,
2019; Richardson et al., 2013) but plant and microbe population and generation times differ by orders of magnitude, so shifts
may occur in different directions, scales and timescales contributing to a net response.

Depending on this net response, CUE_{ECO} may or may not change. For example, plants respond plastically to nutrient
availability (Oldroyd & Leyser, 2020), for example via C allocation to organs and processes. N addition may not alter local
CUE_{ECO} (Ma et al., 2018) but N fertility does regionally (Chen & Yu, 2019) and globally (Fernández-Martínez et al., 2014).
Aridity also drives CUE_{ECO} globally (Migliavacca et al., 2021), because under unfavourable conditions there are greater
costs – respiration - for equivalent function -growth, acquisition of resources such as nutrients and water.

Arid- and semi- arid systems play an important role in the C cycle, switching between net C sources and C sinks (Ahlstrom
et al., 2015). In other words, annual GPP and R_{ECO} are not proportional. Both vegetation growth and microbial activity are
also water limited for part of the year so this is driven by seasons and 'wet' and 'dry' years (El-Madany et al., 2020; Scott et
al., 2015). Hence the timescale at which the ecosystem is observed may not only determine the strength of the net sink, but
whether the net C flux, and CUE_{ECO} observed, is positive or negative at all. In this study, we were interested in how CUE_{ECO}
of a Mediterranean tree-grass ecosystem responded to ecosystem stoichiometry manipulated by long term N (NT) and N and
P (NPT) addition compared to a control treatment (CT). We examined CUE_{ECO} and how it was regulated by interannual
variability in meteorological conditions, and both nutrient treatments and their effects on vegetative phenology.

A great deal of prior work has been published relating to ecosystem function at our study site. In brief: both imbalanced N
fertilization and balanced NP fertilization at ecosystem level increased tree and herbaceous productivity (Bogdanovich et al.,
2021; Nair et al., 2019), drove faster green-up phenology (Y. Luo et al., 2020), but also extra transpiration under NT and
NPT relative to CT. Extra P (NPT) helped plants regulate water losses in the pre-summer drydown via increased water use
efficiency compared to NT (El-Madany et al., 2021). NT shifted rooting distributions towards shallow roots (Nair et al.,

2019) and acquired more N from biomass turnover (Nair et al., 2020). Hence aspects of the system are limited by P. These allometric shifts may alter C cycling and thus CUE_{ECO} through plant C investment to obtain N, P, or water. Microbial

105 biomass also peaks in autumn, capitalizing on release from moisture restrictions (Morris et al., 2019). Overall C and water flux responses are discussed at length in El-Madany et al., (2021), here we focus specifically on CUE_{ECO}, and how this changes based on meteorological and vegetation conditions.

We examined CUE_{ECO} on three timescales: the **annual scale**, where coarse trends could be identified, the **seasonal scale,**

110 where relationship to particular climate anomalies could be identified, and a **biweekly scale**, which allowed the fitting of interpretative models linking both to short term meteorology (e.g. rainy periods, heatwaves), incorporating vegetation change via overall spectral vegetation indexes from above-ground remote sensing. We hypothesised:

- **On the annual scale**, CUE_{ECO} would vary between years, mostly driven by rainfall amount (El-Madany et al., 2020). In general, additions of both N and NP together would increase ecosystem CUE_{ECO}. Known water deficits

115   under NT (El-Madany et al., 2021; Y. Luo et al., 2020) would reduce CUE_{ECO} in this treatment relative to NPT.

- **On the seasonal scale**, CUE_{ECO} would follow phenology – growing seasons would have positive CUE_{ECO} and fallow seasons negative. Within seasons across years, wetter seasons would have higher C losses - due to greater microbial activity - but higher CUE_{ECO} due to concurrent higher C uptake if these were 'growing' seasons – autumn, winter and spring, rather than drydown and summer. Seasons with N-driven water deficits would have

120   lower CUE_{ECO} in NT.

- Finally, at **biweekly** aggregation, we predicted a strong role of water availability on short term CUE_{ECO}. Water stress should affect stomatal gas exchange and thus GPP, but respiration must happen anyway. We expected an effect of energy/light availability as radiation allows more GPP, and as phenology is driven by predictable annual cycles.

2 Methods

2.1 Study Site

We worked at Majadas de Tiétar, in Extremadura, Spain (39 56'24.68"N, 5 46'28.70" W), a typical Mediterranean tree-grass *'dehesa'*, with 20-25 *Quercus ilex* (L.) trees ha^{-1}, ~20 % canopy cover. The MAT (mean annual temperature) is 16.7 °C, and the MAP (mean annual precipitation) is 636 mm (El-Madany et al., 2021). Annual precipitation is very variable (± 50 %), including seasonal timing and distribution. Typically, most rain falls between October and April with a long dry summer interspersed by occasional rains. The growing season begins following major autumn rains, typically in October, lasting until May/June where soil moisture depletion, high radiation, and temperature/vapour pressure deficit (VPD) causes herbaceous layer senescence. A species diverse and seasonal herbaceous layer is used for grazing (< 0.3 cows ha). During summer droughts cattle are usually moved to mountain areas. The soil is an Abruptic Luvisol, with a sandy upper layer (5/20/75 clay/silt/sand) above a thick clay layer which varies across the site beginning between 30 and 60 cm.

At the site, three eddy covariance towers (FLUXNET: ES-LMa, ES-LM1, ES-LM2) and various ancillary instruments are in the same farmland area, with homogeneous topography, meteorology and soil. Pre-treatment, vegetation properties, and carbon, energy and water fluxes did not differ (El-Madany et al., 2018; Nair et al., 2019). Meteorological variables were measured per tower, but we used a unified dataset as cross-footprint spatial variation should be smaller than sensor error. Fertilization treatments were in the 2014/2015 growing season on 20 ha, covering the 80% footprint climatology of respective EC towers (El-Madany et al., 2018; El-Madany et al., 2021). These added N to ES-LM1 (100 kg N ha^{-1} as Ca-ammonium nitrate fertilizer) and N and P (an additional 50 kg P, as triple superphosphate fertilizer and N as ammonium nitrate) to ES-LM2. This load was equivalent to 10 years atmospheric N deposition (Morris et al., 2019), with P sufficient to maintain average ecosystem-scale leaf N:P ratios. A supplementary N and P fertilization was made in 2016/2017 adding 20% of the initial fertilization and a small extra P addition (6 kg ha-1) was made in the 2019 autumn to start a refertilization which was ultimately delayed by the coronavirus pandemic This design was informed by a small-scale fully factorial experiment showing N limitation at plot level, with a limited herbaceous response to P without N (Martini et al., 2019; Perez-Priego et al., 2015). For this reason, along with specific interest in N:P imbalance and limits to space, a P only treatment was omitted. The towers/treatments are henceforth referred to as CT (control tower, ES-LMa), NT (ES-LM1), and NPT (ES-LM2).

Carbon, energy and water vapour fluxes were measured using the eddy covariance (EC) technique described in (El-Madany et al., 2018; El-Madany et al., 2021). Each tower had an EC system at 15m consisting of a 3-dimensional sonic anemometer (R3-50, Gill LTD) and infra-red gas analyser LI-7200, (LI-COR Bioscience) to measure CO_2 and H_2O mixing ratios. Data were collected at 20 Hz and processed using EddyPro 6.2, quality checked, and storage fluxes added using a seven level CO_2 profile system to compute NEE. Friction velocity (u*) thresholds were detected per tower per year and filtered (Papale et al., 2006). Alternate CUE_{ECO} based on fixed u* are presented in S1 with smaller effects than the factors in this analysis. Time series were gap-filled using marginal distribution sampling and NEE was partitioned to GPP and R_{ECO} using night-time

respiration temperature sensitivity (Reichstein et al., 2005). Flux calculation and post processing were according to El-Madany et al. (2018) using REddyProc in R (Wutzler et al., 2018). A single Star-Dot NetCam 5MP digital camera was

160   mounted on each tower providing daily images used for phenology tracking using the 'Phenocam' method (Y. Luo et al., 2018). Soil water content was measured per tower via 4 replicated sensors (ML3, Delta-T Devices Ltd) at 5 cm and a EnviroSCAN (Sentek) profile probe for measurements at 10, 20, 30, 50 and 100 cm below ground per tower site. Flux data was available from March 2014 for all sites. We used data until the end of the 2020 growing season.

165   **2.2 Definition of Carbon Use Efficiency, Scales of Analysis, Aggregation**

We use ecosystem scale CUE_{ECO}, NEP / GPP. CUE_{ECO} can be negative if NEP (i.e. positive NEE) is negative. At short time scales CUE_{ECO} becomes difficult to interpret because of short-term storage of C (e.g. plant non-structural carbon) and autocorrelation in half-hourly data. We analysed three scales: annual, seasonal, and 14-day. This last timescale was most vulnerable to autocorrelation so we did not use a fixed temporal predictor at this scale, as detailed later.

170   We first took the half-hourly GPP, Reco, and NEE daily means, then we aggregate them by computing the average across the period required. We let daily averages contribute to aggregate mean if >75% of the total half hourly EC observations and >8 of the 'night time' (i.e. Potential Radiation < 10 Wm^{-2}) observations were good quality (measured and gap filled). If daily data from any tower was discarded, simultaneous data from the other two towers was also discarded. We removed 50 days over the entire 6-year period. To aggregate at biweekly scales, we assigned a group per 14 days and did not use a rolling

175   average; this was to reduce autocorrelation and preserve independence of observations.

2.3. Water Availability

All metrics relating to water availability were calculated per treatment aside from precipitation, which we handled universally as per other meteorological variables. As soil moisture at deeper soil layers was variable and most root biomass was within 20 cm (Nair et al., 2019), we used a normalized soil water content (SWCn) from 5 to 20 cm sensors. We also

180   used a water availability index, Conservative Surface Wetness Index (CSWI), (Nelson et al., 2018) to account for general ecosystem 'wetness'. CSWI is a bucket model explaining rainwater removal via evaporation, accounting for water evaporated and cloudy periods of low ET after rain events when surfaces stay wet. More negative values indicate drier conditions. CSWI was calculated half-hourly and averaged across each aggregation step. Hence, we accounted for immediate rain inputs, general soil moisture and water available for plants and general wetness of the surface where many

185   active microbes are located, through metrics dependent on different instruments.

2.4. Seasons, Phenology, and Vegetation Indexes

For assignment of seasons, we used PhenoCams. PhenoCams provide daily digital photographs and can provide detailed
190 phenological transition dates ('PTDs') and define phenological periods (e.g., Y. Luo et al., 2018). We extracted PTDs for
each treatment corresponding to five *phenophases* (autumn, winter, spring, drydown, summer). We defined the 'growing
season' as autumn + winter + spring. Alternative calendar year definitions are insensitive to the rhythms of biological
activity driven by changeable meteorology, or nutrient availability at this site (Y. Luo et al., 2020). Consequently, our annual
scale is six *hydrological years*, with the start/end date of the year determined by the summer – autumn transition and
195 identified by the later year, e.g. H2016 started in autumn 2015. The average difference and maximum difference between
PTDs between years is shown in *Table* 1 and definition of individual season PTDs is in S2. Because we allowed the
hydrological year and season to vary between treatments based on vegetation activity, there were minor differences in
treatment level annual/seasonal means.

200 While we could define the seasonal transitions from PhenoCams, the independent site-mounted cameras are not
intercalibrated so biases may result from instruments (Richardson et al., 2018). Therefore we used the normalized difference
vegetation index (NDVI, Rouse et al., 1974) from the Sentinel-2 Mission (Copernicus Programme, European Space
Agency), which provides comparable simultaneous observations of the sites and physical quantities (e.g., surface reflection
factors), at the expenses of lower frequency sampling and 10 m spatial resolution. We used a combined dataset of Sentinel
205 2A imagery beginning on 20th July 2015, shortly after the fertilization, and switched to more frequent Sentinel 2B imagery
when it was available (28th Mar 2018). We used a 100 m radius around each tower. The average gap between good quality
days (cloudy overpasses filtered out) was 11 days although it could be as high as 30 days. We interpolated between points
using a Kalman smoothing filter before averaging on the various scales used for analyses.

Table 1

Mean transition dates (as 1st day of anterior season) for all 5 defined seasons as calendar day of year. Also shown are the
mean difference between seasonal transition dates for the different treatments.

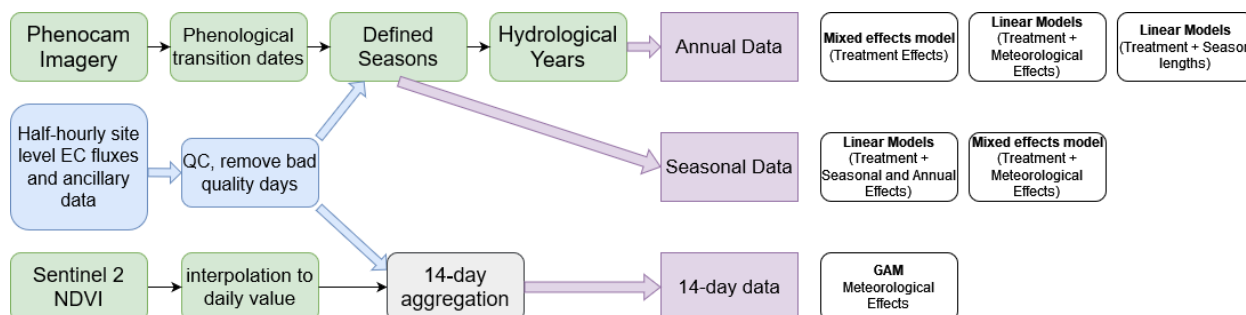| Season Transition		Average DOY	Earliest DOY	Latest DOY	Average CT-NT (Days)	Average CT-NPT (Days)
SUMMER	–	275	274	278	2	2
AUTUMN						
AUTUMN	–	352	341	5	-2	1
WINTER						
WINTER-SPRING		66	29	88	-2	-9
SPRING-DRYDOWN		123	99	142	1	-8
DRYDOWN-		186	175	199	2	1
SUMMER						

2.5 Modelling Approaches

Figure 1

Summary of our data sources and analytical structure. Phenocam imagery only informed season and year transitions, we used Sentinel2 NDVI for the 14-day analysis. We aggregated quality-controlled EC data by season and hydrological years for the annual and seasonal scale while we used a 14 day window for the 14-day scale. Blue boxes and arrows indicate data in the main flux and meteorology dataset, green boxes data from elsewhere and purple arrows and boxes aggregated data. The analyses conducted are shown to the right of each level of data aggregation.

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In summary, the explanatory variables we used were: nutrient fertilization treatment (treatment), season and hydrological year, plus the environmental variables air temperature at 2m (Ta), total precipitation (ppt), number of days with ppt > 0.5 mm ('rainy days'), photosynthetically active radiation (PAR), daytime vapor pressure deficit (VPD), daytime relative humidity (RH), normalized soil moisture content (SWCn), Conservative Surface Wetness Index (CSWI) and sentinel 2 215 NDVI of the whole ecosystem (NDVI). We performed all data analyses in R 4.0.3 (R Core Team, 2022). Because the aggregation steps resulted in data sets of different sizes, we applied different analytical methods at the different scales. We performed linear models, mixed effects models conducted using lme4 (Bates, 2010) and generalized addictive models (GAMs, Hastie & Tibshirani, 1986) via the mgcv package in R (Wood, 2011). We show mixed model R^2 using marginal (R^2_m) and conditional (R^2_C, Nakagawa & Schielzeth, 2013), the former an estimate of goodness of fit with only fixed effects 220 and the latter including random effects. We report deviance explained (the proportion of null deviance explained by the model) for GAMs, appropriate for non-normal errors. In all analyses unless otherwise mentioned, model selection was via AICc. Overall analysis structure is illustrated in Fig. 1.

For the **annual analysis** (six hydrological years), we performed a linear mixed effect model with treatment (fixed effect) and hydrological year (random effect). For environmental controls, we used a linear model, beginning with treatment effect and 225 interaction terms with Ta, ppt, rainy days, SWC, CSWI. We excluded NDVI as PhenoCam data were used to determine season. We also used linear models to explore how year length, growing season length, relative growing season length and

annual productivity (mean GPP per day) and turnover (mean R_{ECO} per day), all interacting with treatments, controlled annual CUE_{ECO}. As we had few prior expectations about which variables were most important, we employed a data dredging approach comparing all possible combinations, with max. 6 terms per model, retaining all predictors in >50 % of models within 4 AICc of the most parsimonious model for the overall best model.

For **seasonal analysis**, across thirty season-hydrological year combinations in the three treatments, we used linear mixed effects models. First, hydrological year was a random effect and season and treatment were fixed effects to test the seasonal controls on CUE_{ECO}. Then we performed a model where treatment and interaction with both environmental variables and the six sequential hydrological years were fixed effects and season a random effect. Environmental variables were Ta, ppt, SWC, CSWI. Season was not nested within hydrological year to avoid a singular fit. We did not include the rainy days, nor NDVI in this analysis because annual weather largely determined the season. We performed a similar model dredging exercise as the annual analysis to arrive at the final models.

For the **biweekly** aggregation, we used mixed effects models when only considering year, season, and treatment, and GAMs (Wood, 2006) to investigate climate, soil water variables and vegetation spectral status effects on biweekly CUE_{ECO}. GAMs were suitable as we were expecting non-linear trends. We fit models for these data where all predictors were available (i.e. autumn 2015 onwards). The potential predictors we used were Ta, PAR, VPD, RH, ppt, SWCn, CSWI, and NDVI. Because of the high covariation of half-hourly atmospheric environmental variables (Ta, PAR, VPD, RH), we performed a principal component analysis to reduce dimensionality. The first PCA axis (93 % of the variance) represented all these variables together. High values of this axis, which we refer to as 'Meteorology' corresponded to high air Ta, PAR and VPD, low values to high RH (S3). We did not use a similar reduction for Rain, SWCn, and CSWI or the spectral indices as these represented processes on incomparable timescales. We used reduced maximum likelihood (REML) estimation and manually penalized out non-informative smooths one by one, refitting the model each time. We applied a smoothness penalty on the whole model to prevent overfitting. We selected models via per-smooth penalties which allowed smooths to penalize to zero, removed non-significant and non-informative effects with *F*-value close to or equal zero, always removing *treatment*factor* interactions before univariate smooths. We compared GCv (generalized cross validation) score at each step. We did not include interaction terms beyond a *treatment*factor* term because of difficulty specifying these fairly across so many variables and the potential for overfitting a model on the aggregated data.

After selecting the most parsimonious GAM, we performed several additional checks. We examined variable concurvity once the model was selected, which never exceeded 0.8. We checked the models performance using the gam.check() function of mgcv. We also evaluated performance of this GAM via model residuals against treatments, seasons and hydrological years.

3. Results

3.1 Annual Carbon Flux Response to Fertilization

260   As observed from the eddy covariance fluxes without nutrient amendment, CT had a weak negative or neutral CUE_{ECO} over the six years. The annual effect varied (Fig. 2) and there was no significant annual trend over time. Nutrient treatments increased annual CUE_{ECO}. Consequently, annual CUE_{ECO} was affected by treatments, both NT and NPT's CUE_{ECO} significantly different ($P < 0.01$) to CT but not to each other ($P = 0.06$). Both GPP (Fig. 2a) and R_{ECO} (Fig. 2b) were increased by fertilization for the entire treatment period, as previously reported (El-Madany et al., 2021). Treating year as a

265   random effect (i.e. no linear trend but intra-annual conditions affected the C cycle), most variation was explained by annual year ($R^2_M = 0.18$, $R^2_C = 0.91$). The marginal treatment effect was stronger for CUE_{ECO} ($R^2_M = 0.25$, $R^2_C = 0.87$) than GPP ($R^2_M = 0.21$, $R^2_C = 0.91$) or R_{ECO} ($R^2_M = 0.06$, $R^2_C = 0.81$).
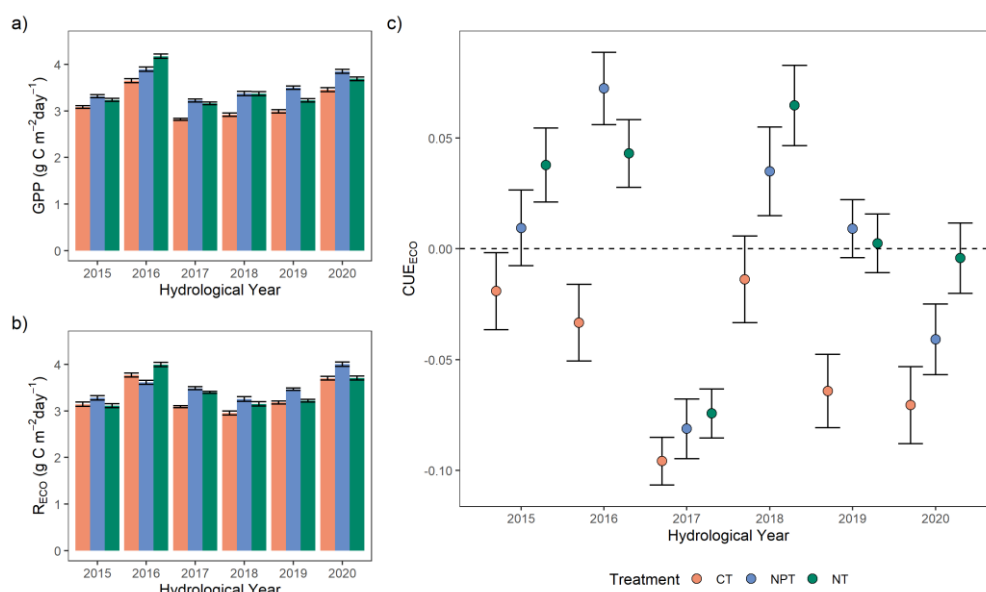

Figure 2. Mean annual a) GPP   b) R_{ECO} and c) annual CUE by hydrological year. In all cases error bars show uncorrelated 95 % CI.

Treating years as independent and using annual means of meteorological variables, we were also able to achieve good fit

270 (R^2adj = 0.79), although not as good as the random annual effect. The most parsimonious model retained MAT ($P < 0.001$),
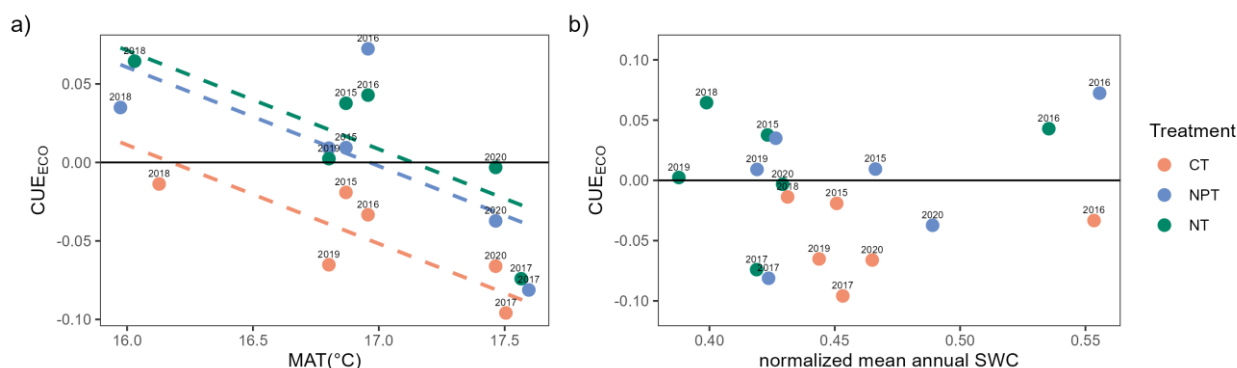

Figure 3. Relationship between a) mean annual temperature and b) normalized mean annual SWC on annual CUE$_{ECO}$. The grey dotted line indicates the best fit of an ANCOVA with treatment as covariate for CUE against the x axis variable (SWCn did not have a significant effect in the ANCOVA). Hotter and drier years tended to reduce CUE$_{ECO}$, but it was clear from b) that annual SWC obscured processes happening at finer scales – consistently low CUE in the N treatment happened at low SWC.

and SWC ($P < 0.01$) alongside treatment ($P < 0.01$) but no *treatment*meteorology* interactions. Both treatments were

significantly different than CT (both $P < 0.005$) but not each other. Generally, hotter, and drier years reduced CUE$_{ECO}$ (Fig.

3).

275 We also tested the effect of total length of the hydrological year, total length of the growing season, the relative length of the

growing season, and average daily GPP on annual CUE$_{ECO}$ (Fig 4.). The best predictors of CUE$_{ECO}$ were relative length of

growing season and treatment (Fig. 4c, R^2_{adj} of 0.85). Shorter absolute vegetative year length or absolute longer growing

seasons also increased annual CUE$_{ECO}$ but were not as good predictors (Fig. 4a, 4b). Similarly, daily mean GPP was less

correlated   despite   the   calculation   of
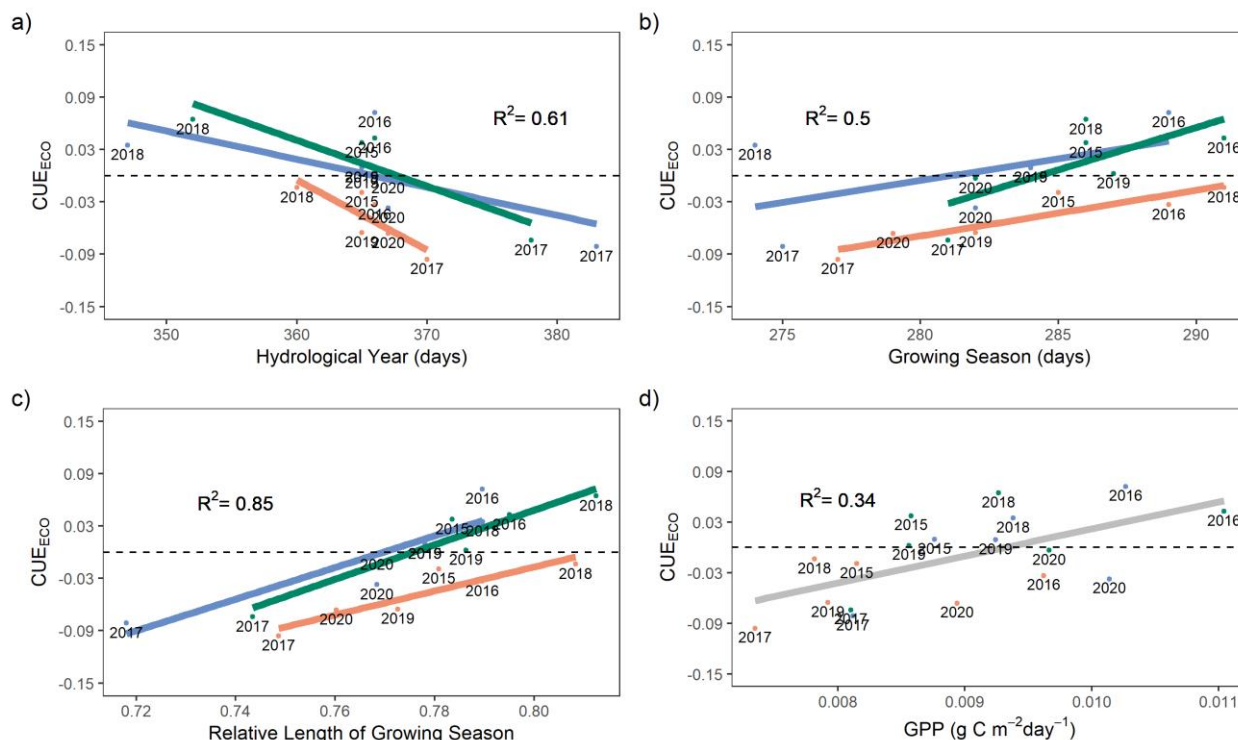
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Figure 4. Relationship between a) absolute length of hydrological year b) absolute length of the growing season (autumn, winter, and spring, c) relative length of the growing season (length growing season / length annual year) and d) mean daily GPP of the year. Both R_{ECO} per day and absolute GPP of the year did not have a significant relationship and are not shown. Lines show significant linear regressions, coloured indicate per-treatment, grey without a treatment effect.

CUE_{ECO} partially from GPP (4d). We also checked for correlations between daily mean R_{ECO} and absolute GPP with CUE_{ECO}, neither of these had significant effects. In other words, CUE_{ECO} was controlled by how long the summer drought lasted (Fig. S4, although note the high leverage of nutrient treatments in 2017 and 2018 in Fig. 4b).

3.2 Seasonal Trends

As expected, there were major differences in CUE_{ECO} between seasons: 'growing period' had positive CUE_{ECO}, and dry periods, where most vegetation is dormant or dead, had strong negative CUE_{ECO} (a). CUE_{ECO} was declining in summers and winters but had a positive trend in the drydown (c).
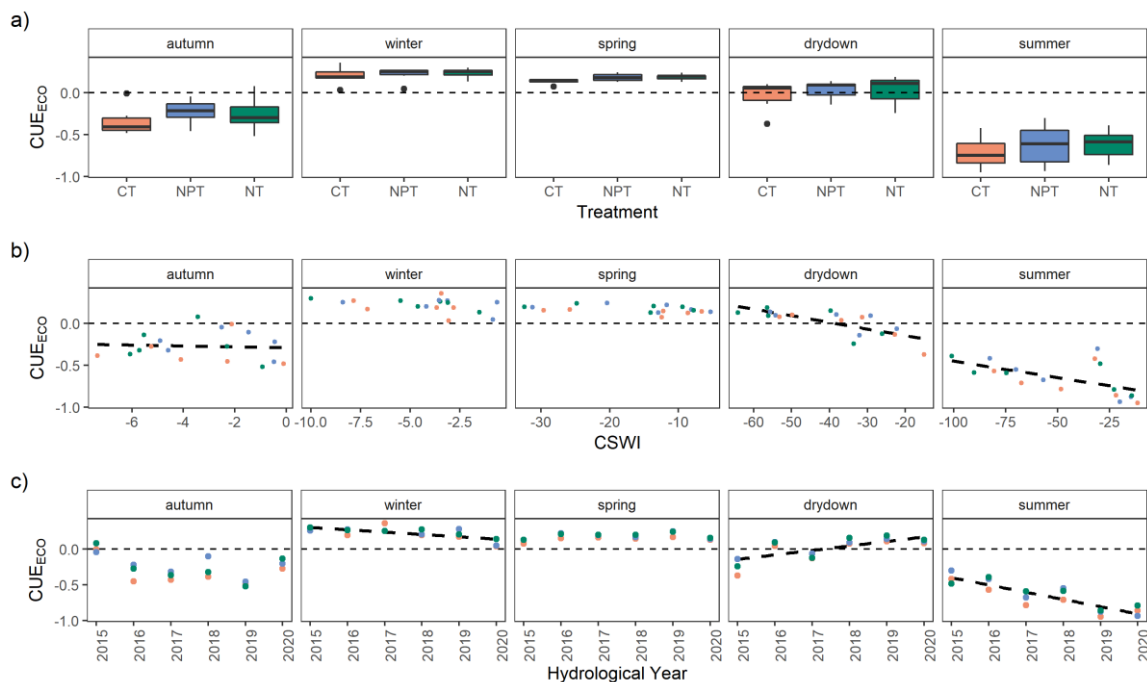
Figure 5. Seasonal CUE$_{ECO}$ between treatments and controls. a) Treatment effects within seasons. NPT had a slightly higher CUE in some seasons. Dashed lines in b) CSWI and c) time trends indicate a significant trend (P < 0.05) within seasons. Overall CUE$_{ECO}$ is strongly driven by the phenological cycle but there were: b) time trends within seasons - CUE$_{ECO}$ was declining in winter and summer; c) the only meteorological variable in the most parsimonious seasonal meteorology model was CSWI; - wetter conditions tended reduced CUE$_{ECO}$.

290

When seasonal variation was controlled as a random effect (R^2_c = 0.91), and we included meteorological variables and the numeric year in the model, the marginal component was small compared to season (R^2_M = 0.07). This effect was due to treatment (n.s. but remaining in the most parsimonious overall model), CSWI (P = 0.001) and the annual trend (P = 0.05). Higher CSWI decreased CUE$_{ECO}$ in winter, drydown, and summer (Fig. 5b).

295 **3.3 Drivers of Sub-seasonal Variation**

Environmental variables followed annual cycles (Fig. S5) and explained around 78 % (dev. Explained) of bi-weekly variation. The most parsimonious model (Fig. 6) had effects of 'Meteorology', SWCn, CSWI and NDVI. The largest effect was NDVI; positive CUE$_{ECO}$ required active vegetation. The NDVI effect started to saturate at around 0.5 (Fig. 6e). The two wetness parameters had different effects: CUE$_{ECO}$ partial residuals were highest at low SWCn and at intermediate CSWI.

300 Overall, variation in SWCn had a larger effect than CSWI. While concurvity assumptions were satisfied and including all three terms improved the model without reducing GCv, we note environmental variables were not independent; CUE$_{ECO}$ cannot be > 1. Conditions where summed partial residuals could achieve this (i.e. low SWC, intermediate CSWI where

partial residuals together result in $CUE_{ECO} > 1$) would not occur together; but both indicate more water. There was also a 'Meteorology' effect, higher values (i.e. warmer, dryer) and lower values (cooler, wetter) raised CUE_{ECO} compared to

305   intermediate values but overall contribution was small. Treatment effects were evident in NDVI (a slight increase in CUE_{ECO} partial residual) of fertilized treatments at high NDVI. There was also a treatment interaction with conditions: NT was slightly drier and CT treatment had an overall lower NDVI. Consequently, effects of the environmental conditions on the partial residual of CUE_{ECO} must be interpreted with reference to actual trends in these conditions between treatments.
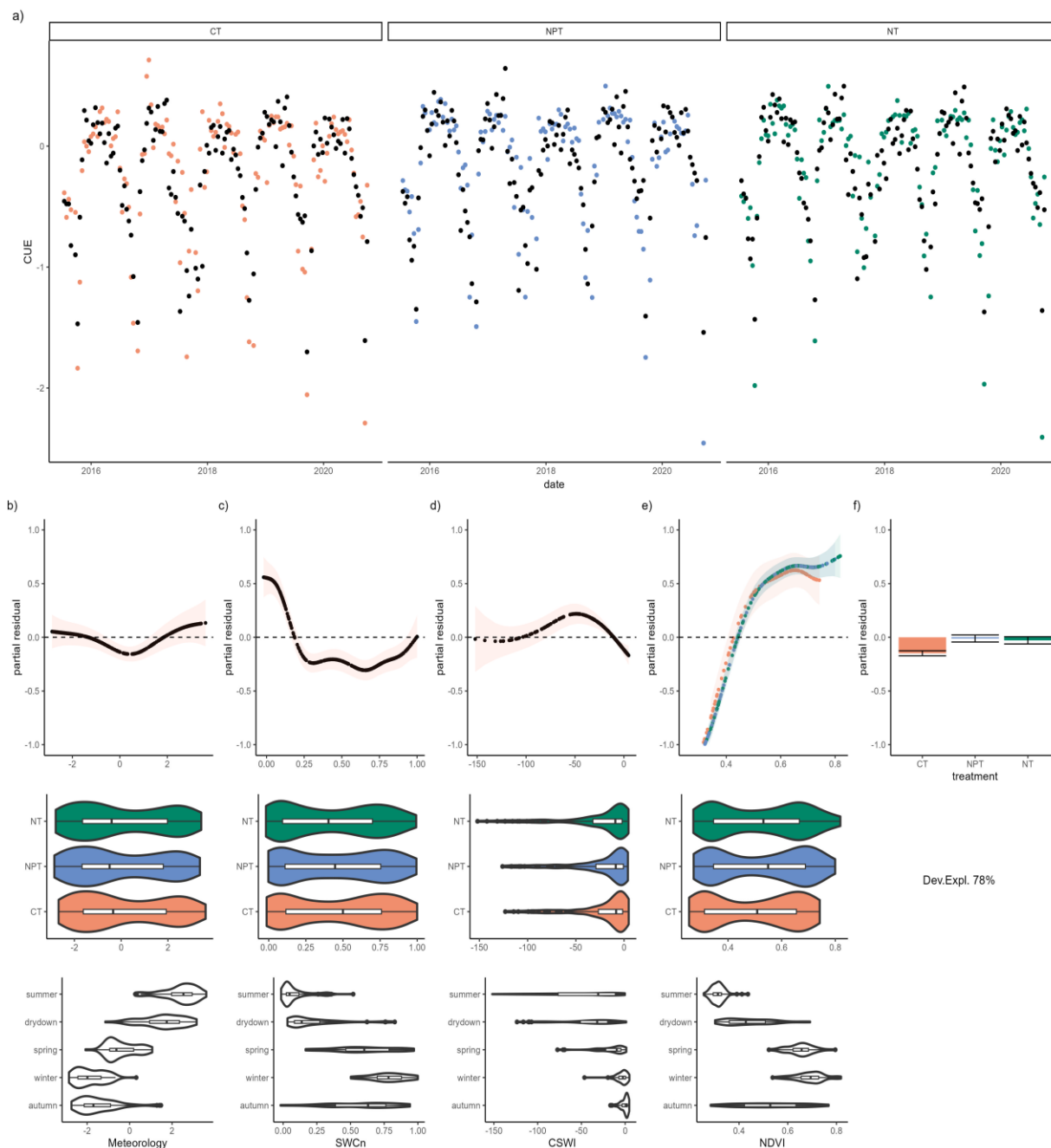
Figure 6. Visual summary of the most parsimonious GAM model. In panel a, CUE$_{ECO}$ (colour) is compared against model predictions (black) with an overall R^2 of 80 % across the five years with all available data. Univariate smooths are shown for b) 'Met. Variables' (higher values are hotter and drier), c) SWCn and d) CSWI. Panel e) shows the smooth with significant treatment-dependent smooth for NDVI. Error bars are 2 x standard errors. Box plots below each curve show the distribution of data between (upper panel) treatments and (lower panel) season; note that as well as a treatment dependent effect *of* the conditions, particularly NDVI, and SWCn, the N treatment was slightly drier, and the control treatment had a lower NDVI. The model had no parametric treatment effect and the relative effect of treatments on the residual is shown in panel f). In panel e), the partial residuals for N and NP treatments overlap substantially.

4 Discussion

We expected nutrient addition of N and NP to increase annual CUE_{ECO} in series because of stronger water limitations with N alone (El-Madany et al., 2021; Y. Luo et al., 2020). We expected these effects to propagate to seasonal scale and in particular increase CUE_{ECO} in wetter 'growing' seasons. We expected, and found, these effects to be relatively small

315 compared to variation in environmental conditions but they nonetheless remained determinants of CUE_{ECO} at all scales. Once we accounted for variation between hydrological years, nutrient treatments increased CUE_{ECO} but NT and NPT did not differ (Fig. 2, Fig. 3, Fig. 6). Annual CUE_{ECO} was more strongly driven by GPP than R_{ECO} (Fig. 3), although both were components of CUE_{ECO}. Cool and wet years increased CUE_{ECO} (Fig. 3) as did both absolute and proportionally long growing seasons, the latter usually corresponding to short hydrological years (Fig. 4). Short hydrological years could also be caused

320 by short summers if the next autumn's rain arrived early. However, at phenophase defined seasonal scale, the treatment effect was not significant, and the wetness effect reversed –the more favourable conditions for a positive effect on CUE_{ECO} were drier drydowns and summers (Fig. 5b). Between years, we also observed decreasing winter and summer CUE_{ECO} and increasing drydown CUE_{ECO} over time (Fig. 5c). These trends illustrate the importance of timescale and context in examining CUE_{ECO}.

325 At shorter timescales, positive CUE_{ECO} effects were under lower soil moisture but not necessarily when ecosystem wetness was lowest (compare SWCn, CSWI, Fig. 6c and 6d). NT and NPT had higher CUE_{ECO} partial residuals in periods with high NDVI and overall higher NDVI – i.e more active vegetation, independent of 'N:P imbalance'. This was offset by increased dryness - evident through CSWI (Fig. 6d). This modulation by water was previously shown in growing season length (Y. Luo et al., 2020) and water fluxes (El-Madany et al., 2021) alongside allocation shifts from above-to-belowground (Nair et

330 al., 2019). Here, we discuss these effects, potential model fits, and potential biases, in the context of nutrient treatments increasing the CUE_{ECO} of the ecosystem.

4.1 Effect of Nutrient Treatments on CUE

The biggest increases in annual CUE_{ECO} in both treatments was in the wet (Fig. 3) H2016, which had a long growing season (Fig. 4). Hence in gross annual carbon cycling terms, there was no effect of N:P imbalance, rather an effect of N fertilization.

335 Moderate increases in N supply increase grassland topsoil C stocks by ~ 20 g C m^{-2} y^{-1} in long term surveys (Soussana et al., 2004). Our N addition was 10x mean annual N deposition, applied irregularly and with a lower N load than most N fertilizations. Thus, chronic N deposition may increase C sinks in N-limited dehesas and N fertilization, i.e. pasture improvement, may be beneficial for overall C balance. This agrees with positives effect on soil organic carbon of over-seeding with legume rich mixtures in dehesas (Moreno et al., 2021).

340 At the biweekly scale, NT and NPT behaved very similarly. There were treatment differences in the smooth for NDVI - treatments enhanced CUE_{ECO} at higher NDVI (Fig. 6e). Otherwise, the major differences between treatments were not on the effects of environmental variables but rather variable distribution. Depletion in water was visible in CSWI from N but less so

in SWCn while NDVI was also slightly higher. Thus compensatory effects via depletion of finite water resources appeared to be counterbalancing vegetative increases between NT and NPT and leading to no overall CUE_{ECO} differences when P is
345   added alongside N.

While Leaf N increased in NT, P also increased in NPT (El-Madany et al., 2021) so it may be surprising that P did not affect CUE_{ECO}. Indeed, leaf N and P relate to photosynthesis in complement (Evans, 1989; Kergoat et al., 2008; Musavi et al., 2016) and P limitation limits responses to ongoing global C fertilization (Jiang et al., 2020) as it co-limits photosynthesis with N (Domingues et al., 2010). At our site, P availability also affects herbaceous photosynthetic parameters (Martini et al.,
350   2019) and fertilization effects on GPP were higher under NPT than NT (El-Madany et al., 2021). The lack of a CUE_{ECO} response may be because CUE_{ECO} is a result of both GPP and R_{ECO}, explained by considering differences between leaf level C exchange and whole system functioning. Although less well studied than photosynthesis, concurrent N and P biochemical limitation of respiration (Crous et al., 2017; Rowland et al., 2017), leading to potential subsequent interactive effects on ecosystem level CUE_{ECO}.

355   Deeper roots in NPT contrasts increase in root:shoot ratio without P (Nair et al., 2019) and P addition slows N-driven water losses (El-Madany et al., 2021; Y. Luo et al., 2020) but water regulation may also cost carbon due to stomatal control. Hence productivity gains in other periods may be offset by R_{ECO} or reduced GPP in dry periods and NPT benefits may cancel out, at least for the C cycle alone. Indeed, investment belowground can have a positive or negative effect on ecosystem C status (Dijkstra et al., 2021) and other C use (e.g. changes in C to exudates or stability in SOC) could compensate and equalise
360   overall CUE_{ECO}. In other systems, chronic N fertilization increases soil C concentrations (Cenini et al., 2015) but P addition effects are less understood (Ding et al., 2021; Jiang et al., 2019). Due to the integrative nature of EC data these distinctions may be invisible in our study. However, a lab experiment using soil collected in February 2018, showed fertilization legacy effects marginally increased microbial CUE and soil microbial communities in NPT had shifted metabolism to older substrates (Morris et al., 2022). Such microbial shifts may contribute to CUE_{ECO} (Tahovská et al., 2020; Tao et al., 2023) and
365   heterotrophic respiration partially compensate for increased GPP. Microbial CUE increases with temperature (Manzoni et al., 2012) and is seasonal (Simon et al., 2020; Ullah et al., 2021). Aspects of the smooths in Fig. 6 may be explained by microbial shifts not represented by the data for model fitting. In the absence of data to fully parameterise this, an alternate approach would be to fit a model with processes based belowground assignment to such sites which can then be tested from frequency limited but targeted measurements of such difficult to observe properties (Caldararu et al., 2023).

370   **4.2 Potential Effects of The Herbaceous Layer Structure on Carbon Exchange**

EC data integrates whole ecosystem responses, but changes result from activity of individual organisms and their communities (e.g. Craine et al., 2001) which respond on their own. Even discounting microbial responses, herbaceous vegetation turns over faster than trees. Dehesa herbaceous communities are diverse and dynamic (Moreno et al., 2016) and ecosystem structure i.e. diversity (Fernández-Moya et al., 2011; Zeng et al., 2015), allometry (Nair et al., 2019; Sainju et al.,
375   2017), and trophic partitioning (Lemanski & Scheu, 2014), all respond to fertilization and N:P imbalance. While a nutrient

content effect was limited to herbaceous biomass (El-Madany et al., 2021), whole system NDVI was higher in fertilized treatments in summer (Fig. S4). This could be explained by structural differences in tree cover in NPT (El-Madany et al., 2018) than CT or NT but not differences between CT and NT. Changes in individual species happened during the experiment, including a higher abundance of the C4 species *Cyanodon dactylon* (L.) in NT which persisted into summers

380 due to its drought adaptions (unpublished plant inventories). Hence a combination of both water cycling and community structure effects may explain why neither treatment reached as absolute low NDVI as CT (Fig. 6), through different paths. In the rest of the year most GPP occurs and the herbaceous layer controls ecosystem spectral dynamics (Pacheco-Labrador et al., 2017). A previous smaller scale experiment showed a shift towards grasses and a decline in legumes under fertilization, affecting whole canopy properties relating to light use efficiency (Migliavacca et al., 2017). Plant types also vary in water

385 use efficiency (Tsialtas et al., 2001), root architecture (Tron et al., 2015) and leaf-level traits (Rumman et al., 2018) and can modify CUE_{ECO} both directly and indirectly via microbes (Van Der Heijden et al., 2008; Schnitzer et al., 2011). Indeed, in longer term experiments, plant species diversity can have higher benefit to soil C than N addition (Pastore et al., 2021) and plant diversity responses to nutrients drive microbial responses (Leff et al., 2015). Hence residual differences between years which we cannot explain may be due to unexpected feedbacks on whole system community structure. An important facet of

390 holistic system understanding in natural ecosystems is quantifying these changes and if, when and how they relate to overall ecosystem function.

4.3 Interannual Variation and Model Performance Between Years and Seasons

Figure 7. Residuals from the GAM model grouped by season and year. In some combinations the model fit better than others. Note in particular the overall worse fit in autumn and summer, and high errors in drydown and summer

Different years had different overall and seasonal CUE_{ECO} between treatments (Fig. 2, Fig. 3, Fig. 4). While our model on biweekly data performed well (overall dev. explained of 78%), there were times when it did not. The largest residuals (Fig. 395 7) were in summer and autumn where rain pulses control respiration (Casals et al., 2011) at even shorter timescales. This effect is particularly strong in the 2017/2018 transition. 2017 had the shortest growing year (Fig. 3), lowest CUE_{ECO} and followed the year with highest GPP (Fig. 2a). Respiration fluxes are more difficult to partition and in these negative CUE_{ECO} conditions were the dominant flux. The model was notably bad at predicting the 2017 drydown and summer CUE_{ECO}. We did, however, offset the influence of difficult to predict events via a PhenoCam-derived hydrological year; the onset autumn 400 rains drive a pulse of CO_2 efflux after the water-limited summer consuming C fixed in the previous growing seasons but was broadly always captured by starting the new hydrological year based on rain-dependent vegetation green up rather than date. As we used PTDs rather than fixed calendar dates to start autumn, we were able to assign this R_{ECO} response to the closing hydrological year/summer (Fig. S6). However, rain in semi-arid systems is only a part of overall water inputs (Uclés et al., 2014) and dry season non-rainfall water inputs occur (Paulus et al., 2021). Intermittent summer rainfall may also mean dry 405 season C dynamics differ from coupled day-night shifts between photochemical-thermal and microbial degradation in dryer

arid lands (Gliksman et al., 2017) and also not resemble temperate winter-fallow systems. Likewise intermittent summer storms during periods of high evaporation may not always surpass a threshold for long term responses. Longer datasets lasting multiple years with well quantified rainfall and non-rainfall inputs may be the route to understanding such anomalies and/or fitting models which perform better in these conditions.

410 **4.4 Potential Long Term Effects**

Finally, ongoing global change effects may alter CUE_{ECO}. Long term aridification trends (Cos et al., 2022; Paniagua et al., 2019) would provide progressively worse summers and warmer winters (Fig. 4) and hence cause a long term decrease in CUE_{ECO} . Drier summers are usually long and so reduce the fraction of year suitable for growth (Fig. 4c) and consequently annual GPP. Concurrently we observe decreasing CUE_{ECO} in winter and summer (Fig. 5c). CUE_{ECO} normalizes for GPP and

415 is not offset by increasing drydown CUE_{ECO} because this season is shorter (Table 1). At annual scales, GPP is also a stronger predictor of CUE_{ECO} than R_{ECO} (Fig. 3). Thus, CUE_{ECO} is in addition to productivity changes induced by the fertilization. From our GAM results, dry summers have low NDVI, and would move CSWI towards a less positive effect. While SWCn increases its partial residual at low values, this is both more dynamic and affected by rain pulses. As already mentioned, summer and autumn soil C effluxes in our system are inevitable but occur when sufficient precipitation enables

420 mineralization of dead C stocks (Fig. 5b) – if not in summer, then at the autumn rains. However, because we used PTD-derived seasonal dates, these losses are mostly assigned to summer rather than autumn (Fig. S6) so the trend in declining CUE_{ECO} is not because of lag in this efflux but an actual decrease in summer. We were able to correctly assign this R_{ECO} pulse to the annual year scale, allowing the annual CUE_{ECO} to be shown to be more dependent on the GPP (Fig. 3). This distinction is possible because microbes respond faster than vegetation to breaking drought. This response is not possible in

425 the drydown-summer transition so increasing CUE_{ECO} here may be because microbial respiration is delayed as the season becomes dryer (Fig. 5b). In a similar Spanish tree-grass ecosystem, Matías et al., (2021) observed experimental warming consistently increased total soil respiration, while drought reduced it, through point measurements in May and October. But over summer, warmer and harsher conditions may reduce CUE_{ECO} (Fig. 3) because these conditions cause decomposition of more non-labile SOC (Knorr et al., 2005) through more extreme shifts between warm dry and warm wet conditions. Indeed

430 in the aforementioned experiment, summer warming and drought together accelerate SOM decomposition (San-Emeterio et al., 2023). This fraction is likely older carbon from previous years compared to the predominantly labile current year inputs (Knorr et al., 2005). On the other hand, winter is not typically water limited in relatively 'continental' site in central Iberia, midway through the phenological year. Interpreting the water effect must be done with care because as well as removing water limitations, wetter periods increase respiration. Even though we tried to control for concurvity via model checks, this

435 could also be due to suppression of GPP through clouds associated with rain. Cloudiness should have affected radiation and air temperature in 'Meteorology' in the GAM, although this had minimal effects, perhaps because a finer timescale was necessary to see short term effects. All together these trends suggest future semi-arid Mediterranean systems may shift to

being stronger ecosystem C sources through aridity or rain-pulse-driven losses of C from organic matter in longer summers and a concurrent reduction in pasture productivity.

440 Aside from direct carbon cycle effects, a drying and lengthening summer may also interact with nutrient availability, particularly N. Nutrient availability can shift during growing seasons –turnover of dead biomass is not necessarily linked to immediate productivity (Klaus et al., 2016) and microbes are better competitors for immediate N availability than plants (Templer et al., 2012). More turnover delayed until autumn and mobility in wet conditions mean N limits on productivity in Mediterranean systems may be stronger in the early season, in addition to potential progressive limitation (Y. Luo et al.,

445 2004) from CO_2 fertilization effects. With simultaneous increases in demand and reductions in supply of N, such systems may move to stronger N limitation. From our fertilization results, this also suggests CUE_{ECO} would decrease. On the other hand, delayed turnover may decrease leaching because as the wet autumn begins plant N demand is low (Llorens et al., 2011) and so actually have long tern positive effects on N availability. N fertilization both increases GPP and water transpired, shortening growing seasons by intensifying drydowns (Y. Luo et al., 2020), this N limitation may increase late

450 season productivity and shorten phenological summers. However, because of climate-driven summer intensification, and inevitable water losses at season's end, hypothetical longer growing seasons are unlikely to fully compensate if this is driven by N-limited lower productivity. Thus long term N feedbacks are complex and still unclear, interacting with growing season phenology and could be in either direction. Understanding nutrient cycling and whole system phenology responses under dynamic shifts in function in these dry summer systems is thus of great importance. Insight from winter-fallow systems

455 without strong seasonal shifts may not be sufficient to understand such responses.

4.5 Conclusion

N fertilization increased ecosystem CUE in our Mediterranean tree-grass site, and additional P fertilization had no distinct effects on whole system CUE_{ECO} despite several previously observed plant effects which could have been interpreted to lead to a more productive ecosystem. Hence N:P imbalance effects on allometry, stoichiometry, water-use and stress responses

460 appeared to have been completely obscured on ecosystem level by compensatory responses, intrinsic variability, and noise at three different levels of temporal aggregation. We also observed trends over the study period of decreasing CUE_{ECO} in winter and summers, and higher CUE_{ECO} in years with longer growing seasons and shorter summers, suggesting a potential long-term trend towards more C losses as the climate warms and duration of summer dry period increases. Seasonal trends were governed by water availability and water effects both became more important and became more complex the finer the

465 timescale we considered. Consideration of phenology, weather, and timescale on CUE_{ECO} is critically important for understanding this ecosystem parameter.

5 Acknowledgements

480 ## 6 Data Availability Statement

The main dataset used in this paper was the gap filled, quality controlled master dataset from the Majadas del Tietar 'MANIP' experiment. We will upload variables used at the point to a publicly accessible open repository once this manuscript reaches peer review acceptance.

Phenocam imagery used is available from the phenocam network (site names: CT: eslma, NT: eslma1, NPT: eslma2, scripts
485   to extract the PTDs are available from Yunpeng Luo on request)

7 References

Ahlstrom, A., Raupach, M. R., Schurgers, G., Smith, B., Arneth, A., Jung, M., Reichstein, M., Canadell, J. G., Friedlingstein, P., Jain, A. K., Kato, E., Poulter, B., Sitch, S., Stocker, B. D., Viovy, N., Wang, Y. P., Wiltshire, A.,
490   Zaehle, S., & Zeng, N. (2015). The Dominant Role of Semi-Arid Ecosystems in the Trend and Variability of the Land CO2 Sink. *Science*, *348*(6237), 895–899. https://doi.org/10.1126/science.aaa1668

Bahn, M., Schmitt, M., Siegwolf, R., Richter, A., & Brüggemann, N. (2009). Does photosynthesis affect grassland soil-respired CO2 and its carbon isotope composition on a diurnal timescale? *New Phytologist*, *182*(2), 451–460. https://doi.org/10.1111/j.1469-8137.2008.02755.x

495   Bates, D. M. (2010). *Lme4: Mixed-Effects Modeling with R*. URL Http://Lme4. r-Forge. r-Project. Org/Book.

Bogdanovich, E., Perez-Priego, O., El-Madany, T. S., Guderle, M., Pacheco-Labrador, J., Levick, S. R., Moreno, G., Carrara, A., Pilar Martín, M., & Migliavacca, M. (2021). Using terrestrial laser scanning for characterizing tree structural parameters and their changes under different management in a Mediterranean open woodland. *Forest Ecology and Management*, *486*, 118945. https://doi.org/10.1016/j.foreco.2021.118945

500   Bond-Lamberty, B., Bailey, V. L., Chen, M., Gough, C. M., & Vargas, R. (2018). Globally rising soil heterotrophic respiration over recent decades. *Nature*, *560*(7716), 80–83. https://doi.org/10.1038/s41586-018-0358-x

Bradford, M. A., & Crowther, T. W. (2013). Carbon use efficiency and storage in terrestrial ecosystems. *New Phytologist*, *199*(1), 7–9. https://doi.org/10.1111/nph.12334

Butterfield, H. S., & Malmström, C. M. (2009). The effects of phenology on indirect measures of aboveground biomass in
505   annual grasses. *International Journal of Remote Sensing*, *30*(12), 3133–3146. https://doi.org/10.1080/01431160802558774

Caldararu, S., Purves, D. W., & Palmer, P. I. (2014). Phenology as a Strategy for Carbon Optimality: A Global Model. *Biogeosciences*, *11*(3), 763–778. https://doi.org/10.5194/bg-11-763-2014

Caldararu, S., Rolo, V., Stocker, B. D., Gimeno, T. E., & Nair, R. (2023). Beyond model evaluation – combining
510   experiments and models to advance terrestrial ecosystem science. *Biogeosciences*, *20*(17), 3637–3649. https://doi.org/10.5194/bg-20-3637-2023

Cao, M., & Woodward, F. I. (1998). Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. *Nature*, *393*(6682), Article 6682. https://doi.org/10.1038/30460

Casals, P., Lopez-Sangil, L., Carrara, A., Gimeno, C., & Nogués, S. (2011). Autotrophic and Heterotrophic Contributions to
515   Short-Term Soil CO 2 Efflux Following Simulated Summer Precipitation Pulses in a Mediterranean Dehesa. *Global Biogeochemical Cycles*, *25*(3), n/a-n/a. https://doi.org/10.1029/2010GB003973

Cenini, V. L., Fornara, D. A., McMullan, G., Ternan, N., Lajtha, K., & Crawley, M. J. (2015). Chronic nitrogen fertilization and carbon sequestration in grassland soils: Evidence of a microbial enzyme link. *Biogeochemistry*, *126*(3), 301–313. https://doi.org/10.1007/s10533-015-0157-5

520   Chen, Z., & Yu, G. (2019). Spatial variations and controls of carbon use efficiency in China's terrestrial ecosystems. *Scientific Reports*, *9*(1), 19516. https://doi.org/10.1038/s41598-019-56115-5

Cos, J., Doblas-Reyes, F., Jury, M., Marcos, R., Bretonnière, P.-A., & Samsó, M. (2022). The Mediterranean climate change hotspot in the CMIP5 and CMIP6 projections. *Earth System Dynamics*, *13*(1), 321–340. https://doi.org/10.5194/esd-13-321-2022

525   Coskun, D., Britto, D. T., & Kronzucker, H. J. (2016). Nutrient constraints on terrestrial carbon fixation: The role of nitrogen. *Journal of Plant Physiology*, *203*, 95–109. https://doi.org/10.1016/j.jplph.2016.05.016

Craine, J. M., Elmore, A. J., Wang, L., Aranibar, J., Bauters, M., Boeckx, P., Crowley, B. E., Dawes, M. A., Delzon, S., Fajardo, A., Fang, Y., Fujiyoshi, L., Gray, A., Guerrieri, R., Gundale, M. J., Hawke, D. J., Hietz, P., Jonard, M., Kearsley, E., … Zmudczyńska-Skarbek, K. (2018). Isotopic evidence for oligotrophication of terrestrial ecosystems.
530   *Nature Ecology & Evolution*, *2*(11), 1735–1744. https://doi.org/10.1038/s41559-018-0694-0

Craine, J. M., Wedin, D. A., & Reich, P. B. (2001). The response of soil CO2 flux to changes in atmospheric CO2, nitrogen supply and plant diversity. *Global Change Biology*, *7*(8), 947–953. https://doi.org/10.1046/j.1354-1013.2001.00455.x

Crous, K. Y., O'Sullivan, O. S., Zaragoza-Castells, J., Bloomfield, K. J., Negrini, A. C. A., Meir, P., Turnbull, M. H.,
535   Griffin, K. L., & Atkin, O. K. (2017). Nitrogen and phosphorus availabilities interact to modulate leaf trait scaling relationships across six plant functional types in a controlled-environment study. *New Phytologist*, *215*(3), 992–1008. https://doi.org/10.1111/nph.14591

DeLucia, E. H., Drake, J. E., Thomas, R. B., & Gonzalez-Meler, M. (2007). Forest carbon use efficiency: Is respiration a constant fraction of gross primary production? *Global Change Biology*, *13*(6), 1157–1167.
540   https://doi.org/10.1111/j.1365-2486.2007.01365.x

Dijkstra, F. A., Zhu, B., & Cheng, W. (2021). Root effects on soil organic carbon: A double-edged sword. *New Phytologist*, *230*(1), 60–65. https://doi.org/10.1111/nph.17082

Ding, W., Cong, W.-F., & Lambers, H. (2021). Plant phosphorus-acquisition and -use strategies affect soil carbon cycling. *Trends in Ecology & Evolution*, *36*(10), 899–906. https://doi.org/10.1016/j.tree.2021.06.005

545 Domingues, T. F., Meir, P., Feldpausch, T. R., Saiz, G., Veenendaal, E. M., Schrodt, F., Bird, M., Djagbletey, G., Hien, F., Compaore, H., Diallo, A., Grace, J., & Lloyd, J. (2010). Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant, Cell & Environment*, *33*(6), 959–980. https://doi.org/10.1111/j.1365-3040.2010.02119.x

Du, E., Terrer, C., Pellegrini, A. F. A., Ahlström, A., van Lissa, C. J., Zhao, X., Xia, N., Wu, X., & Jackson, R. B. (2020).
550 Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience*, *13*(3), Article 3. https://doi.org/10.1038/s41561-019-0530-4

El-Madany, T. S., Carrara, A., Martín, M. P., Moreno, G., Kolle, O., Pacheco-Labrador, J., Weber, U., Wutzler, T., Reichstein, M., & Migliavacca, M. (2020). Drought and heatwave impacts on semi-arid ecosystems' carbon fluxes along a precipitation gradient. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1810),
555 20190519. https://doi.org/10.1098/rstb.2019.0519

El-Madany, T. S., Reichstein, M., Carrara, A., Martín, M. P., Moreno, G., Gonzalez-Cascon, R., Peñuelas, J., Ellsworth, D. S., Burchard-Levine, V., Hammer, T. W., Knauer, J., Kolle, O., Luo, Y., Pacheco-Labrador, J., Nelson, J. A., Perez-Priego, O., Rolo, V., Wutzler, T., & Migliavacca, M. (2021). How Nitrogen and Phosphorus Availability Change Water Use Efficiency in a Mediterranean Savanna Ecosystem. *Journal of Geophysical Research: Biogeosciences*,
560 *126*(5), e2020JG006005. https://doi.org/10.1029/2020JG006005

El-Madany, T. S., Reichstein, M., Perez-Priego, O., Carrara, A., Moreno, G., Pilar Martín, M., Pacheco-Labrador, J., Wohlfahrt, G., Nieto, H., Weber, U., Kolle, O., Luo, Y. P., Carvalhais, N., & Migliavacca, M. (2018). Drivers of Spatio-Temporal Variability of Carbon Dioxide and Energy Fluxes in a Mediterranean Savanna Ecosystem. *Agricultural and Forest Meteorology*, *262*(July 2017), 258–278. https://doi.org/10.1016/j.agrformet.2018.07.010

565 Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, *78*(1), 9–19. https://doi.org/10.1007/BF00377192

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Sardans, J., Luyssaert, S., Campioli, M., Chapin III, F. S., Ciais, P., Malhi, Y., Obersteiner, M., Papale, D., Piao, S. L., Reichstein, M., Rodà, F., & Peñuelas, J. (2014). Nutrient

Availability as the Key Regulator of Global Forest Carbon Balance. *Nature Climate Change*, *4*(6), 471–476.

570   https://doi.org/10.1038/nclimate2177

Fernández-Moya, J., Miguel-Ayanz, A. S., Cañellas, I., & Gea-Izquierdo, G. (2011). Variability in Mediterranean Annual

Grassland Diversity Driven by Small-Scale Changes in Fertility and Radiation. *Plant Ecology*, *212*(5), 865–877.

https://doi.org/10.1007/s11258-010-9869-8

Friedlingstein, P., O'Sullivan, M., Jones, M. W., Andrew, R. M., Gregor, L., Hauck, J., Le Quéré, C., Luijkx, I. T., Olsen,

575   A., Peters, G. P., Peters, W., Pongratz, J., Schwingshackl, C., Sitch, S., Canadell, J. G., Ciais, P., Jackson, R. B.,

Alin, S. R., Alkama, R., … Zheng, B. (2022). Global Carbon Budget 2022. *Earth System Science Data*, *14*(11),

4811–4900. https://doi.org/10.5194/essd-14-4811-2022

Gang, C., Wang, Z., You, Y., Liu, Y., Xu, R., Bian, Z., Pan, N., Gao, X., Chen, M., & Zhang, M. (2022). Divergent

responses of terrestrial carbon use efficiency to climate variation from 2000 to 2018. *Global and Planetary Change*,

580   *208*, 103709. https://doi.org/10.1016/j.gloplacha.2021.103709

Gang, C., Zhang, Y., Guo, L., Gao, X., Peng, S., Chen, M., & Wen, Z. (2019). Drought-Induced Carbon and Water Use

Efficiency Responses in Dryland Vegetation of Northern China. *Frontiers in Plant Science*, *10*, 224.

https://doi.org/10.3389/fpls.2019.00224

Gentine, P., Green, J. K., Guérin, M., Humphrey, V., Seneviratne, S. I., Zhang, Y., & Zhou, S. (2019). Coupling between the

585   terrestrial carbon and water cycles—A review. *Environmental Research Letters*, *14*(8), 083003.

https://doi.org/10.1088/1748-9326/ab22d6

Gliksman, D., Rey, A., Seligmann, R., Dumbur, R., Sperling, O., Navon, Y., Haenel, S., De Angelis, P., Arnone, J. A., &

Grünzweig, J. M. (2017). Biotic degradation at night, abiotic degradation at day: Positive feedbacks on litter

decomposition in drylands. *Global Change Biology*, *23*(4), 1564–1574. https://doi.org/10.1111/gcb.13465

590 Hastie, T., & Tibshirani, R. (1986). Generalized Additive Models. *Statistical Science*, *1*(3), 297–310.

https://doi.org/10.1214/ss/1177013604

Hicks, L. C., Lajtha, K., & Rousk, J. (2021). Nutrient limitation may induce microbial mining for resources from persistent

soil organic matter. *Ecology*, *102*(6), e03328. https://doi.org/10.1002/ecy.3328

Janssens, I., Dieleman, W., Luyssaert, S., Subke, J., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, a. J., Grace, J.,

595   Matteucci, G., Papale, D., Piao, S. L., Schulze, E.-D., Tang, J., & Law, B. E. E. (2010). Reduction of Forest Soil

        Respiration in Response to Nitrogen Deposition. *Nature Geoscience*, *3*(5), 315–322.

        https://doi.org/10.1038/ngeo844

Jeong, S.-J., Medvigy, D., Shevliakova, E., & Malyshev, S. (2012). Uncertainties in terrestrial carbon budgets related to

        spring phenology. *Journal of Geophysical Research: Biogeosciences*, *117*(G1).

600   https://doi.org/10.1029/2011JG001868

Jiang, M., Caldararu, S., Zaehle, S., Ellsworth, D. S., & Medlyn, B. E. (2019). Towards a more physiological representation

        of vegetation phosphorus processes in land surface models. *The New Phytologist*, *222*(3), 1223–1229.

        https://doi.org/10.1111/nph.15688

Jiang, M., Caldararu, S., Zhang, H., Fleischer, K., Crous, K. Y., Yang, J., De Kauwe, M. G., Ellsworth, D. S., Reich, P. B.,

605   Tissue, D. T., Zaehle, S., & Medlyn, B. E. (2020). Low phosphorus supply constrains plant responses to elevated

        CO2: A meta-analysis. *Global Change Biology*, *26*(10), 5856–5873. https://doi.org/10.1111/gcb.15277

Joffre, R. (1990). Plant and soil nitrogen dynamics in mediterranean grasslands: A comparison of annual and perennial

        grasses. *Oecologia*, *85*(1), 142–149. https://doi.org/10.1007/BF00317355

Keenan, T. F., Gray, J., Friedl, M. A., Toomey, M., Bohrer, G., Hollinger, D. Y., Munger, J. W., O'Keefe, J., Schmid, H. P.,

610   Wing, I. S., Yang, B., & Richardson, A. D. (2014). Net carbon uptake has increased through warming-induced

        changes in temperate forest phenology. *Nature Climate Change*, *4*(7), 598–604.

        https://doi.org/10.1038/nclimate2253

Kergoat, L., Lafont, S., Arneth, A., Le Dantec, V., & Saugier, B. (2008). Nitrogen controls plant canopy light-use efficiency

        in temperate and boreal ecosystems. *Journal of Geophysical Research: Biogeosciences*, *113*(G4).

615   https://doi.org/10.1029/2007JG000676

Klaus, V. H., Boch, S., Boeddinghaus, R. S., Hölzel, N., Kandeler, E., Marhan, S., Oelmann, Y., Prati, D., Regan, K. M.,

        Schmitt, B., Sorkau, E., & Kleinebecker, T. (2016). Temporal and small-scale spatial variation in grassland

productivity, biomass quality, and nutrient limitation. *Plant Ecology*, *217*(7), 843–856. https://doi.org/10.1007/s11258-016-0607-8

620 Knorr, W., Prentice, I. C., House, J. I., & Holland, E. A. (2005). Long-term sensitivity of soil carbon turnover to warming. *Nature*, *433*(7023), Article 7023. https://doi.org/10.1038/nature03226

Lapointe, L. (2001). How phenology influences physiology in deciduous forest spring ephemerals. *Physiologia Plantarum*, *113*(2), 151–157. https://doi.org/10.1034/j.1399-3054.2001.1130201.x

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K.
625 S., Knops, J. M. H., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., & Fierer, N. (2015). Consistent Responses of Soil Microbial Communities to Elevated Nutrient Inputs in Grasslands across the Globe. *Proceedings of the National Academy of Sciences*, *112*(35), 10967–10972. https://doi.org/10.1073/pnas.1508382112

Leibold, M. A., Chase, J. M., & Ernest, S. K. M. (2017). Community assembly and the functioning of ecosystems: How
630 metacommunity processes alter ecosystems attributes. *Ecology*, *98*(4), 909–919. https://doi.org/10.1002/ecy.1697

Lemanski, K., & Scheu, S. (2014). Fertilizer addition lessens the flux of microbial carbon to higher trophic levels in soil food webs of grassland. *Oecologia*, *176*(2), 487–496. https://doi.org/10.1007/s00442-014-3037-0

Liu, Y., Yang, Y., Wang, Q., Du, X., Li, J., Gang, C., Zhou, W., & Wang, Z. (2019). Evaluating the responses of net primary productivity and carbon use efficiency of global grassland to climate variability along an aridity gradient. *Science of*
635 *The Total Environment*, *652*, 671–682. https://doi.org/10.1016/j.scitotenv.2018.10.295

Llorens, P., Latron, J., Álvarez-Cobelas, M., Martínez-Vilalta, J., & Moreno, G. (2011). Hydrology and Biogeochemistry of Mediterranean Forests. In D. F. Levia, D. Carlyle-Moses, & T. Tanaka (Eds.), *Forest Hydrology and Biogeochemistry: Synthesis of Past Research and Future Directions* (pp. 301–319). Springer Netherlands. https://doi.org/10.1007/978-94-007-1363-5_14

640 Luo, W., Jiang, Y., Lü, X., Wang, X., Li, M.-H., Bai, E., Han, X., & Xu, Z. (2013). Patterns of Plant Biomass Allocation in Temperate Grasslands across a 2500-km Transect in Northern China. *PLoS ONE*, *8*(8), e71749. https://doi.org/10.1371/journal.pone.0071749

Luo, Y., El-Madany, T., Ma, X., Nair, R., Jung, M., Weber, U., Filippa, G., Bucher, S. F., Moreno, G., Cremonese, E., Carrara, A., Gonzalez-Cascon, R., Escudero, Y. C., Galvagno, M., Pacheco-Labrador, J., Martín, M. P., Perez-Priego, O., Reichstein, M., Richardson, A. D., … Migliavacca, M. (2020). Nutrients and water availability constrain the seasonality of vegetation activity in a Mediterranean ecosystem. *Global Change Biology*, *26*(8), 4379–4400. https://doi.org/10.1111/gcb.15138

Luo, Y., El-Madany, T. S., Filippa, G., Ma, X., Ahrens, B., Carrara, A., Gonzalez-Cascon, R., Cremonese, E., Galvagno, M., Hammer, T. W., Pacheco-Labrador, J., Martín, M. P., Moreno, G., Perez-Priego, O., Reichstein, M., Richardson, A. D., Römermann, C., & Migliavacca, M. (2018). Using Near-Infrared-Enabled Digital Repeat Photography to Track Structural and Physiological Phenology in Mediterranean Tree–Grass Ecosystems. *Remote Sensing*, *10*(8), Article 8. https://doi.org/10.3390/rs10081293

Luo, Y., Su, B., Currie, W. S., Dukes, J. S., Finzi, A., Hartwig, U., Hungate, B., McMurtrie, R. E., Oren, R., Parton, W. J., Pataki, D. E., Shaw, R. M., Zak, D. R., & Field, C. B. (2004). Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *BioScience*, *54*(8), 731–739. https://doi.org/10.1641/0006-3568(2004)054[0731:PNLOER]2.0.CO;2

Ma, F., Song, B., Zhang, F., Quan, Q., Zhou, Q., & Niu, S. (2018). Ecosystem Carbon Use Efficiency Is Insensitive to Nitrogen Addition in an Alpine Meadow. *Journal of Geophysical Research: Biogeosciences*, *123*(8), 2388–2398. https://doi.org/10.1029/2018JG004530

Manzoni, S., Čapek, P., Porada, P., Thurner, M., Winterdahl, M., Beer, C., Brüchert, V., Frouz, J., Herrmann, A. M., Lindahl, B. D., Lyon, S. W., Šantrůčková, H., Vico, G., & Way, D. (2018). Reviews and syntheses: Carbon use efficiency from organisms to ecosystems – definitions, theories, and empirical evidence. *Biogeosciences*, *15*(19), 5929–5949. https://doi.org/10.5194/bg-15-5929-2018

Manzoni, S., Taylor, P., Richter, A., Porporato, A., & ??gren, G. I. G. I. (2012). Environmental and Stoichiometric Controls on Microbial Carbon-Use Efficiency in Soils. *New Phytologist*, *196*(1), 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

Martini, D., Pacheco-Labrador, J., Perez-Priego, O., van der Tol, C., El-Madany, T. S., Julitta, T., Rossini, M., Reichstein, M., Christiansen, R., Rascher, U., Moreno, G., Martín, M. P., Yang, P., Carrara, A., Guan, J., González-Cascón, R., & Migliavacca, M. (2019). Nitrogen and Phosphorus Effect on Sun-Induced Fluorescence and Gross Primary Productivity in Mediterranean Grassland. *Remote Sensing*, *11*(21), Article 21. https://doi.org/10.3390/rs11212562

Maseyk, K. S., Lin, T., Rotenberg, E., Grünzweig, J. M., Schwartz, A., & Yakir, D. (2008). Physiology–phenology interactions in a productive semi-arid pine forest. *New Phytologist*, *178*(3), 603–616. https://doi.org/10.1111/j.1469-8137.2008.02391.x

Matías, L., Hidalgo-Galvez, M. D., Cambrollé, J., Domínguez, M. T., & Pérez-Ramos, I. M. (2021). How will forecasted warming and drought affect soil respiration in savannah ecosystems? The role of tree canopy and grazing legacy. *Agricultural and Forest Meteorology*, *304–305*, 108425. https://doi.org/10.1016/j.agrformet.2021.108425

Meunier, C. L., Boersma, M., El-Sabaawi, R., Halvorson, H. M., Herstoff, E. M., Van de Waal, D. B., Vogt, R. J., & Litchman, E. (2017). From Elements to Function: Toward Unifying Ecological Stoichiometry and Trait-Based Ecology. *Frontiers in Environmental Science*, *5*. https://doi.org/10.3389/fenvs.2017.00018

Migliavacca, M., Musavi, T., Mahecha, M. D., Nelson, J. A., Knauer, J., Baldocchi, D. D., Perez-Priego, O., Christiansen, R., Peters, J., Anderson, K., Bahn, M., Black, T. A., Blanken, P. D., Bonal, D., Buchmann, N., Caldararu, S., Carrara, A., Carvalhais, N., Cescatti, A., … Reichstein, M. (2021). The three major axes of terrestrial ecosystem function. *Nature*, *598*(7881), 468–472. https://doi.org/10.1038/s41586-021-03939-9

Migliavacca, M., Perez-Priego, O., Rossini, M., El-Madany, T. S., Moreno, G., van der Tol, C., Rascher, U., Berninger, A., Bessenbacher, V., Burkart, A., Carrara, A., Fava, F., Guan, J. H., Hammer, T. W., Henkel, K., Juarez-Alcalde, E., Julitta, T., Kolle, O., Martín, M. P., … Reichstein, M. (2017). Plant functional traits and canopy structure control the relationship between photosynthetic CO2 uptake and far-red sun-induced fluorescence in a Mediterranean grassland under different nutrient availability. *New Phytologist*, *214*(3), 1078–1091. https://doi.org/10.1111/nph.14437

Migliavacca, M., Reichstein, M., Richardson, A. D., Mahecha, M. D., Cremonese, E., Delpierre, N., Galvagno, M., Law, B. E., Wohlfahrt, G., Andrew Black, T., Carvalhais, N., Ceccherini, G., Chen, J., Gobron, N., Koffi, E., William

Munger, J., Perez-Priego, O., Robustelli, M., Tomelleri, E., & Cescatti, A. (2015). Influence of physiological phenology on the seasonal pattern of ecosystem respiration in deciduous forests. *Global Change Biology*, *21*(1), 363–376. https://doi.org/10.1111/gcb.12671

695   Moreno, G., Gonzalez-Bornay, G., Pulido, F., Lopez-Diaz, M. L., Bertomeu, M., Juárez, E., & Diaz, M. (2016). Exploring the Causes of High Biodiversity of Iberian Dehesas: The Importance of Wood Pastures and Marginal Habitats. *Agroforestry Systems*, *90*(1), 87–105. https://doi.org/10.1007/s10457-015-9817-7

Moreno, G., Hernández-Esteban, A., Rolo, V., & Igual, J. M. (2021). The enduring effects of sowing legume-rich mixtures on the soil microbial community and soil carbon in semi-arid wood pastures. *Plant and Soil*, *465*(1), 563–582.

700   https://doi.org/10.1007/s11104-021-05023-7

Morris, K. A., Nair, R. K. F., Moreno, G., Schrumpf, M., & Migliavacca, M. (2019). Fate of N additions in a multiple resource-limited Mediterranean oak savanna. *Ecosphere*, *10*(11), e02921. https://doi.org/10.1002/ecs2.2921

Morris, K. A., Richter, A., Migliavacca, M., & Schrumpf, M. (2022). Growth of soil microbes is not limited by the availability of nitrogen and phosphorus in a Mediterranean oak-savanna. *Soil Biology and Biochemistry*, 108680.

705   https://doi.org/10.1016/j.soilbio.2022.108680

Musavi, T., Migliavacca, M., van de Weg, M. J., Kattge, J., Wohlfahrt, G., van Bodegom, P. M., Reichstein, M., Bahn, M., Carrara, A., Domingues, T. F., Gavazzi, M., Gianelle, D., Gimeno, C., Granier, A., Gruening, C., Havránková, K., Herbst, M., Hrynkiw, C., Kalhori, A., … Mahecha, M. D. (2016). Potential and limitations of inferring ecosystem photosynthetic capacity from leaf functional traits. *Ecology and Evolution*, *6*(20), 7352–7366.

710   https://doi.org/10.1002/ece3.2479

Nair, R. K. F., Morris, K. A., Hertel, M., Luo, Y., Moreno, G., Reichstein, M., Schrumpf, M., & Migliavacca, M. (2019). N:P stoichiometry and habitat effects on Mediterranean savanna seasonal root dynamics. *Biogeosciences*, *16*(9), 1883–1901. https://doi.org/10.5194/bg-16-1883-2019

Nair, R. K. F., Morris, K. A., Migliavacca, M., Moreno, G., & Schrumpf, M. (2020). Plant-available N:P alters root litter N

715   recycling in a Mediterranean tree–grass ecosystem. *Journal of Plant Nutrition and Soil Science*, *18*(3), 517–529. https://doi.org/10.1002/jpln.202000097

Nakagawa, S., & Schielzeth, H. (2013). A General and Simple Method for Obtaining R 2 from Generalized Linear Mixed-Effects Models. *Methods in Ecology and Evolution*, *4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

720   Nelson, J. A., Carvalhais, N., Cuntz, M., Delpierre, N., Knauer, J., Ogée, J., Migliavacca, M., Reichstein, M., & Jung, M. (2018). Coupling Water and Carbon Fluxes to Constrain Estimates of Transpiration: The TEA Algorithm. *Journal of Geophysical Research: Biogeosciences*, *123*(12), 3617–3632. https://doi.org/10.1029/2018JG004727

Oldroyd, G. E. D., & Leyser, O. (2020). A plant's diet, surviving in a variable nutrient environment. *Science*, *368*(6486). https://doi.org/10.1126/science.aba0196

725   Pacheco-Labrador, J., El-Madany, T. S., Martín, M. P., Migliavacca, M., Rossini, M., Carrara, A., & Zarco-Tejada, P. J. (2017). Spatio-Temporal Relationships between Optical Information and Carbon Fluxes in a Mediterranean Tree-Grass Ecosystem. *Remote Sensing*, *9*(6), Article 6. https://doi.org/10.3390/rs9060608

Paniagua, L. L., García-Martín, A., Moral, F. J., & Rebollo, F. J. (2019). Aridity in the Iberian Peninsula (1960–2017): Distribution, tendencies, and changes. *Theoretical and Applied Climatology*, *138*(1), 811–830.

730   https://doi.org/10.1007/s00704-019-02866-0

Papale, D., Reichstein, M., Aubinet, M., Canfora, E., Bernhofer, C., Kutsch, W., Longdoz, B., Rambal, S., Valentini, R., Vesala, T., & Yakir, D. (2006). Towards a standardized processing of Net Ecosystem Exchange measured with eddy covariance technique: Algorithms and uncertainty estimation. *Biogeosciences*, *3*(4), 571–583. https://doi.org/10.5194/bg-3-571-2006

735   Pastore, M. A., Hobbie, S. E., & Reich, P. B. (2021). Sensitivity of grassland carbon pools to plant diversity, elevated CO2, and soil nitrogen addition over 19 years. *Proceedings of the National Academy of Sciences*, *118*(17). https://doi.org/10.1073/pnas.2016965118

Paulus, S., El-Madany, T. S., Orth, R., Hildebrandt, A., Wutzler, T., Carrara, A., Moreno, G., Perez-Priego, O., Kolle, O., Reichstein, M., & Migliavacca, M. (2021). Lysimeter based evaporation and condensation dynamics in a

740   Mediterranean ecosystem. *Hydrology and Earth System Sciences Discussions*, 1–29. https://doi.org/10.5194/hess-2021-519

Peñuelas, J., Janssens, I. A., Ciais, P., Obersteiner, M., & Sardans, J. (2020). Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Global Change Biology*, *26*(4), 1962–1985. https://doi.org/10.1111/gcb.14981

745   Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., & Janssens, I. A. (2013). Human-Induced Nitrogen–Phosphorus Imbalances Alter Natural and Managed Ecosystems across the Globe. *Nature Communications*, *4*. https://doi.org/10.1038/ncomms3934

Peñuelas, J., Sardans, J., Rivas-Ubach, A., & Janssens, Ivan. A. (2012). The Human-Induced Imbalance between C, N and P

750   in Earth's Life System. *Global Change Biology*, *18*(1), 3–6. https://doi.org/10.1111/j.1365-2486.2011.02568.x

Pereira, J. S., Mateus, J. A., Aires, L. M., Pita, G., Pio, C., David, J. S., Andrade, V., Banza, J., David, T. S., Paço, T. A., & Rodrigues, A. (2007). Net ecosystem carbon exchange in three contrasting Mediterranean ecosystems – the effect of drought. *Biogeosciences*, *4*(5), 791–802. https://doi.org/10.5194/bg-4-791-2007

Perez-Priego, O., Guan, J., Rossini, M., Fava, F., Wutzler, T., Moreno, G., Carvalhais, N., Carrara, A., Kolle, O., Julitta, T.,

755   Schrumpf, M., Reichstein, M., & Migliavacca, M. (2015). Sun-Induced Chlorophyll Fluorescence and Photochemical Reflectance Index Improve Remote-Sensing Gross Primary Production Estimates under Varying Nutrient Availability in a Typical Mediterranean Savanna Ecosystem. *Biogeosciences*, *12*(21), 6351–6367. https://doi.org/10.5194/bg-12-6351-2015

Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., & Zhu, X. (2019). Plant phenology

760   and global climate change: Current progresses and challenges. *Global Change Biology*, *25*(6), 1922–1940. https://doi.org/10.1111/gcb.14619

Qiao, Y., Wang, J., Liang, G., Du, Z., Zhou, J., Zhu, C., Huang, K., Zhou, X., Luo, Y., Yan, L., & Xia, J. (2019). Global variation of soil microbial carbon-use efficiency in relation to growth temperature and substrate supply. *Scientific Reports*, *9*(1), 5621. https://doi.org/10.1038/s41598-019-42145-6

765   R Core Team. (2018). *R: A Language and Environment for Statistical Computing* [Computer software].

Reichstein, M., Falge, E., Baldocchi, D., Papale, D., Aubinet, M., Berbigier, P., Bernhofer, C., Buchmann, N., Gilmanov, T., Granier, A., Grünwald, T., Havránková, K., Ilvesniemi, H., Janous, D., Knohl, A., Laurila, T., Lohila, A., Loustau, D., Matteucci, G., … Valentini, R. (2005). On the separation of net ecosystem exchange into assimilation and ecosystem respiration: Review and improved algorithm. *Global Change Biology*, *11*(9), 1424–1439. https://doi.org/10.1111/j.1365-2486.2005.001002.x

Richardson, A. D., Hufkens, K., Milliman, T., Aubrecht, D. M., Chen, M., Gray, J. M., Johnston, M. R., Keenan, T. F., Klosterman, S. T., Kosmala, M., Melaas, E. K., Friedl, M. A., & Frolking, S. (2018). Tracking Vegetation Phenology across Diverse North American Biomes Using PhenoCam Imagery. *Tracking Vegetation Phenology across Diverse North American Biomes Using PhenoCam Imagery. Scientific Data*, *5*, 1–24. https://doi.org/10.1038/sdata.2018.28

Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology*, *169*, 156–173. https://doi.org/10.1016/j.agrformet.2012.09.012

Rouse, J. W., Haas, R. H., Deering, D. W., Schell, J. A., & Harlan, J. C. (1974). *Monitoring the Vernal Advancement and Retrogradation (Green Wave Effect) of Natural Vegetation* (E75-10354). https://ntrs.nasa.gov/citations/19750020419

Rowland, L., Zaragoza-Castells, J., Bloomfield, K. J., Turnbull, M. H., Bonal, D., Burban, B., Salinas, N., Cosio, E., Metcalfe, D. J., Ford, A., Phillips, O. L., Atkin, O. K., & Meir, P. (2017). Scaling leaf respiration with nitrogen and phosphorus in tropical forests across two continents. *New Phytologist*, *214*(3), 1064–1077. https://doi.org/10.1111/nph.13992

Rumman, R., Atkin, O. K., Bloomfield, K. J., & Eamus, D. (2018). Variation in bulk-leaf 13C discrimination, leaf traits and water-use efficiency–trait relationships along a continental-scale climate gradient in Australia. *Global Change Biology*, *24*(3), 1186–1200. https://doi.org/10.1111/gcb.13911

Sainju, U. M., Allen, B. L., Lenssen, A. W., & Ghimire, R. P. (2017). Root biomass, root/shoot ratio, and soil water content under perennial grasses with different nitrogen rates. *Field Crops Research*, *210*, 183–191. https://doi.org/10.1016/j.fcr.2017.05.029

San-Emeterio, L. M., Jiménez-Morillo, N. T., Pérez-Ramos, I. M., Domínguez, M. T., & González-Pérez, J. A. (2023). Changes in soil organic matter molecular structure after five-years mimicking climate change scenarios in a Mediterranean savannah. *Science of The Total Environment*, *857*, 159288. https://doi.org/10.1016/j.scitotenv.2022.159288

Schnitzer, S. A., Klironomos, J. N., HilleRisLambers, J., Kinkel, L. L., Reich, P. B., Xiao, K., Rillig, M. C., Sikes, B. A., Callaway, R. M., Mangan, S. A., van Nes, E. H., & Scheffer, M. (2011). Soil microbes drive the classic plant diversity–productivity pattern. *Ecology*, *92*(2), 296–303. https://doi.org/10.1890/10-0773.1

Scott, R. L., Biederman, J. A., Hamerlynck, E. P., & Barron-Gafford, G. A. (2015). The carbon balance pivot point of southwestern U.S. semiarid ecosystems: Insights from the 21st century drought. *Journal of Geophysical Research: Biogeosciences*, *120*(12), 2612–2624. https://doi.org/10.1002/2015JG003181

Simon, E., Canarini, A., Martin, V., Séneca, J., Böckle, T., Reinthaler, D., Pötsch, E. M., Piepho, H.-P., Bahn, M., Wanek, W., & Richter, A. (2020). Microbial growth and carbon use efficiency show seasonal responses in a multifactorial climate change experiment. *Communications Biology*, *3*(1), Article 1. https://doi.org/10.1038/s42003-020-01317-1

Sinsabaugh, R. L., Moorhead, D. L., Xu, X., & Litvak, M. E. (2017). Plant, microbial and ecosystem carbon use efficiencies interact to stabilize microbial growth as a fraction of gross primary production. *New Phytologist*, *214*(4), 1518–1526. https://doi.org/10.1111/nph.14485

Soong, J. L., Fuchslueger, L., Marañon-Jimenez, S., Torn, M. S., Janssens, I. A., Penuelas, J., & Richter, A. (2020). Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biology*, *26*(4), 1953–1961. https://doi.org/10.1111/gcb.14962

Soussana, J.-F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., & Arrouays, D. (2004). Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management*, *20*(2), 219–230. https://doi.org/10.1111/j.1475-2743.2004.tb00362.x

Stoner, S. W., Hoyt, A. M., Trumbore, S., Sierra, C. A., Schrumpf, M., Doetterl, S., Baisden, W. T., & Schipper, L. A.

815   (2021). Soil organic matter turnover rates increase to match increased inputs in grazed grasslands. *Biogeochemistry*,

           156(1), 145–160. https://doi.org/10.1007/s10533-021-00838-z

Tahovská, K., Choma, M., Kaštovská, E., Oulehle, F., Bárta, J., Šantrůčková, H., & Moldan, F. (2020). Positive response of

           soil microbes to long-term nitrogen input in spruce forest: Results from Gårdsjön whole-catchment N-addition

           experiment. *Soil Biology and Biochemistry*, *143*, 107732. https://doi.org/10.1016/j.soilbio.2020.107732

820   Tao, F., Huang, Y., Hungate, B. A., Manzoni, S., Frey, S. D., Schmidt, M. W. I., Reichstein, M., Carvalhais, N., Ciais, P.,

           Jiang, L., Lehmann, J., Wang, Y.-P., Houlton, B. Z., Ahrens, B., Mishra, U., Hugelius, G., Hocking, T. D., Lu, X.,

           Shi, Z., … Luo, Y. (2023). Microbial carbon use efficiency promotes global soil carbon storage. *Nature*, 1–5.

           https://doi.org/10.1038/s41586-023-06042-3

Templer, P. H., Mack, M. C., Chapin, F. S., Christenson, L. M., Compton, J. E., Crook, H. D., Currie, W. S., Curtis, C. J.,

825   Dail, D. B., D'Antonio, C. M., a Emmett, B., Epstein, H. E., Goodale, C. L., Gundersen, P., Hobbie, S. E., Holland,

           K., Hooper, D. U., Hungate, B. A., Lamontagne, S., … Zak, D. R. (2012). Sinks for Nitrogen Inputs in Terrestrial

           Ecosystems: A Meta-Analysis of 15N Tracer Field Studies. *Ecology*, *93*(8), 1816–1829.

Tron, S., Bodner, G., Laio, F., Ridolfi, L., & Leitner, D. (2015). Can diversity in root architecture explain plant water use

           efficiency? A modeling study. *Ecological Modelling*, *312*, 200–210.

830   https://doi.org/10.1016/j.ecolmodel.2015.05.028

Tsialtas, J. T., Handley, L. L., Kassioumi, M. T., Veresoglou, D. S., & Gagianas, A. A. (2001). Interspecific Variation in

           Potential Water-Use Efficiency and Its Relation to Plant Species Abundance in a Water-Limited Grassland.

           Functional Ecology, *15*(5), 605–614.

Uclés, O., Villagarcía, L., Moro, M. J., Canton, Y., & Domingo, F. (2014). Role of dewfall in the water balance of a semiarid

835   coastal steppe ecosystem. *Hydrological Processes*, *28*(4), 2271–2280. https://doi.org/10.1002/hyp.9780

Ullah, M. R., Carrillo, Y., & Dijkstra, F. A. (2021). Drought-induced and seasonal variation in carbon use efficiency is

           associated with fungi:bacteria ratio and enzyme production in a grassland ecosystem. *Soil Biology and*

           Biochemistry, *155*, 108159. https://doi.org/10.1016/j.soilbio.2021.108159

Van Der Heijden, M. GA., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of

840   plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(3), 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

von Buttlar, J., Zscheischler, J., Rammig, A., Sippel, S., Reichstein, M., Knohl, A., Jung, M., Menzer, O., Arain, M. A., Buchmann, N., Cescatti, A., Gianelle, D., Kiely, G., Law, B. E., Magliulo, V., Margolis, H., McCaughey, H., Merbold, L., Migliavacca, M., … Mahecha, M. D. (2018). Impacts of droughts and extreme-temperature events on

845   gross primary production and ecosystem respiration: A systematic assessment across ecosystems and climate zones. *Biogeosciences*, *15*(5), 1293–1318. https://doi.org/10.5194/bg-15-1293-2018

Weiner, J. (2004). Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, *6*(4), 207–215. https://doi.org/10.1078/1433-8319-00083

Wood, S. N. (2006). *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC.

850   https://doi.org/10.1201/9781420010404

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models: Estimation of Semiparametric Generalized Linear Models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*(1), 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

855 Wutzler, T., Lucas-Moffat, A., Migliavacca, M., Knauer, J., Sickel, K., Šigut, L., Menzer, O., & Reichstein, M. (2018). Basic and extensible post-processing of eddy covariance flux data with REddyProc. *Biogeosciences*, *15*(16), 5015–5030. https://doi.org/10.5194/bg-15-5015-2018

Xu, L., Baldocchi, D. D., & Tang, J. (2004). How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. *Global Biogeochemical Cycles*, *18*(4). https://doi.org/10.1029/2004GB002281

860 Zaehle, S. (2013). Terrestrial nitrogen–carbon cycle interactions at the global scale. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130125. https://doi.org/10.1098/rstb.2013.0125

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., & Shen, C. (2015). Soil Biology & Biochemistry Nitrogen Fertilization Directly Affects Soil Bacterial Diversity and Indirectly Affects Bacterial Community Composition. *Soil Biology and Biochemistry*, *October*, 1–9. https://doi.org/10.1016/j.soilbio.2015.09.018

865 Zhang, Y., Xu, M., Chen, H., & Adams, J. (2009). Global pattern of NPP to GPP ratio derived from MODIS data: Effects of ecosystem type, geographical location and climate. *Global Ecology and Biogeography*, *18*(3), 280–290. https://doi.org/10.1111/j.1466-8238.2008.00442.x

Zhang, Y., Yu, G., Yang, J., Wimberly, M. C., Zhang, X., Tao, J., Jiang, Y., & Zhu, J. (2014). Climate-driven global changes in carbon use efficiency. *Global Ecology and Biogeography*, *23*(2), 144–155. https://doi.org/10.1111/geb.12086

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