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Phenology and plant functional type dominance drive CO₂ exchange in seminatural grasslands in the Pyrenees

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Abstract

Understanding the mechanisms underlying net ecosystem CO₂ exchange (NEE) in mountain grasslands is important to quantify their relevance in the global carbon budget. However, complex interactions between environmental variables and vegetation on NEE remain unclear; and there is a lack of empirical data, especially from the high elevations and the Mediterranean region. A chamber-based survey of CO₂ exchange measurements was carried out in two climatically contrasted grasslands (montane *v.* subalpine) of the Pyrenees; assessing the relative contribution of phenology and environmental variables on CO₂ exchange at the seasonal scale, and the influence of plant functional type dominance (grasses, forbs and legumes) on the NEE light response. Results show that phenology plays a crucial role as a CO₂ exchange driver, suggesting a differential behaviour of the vegetation community depending on the environment. The subalpine grassland had a more delayed phenology compared to the montane, being more temperature than water constrained. However, temperature increased net CO₂ uptake at a higher rate in the subalpine than in the montane grassland. During the peak biomass, productivity (+74%) and net CO₂ uptake (NEE +48%) were higher in the subalpine grassland than in the montane grassland. The delayed phenology at the subalpine grassland reduced vegetation's sensitivity to summer dryness, and CO₂ exchange fluxes were less constrained by low soil water content. The NEE light response suggested that legume dominated plots had higher net CO₂ uptake per unit of biomass than grasses. Detailed information on phenology and vegetation composition is essential to understand elevation and climatic differences in CO₂ exchange.

Introduction

Grasslands are the most widespread habitat in the world and provide crucial goods and services for human population, including animal feeding, climate regulation and carbon cycling (Hooper *et al.*, 2005). Extensively managed mountain grasslands in particular, are some of the most species-rich ecosystems (Wilson *et al.*, 2012), store about 100 t/ha of soil carbon (Sjögersten *et al.*, 2011), and their net ecosystem exchange (NEE, Woodwell and Whittaker 1968) is mostly dominated by assimilation (Gilmanov *et al.*, 2007; Soussana *et al.*, 2007; Berninger *et al.*, 2015).

However, there is still a lack of empirical data, mainly from the high elevations and from some regions, including the Mediterranean basin, in which climate change impacts are projected to be very severe (García-Ruiz *et al.*, 2011). In the particular case of the Pyrenees, despite the few corresponding studies (Wohlfahrt *et al.*, 2008a; Sjögersten *et al.*, 2012; Berninger *et al.*, 2015), NEE datasets are very limited, and knowing the particularities of these systems may provide some guidelines to adapt and mitigate climate change effects in this region.

Moreover, mountain grasslands are especially vulnerable to climate and land use changes, and mid- to long-term effects on the carbon budget still remain controversial (Wu *et al.*, 2011), partly due to complex interactions between environmental variables and vegetation. Indeed, although the role of main environmental CO₂ exchange drivers, such as photosynthetically active radiation (Wohlfahrt *et al.*, 2008b), temperature and soil moisture (Davidson and Janssens, 2006; Albergel *et al.*, 2010; Yvon-Durocher *et al.*, 2012) has been widely assessed, how they interact with phenology and vegetation composition still needs deeper understanding.

Vegetation in mountain grasslands is highly dynamic, changing its structure and composition over time and space (Faurie *et al.*, 1996; Giunta *et al.*, 2009; Mitchell and Bakker, 2016), resulting in a variable patchy configuration of species (Schwinning and Parsons, 1996), and generating differences in biogeochemical cycles and CO₂ exchange (Reich *et al.*, 1997).

While it is known that the aboveground living biomass (AGLB) directly takes-up (Wohlfahrt *et al.*, 2008b; Nakano and Shinoda, 2014) and releases CO₂ (Kardol *et al.*, 2010; Thakur and Eisenhauer, 2015), phenology and vegetation structure may also be determinant for the NEE. Indices of phenological development related to plant productivity, including total green biomass and normalized difference vegetation index (Gao *et al.*, 2016; Zhou *et al.*, 2016) have already been used to estimate gross primary production (GPP, Filippa *et al.*, 2015) and ecosystem respiration (R_{eco}, Ryan and Law 2005).

However, when assessing mountain grasslands there are differences in phenological cycles between elevation belts (Liu *et al.*, 2014; Leifeld *et al.*, 2015), which may result in more complex vegetation–CO₂ exchange interactions than expected. In addition, there are other vegetation fractions, such as standing dead biomass (SDB, dead biomass attached to the plant) and litter (dead plant material, detached from the plant and laying on the soil surface), which are present in considerable amounts in grasslands, and whose specific role as CO₂ exchange drivers has been barely considered (Leitner *et al.*, 2016; Gliksmann *et al.*, 2018).

On the other hand, vegetation composition has also been reported to drive CO₂ exchange fluxes (De Deyn *et al.*, 2009; Metcalfe *et al.*, 2011; Ribas *et al.*, 2015). A common approximation to assess this vegetation–CO₂ exchange relationship is to separate plant species into plant functional types (PFT) that share a common response to an environmental factor, ‘response traits’, and/or a common effect on ecosystem processes, ‘effect traits’ (Lavorel and Garnier, 2002). In the specific case of grasslands, species are often classified in grasses, non-legume forbs (hereafter ‘forbs’) and legume forbs (hereafter ‘legumes’), classification that is based on nitrogen and light (and therefore CO₂) acquisition and use (Tilman, 1997; Symstad, 2000; Díaz *et al.*, 2007; defined as ‘guilds’ in Sebastià, 2007). Thus, legumes have the capacity to fix symbiotic nitrogen, while grasses have some advantages when competing for light as they are usually taller than legumes and forbs, and have erect high-density leaves that ensure good light penetration (Craine *et al.*, 2001). However, there is still some uncertainty about how these PFT can differentially influence CO₂ exchange at a plot scale.

Accordingly, in the present study we investigate the interaction between environmental variables and vegetation on CO₂ exchange fluxes, and more specifically we aim to: (1) compare the contribution of vegetation phenology and environmental variables in two climatically contrasted mountain grasslands in the Pyrenees, and (2) assess the influence of vegetation composition, in terms of the dominant PFT (forbs, grasses and legumes), on light response and therefore on NEE. For that purpose, we performed a survey of CO₂ exchange measurements with a non-steady state chamber, aboveground biomass sampling and environmental variables recording in two extensively managed mountain grasslands in the Pyrenees, located in the montane and subalpine elevation belts, respectively.

Material and methods

Study sites

The study sites were two grazed mountain grasslands in the south-eastern Pyrenees: La Bertolina (BERT), located in Pla de Busa (42°05'N, 1°39'E, 1276 m a. s. l.), and Castellar de n'Hug (CAST) in Plans del Ginebrar (42°18'N, 2°02'E, 1900 m a. s. l.). Both sites were characterized by a Mediterranean climate regime,

with spring and autumn precipitations and relatively high summer temperatures (Fig. 1(a)). However, each grassland had its own specific climatic characteristics and phenological particularities, respective to the given elevation belt.

BERT was a typical montane grassland, with a mean annual temperature of 9 °C and a mean annual precipitation of 870 mm (Fig. 1(a)). In BERT, vegetation started to grow (Fig. 1(c)) as soon as soil water content (SWC, Fig. 1(b)) increased, and senescence started (Fig. 1(c)) as soon as SWC dropped and summer temperatures became high (T_a ~ 18 °C, Fig. 1(b)). On the other hand, CAST was a subalpine grassland, with a mean annual temperature of 5.1 °C and a mean annual precipitation of 1189 mm (Fig. 1(a)). CAST was more temperature limited, and vegetation did not start to grow (Fig. 1(c)) until temperatures increased up-to ≥ 5 °C, irrespective of the highest spring SWC, which coincided with the snowmelt period and cold temperatures (T_a ≤ 5 °C). Senescence started later at CAST than at BERT, and progressed more slowly (Fig. 1(c)), despite the low-mid-summer SWC (Fig. 1(b)).

Vegetation composition at BERT was characteristic of a montane meso-xerophytic grassland, and CAST was a mesic subalpine grassland. Both sites were extensively grazed, by cattle at BERT, with an average stocking rate of 0.44 livestock units (LSU)/ha, from May to November; and by cattle and sheep at CAST, with an average stocking rate of 0.74 LSU/ha, from late June to November (according to the corresponding site managers). The montane grassland (BERT) sustained a lower livestock density, although during a longer time period (~3.1 LSU month/ha/year). On the contrary, the subalpine grassland (CAST) was highly productive during the summer and sustained a higher livestock density, but during a shorter time (~4.4 LSU month/ha/year). Farmers' expectation of the carrying capacity was ~44% higher at CAST than at BERT. Grazing calendar and stocking rates were provided by the farmers and later confirmed during sampling visits. Soil at BERT was udic calciustept and at CAST was lithic udorthent (FAO, 1998).

Sampling design

Two sampling designs were established to achieve the aims of the current paper: a seasonal and a diel sampling. The aim of the seasonal sampling was to record temporal CO₂ variability over the growing season and its relationship with environmental variables and vegetation phenology. The seasonal sampling was carried out from April to December of 2012, at 3-weekly intervals. Every sampling day, sampling points of grassland patches (*n* = 10 at BERT and *n* = 8 at CAST) were systematically placed within the footprint of the respective eddy covariance flux stations previously installed at each site (Fig. 2), which provided ancillary meteorological variables.

At each sampling point, complete CO₂ exchange measurements (NEE and ecosystem respiration, R_{eco}, see CO₂ exchange flux calculations) were recorded twice during daytime (08:00–16:30 UTC). After CO₂ exchange measurement, total aboveground biomass was harvested at the ground level. Total aboveground biomass was separated into the different vegetation fractions: AGLB, SDB (dead biomass attached to the plant) and litter (dead plant material, detached from the plant and on the soil surface) to characterize vegetation phenological changes. Dry weight (DW, g/m²) of all vegetation fractions was determined after oven drying at 60 °C until constant weight.

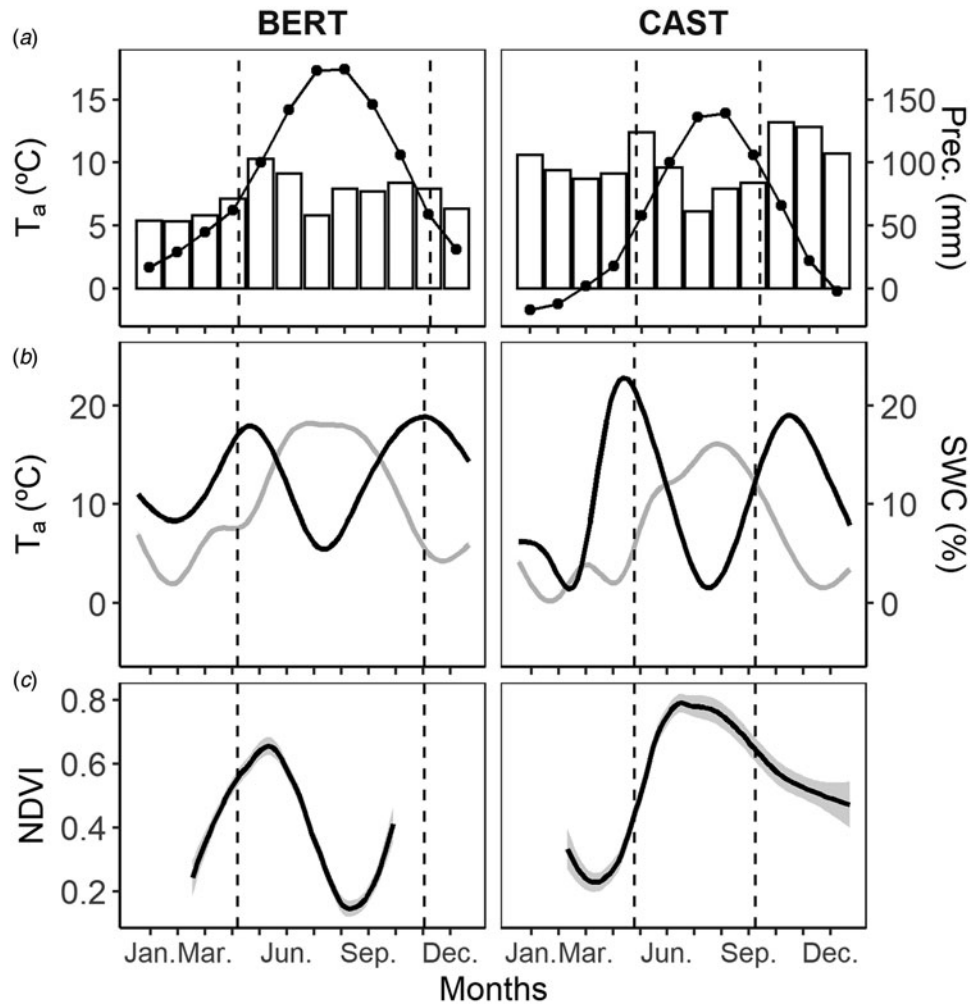


Fig. 1. Climatic and environmental variables of the study sites: Bertolina (BERT) and Castellar (CAST). (a) Mean climatic (1970–2000) monthly air temperature (T_a , solid symbols and line) and monthly precipitation (bars), source: WorldClim (Fick and Hijmans, 2017); (b) 2012 meteorological data: T_a (grey line), and soil water content at 5 cm depth (SWC, black line), lines fitted using generalized additive models with integrated smoothness estimation (gam), mgcv package (Wood, 2004), source: eddy covariance flux stations; (c) 2012 normalized difference vegetation index (NDVI, black line) and its 0.95 confidence interval (grey band), line fitted using local polynomial regression fitting (loess), source: eddy covariance flux stations. Vertical black dashed lines indicate the beginning and the end of the study period.

The aim of the diel sampling was to assess the effect of the dominant PFT on NEE, via the PFT-specific light response. A campaign of intensive CO_2 exchange measurements was carried out at each site, coinciding with the peak biomass (end of May at BERT, day of year (DOY) 150–152 and end of June at CAST, DOY 172–173), to reduce the variability related to different phenological stages and/or environmental conditions, and focusing on the effect of the PFT dominance. Sampling points were selected to ensure the presence of patches with dominance of forbs (F-dominated), grasses (G-dominated) and legumes (L-dominated), selecting three replicates for each PFT ($n = 9$ in both sites). CO_2 exchange complete measurements (NEE and R_{eco}) were done intensively during 48 h at BERT and 24 h at CAST, resulting in 75 complete CO_2 exchange measurements in BERT and 46 at CAST.

As in the seasonal sampling, total aboveground biomass was harvested after CO_2 exchange measurements, and processed in the same way. However, to verify that the PFT dominance classification (F-dominated, G-dominated, L-dominated) given in the field was correct, the AGLB was separated into PFT (forbs, grasses and legumes) to determine the fraction of each PFT, after oven drying at 60 °C until constant weight.

Afterwards, the evenness index was calculated according to Kirwan *et al.* (2007), which has been defined as a measure of the distribution of the relative abundance of each PFT or species, and lies between 0, for mono-specific plots, and 1 when all species or PFT are equally represented (Kirwan *et al.*, 2007). A cluster analysis (Ward's method) was performed based on the PFT proportions and the evenness index, confirming the PFT dominance classification given in the field. Plots G-dominated had generally very low evenness and very high grass proportion, while F-dominated and L-dominated plots had higher values of evenness and the proportion of forbs and legumes, was not so high (Fig. S1).

Carbon dioxide exchange flux calculations

Carbon dioxide exchange measurements were carried out using a self-made non-steady state chamber, connected to an infrared gas analyser (LI-840, LI-COR, USA). Resulting CO_2 mixing ratios (ppm) were recorded at five seconds intervals by a laptop computer connected to the gas analyser (Fig. 3).

Carbon dioxide exchange measurements were performed closing the chamber during 30 s in light conditions (NEE), and shading the

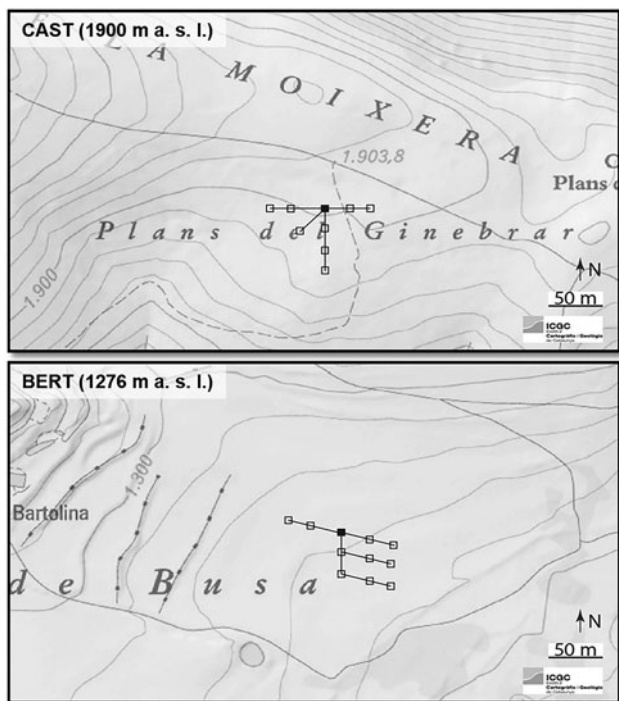


Fig. 2. Map of the study sites, Bertolina (BERT) and Castellar (CAST), and scheme of the seasonal sampling design. White blocks: sampling points, black blocks: eddy covariance stations. Every sampling day new sampling points were selected. Contour line interval 10 m.

chamber to create dark conditions (R_{eco}). GPP was estimated as the sum of both fluxes. Prior to flux calculation, mixing ratios were converted to molar densities (in mol/m^3 , termed as concentration in what follows) using the ideal gas law. Afterwards, CO_2 fluxes were calculated based on the concentration change, following the mass balance equation (Eqn (1), Altimir et al., 2002):

$$\text{CO}_2\text{flux} = q \frac{C_t - C_a}{A} + \frac{V}{A} \frac{dC}{dt} \quad (1)$$

where q is the air flow rate ($1.67 \cdot 10^{-5} \text{ m}^3/\text{s}$, which is 1 litres/min), C_a the atmospheric CO_2 concentration, C_t the CO_2 concentration inside the chamber at time t (s), V the chamber volume (0.019 m^3), A the sampling surface (0.049 m^2) and (dC/dt) the first derivative of the CO_2 concentration in relation to time ($\text{mol m}^3/\text{s}$). Fluxes from the atmosphere to the biosphere were considered negative, and from the biosphere to the atmosphere positive, according to the micrometeorological sign convention.

Finally, data quality was checked based on the flux detection limit, calculated from the standard deviation of the ambient concentration observed over the measuring time, and on linearity (R^2) of the concentration change during the chamber closure. Fluxes with an adjusted $R^2 < 0.8$ and/or below the detection limit were excluded from further analysis (Debouk et al., 2018).

In addition, the eddy covariance flux stations previously installed at each site provided 30 min averaged meteorological data used in the site description (see Study sites section) and CO_2 exchange modelling (see Data analysis section): air temperature (T_a , HMP45C, Vaisala Inc, Helsinki, Finland); volumetric soil water content at 5 cm depth (SWC, CS616, Campbell Scientific, Logan UT, USA); photosynthetically active radiation (PAR, SKP215, Skye

Instruments Ltd, Powys, UK) and normalized difference vegetation index, calculated as $\text{NDVI} = (\text{NIR} - \text{Red})/(\text{NIR} + \text{Red})$, where 'Red' and 'NIR' are the spectral reflectance measurements acquired in the red and near-infrared regions, respectively.

Data analysis

Seasonal carbon dioxide dynamics

All data analyses were performed using the R software (R core Team, 2015). To describe seasonal CO_2 dynamics, average daytime CO_2 exchange fluxes were calculated using data obtained between 8:00 and 16:30 UTM. To investigate the influence of phenology and environmental variables on CO_2 exchange fluxes in the two climatically contrasted grasslands, linear models were run with the given CO_2 flux (NEE, GPP or R_{eco}), as function of vegetation fractions (AGLB, SDB and litter) as a proxy of phenological changes, and abiotic variables (T_a , SWC, PAR), in interaction with site (Eqn (2)). The inclusion of 'site' into the model incorporated the variability due to each specific grassland do not assume by the rest of explanatory variables, such as management for instance.

$$\text{CO}_2\text{flux} = \text{Site}(\beta_{\text{AGLB}}\text{AGLB} + \beta_{\text{SDB}}\text{SDB} + \beta_{\text{Litter}}\text{Litter} + \beta_{T_a}T_a + \beta_{\text{SWC}}\text{SWC}) \quad (2)$$

Collinearity among variables was tested by the variance inflation factors (VIF) tests, using the `vif` function, `car` package (Fox and Weisberg, 2011). Collinearities between variables were found to be not relevant ($\text{VIF} < 5$, Zuur et al., 2007). Final models were selected by a stepwise procedure based on the Akaike information criterion (AIC) using the `stepAIC` function, `MASS` package (Venables and Ripley, 2002). The relative importance of each predictive variable was determined by the `calc.relimp` function, `relaimpo` package (Groemping, 2006).

Plant functional type dominance on net ecosystem exchange light response

To assess the influence of PFT dominance on NEE, the NEE *v.* PAR relationship was modelled using a logistic sigmoid light response function (Eqn (3), Moffat, 2012).

$$\text{NEE} = -2\text{GPP}_{\text{sat}} \left(-0.5 + \frac{1}{1 + e^{(-2\alpha\text{PAR}/\text{GPP}_{\text{sat}})}} \right) + R_{\text{ecoday}} \quad (3)$$

where GPP_{sat} is the asymptotic GPP, α is the apparent quantum yield, defined as the initial slope of the light-response curve and $R_{\text{eco,day}}$ the average daytime ecosystem respiration (Eqn (3)). Two variants of NEE *v.* PAR relationships were fitted: (1) using flux densities per grassland ground area (NEE, $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) and (2) using NEE normalized by AGLB (NEE_{AGLB} , $\mu\text{mol CO}_2/\text{g}/\text{s}$).

Afterwards, the PFT dominance effect was tested on light response parameters in both cases, using nonlinear mixed-effects models (Pinheiro and Bates, 2000), by the `nlme` function of the `nlme` package (Pinheiro et al., 2015). For that purpose, null models in each case ($\text{NEE} \sim \text{PAR}$, Model 1.1, and $\text{NEE}_{\text{AGLB}} \sim \text{PAR}$, Model 2.1) were performed, with site as a random factor and light response parameters (Eqn (3): α , GPP_{sat} and $R_{\text{eco,day}}$) as fixed effects. Afterwards, corresponding models with PFT dominance as covariates of the parameters, α , GPP_{sat} and $R_{\text{eco,day}}$ (Model 1.2 and Model 2.2) were also run. Null models and models

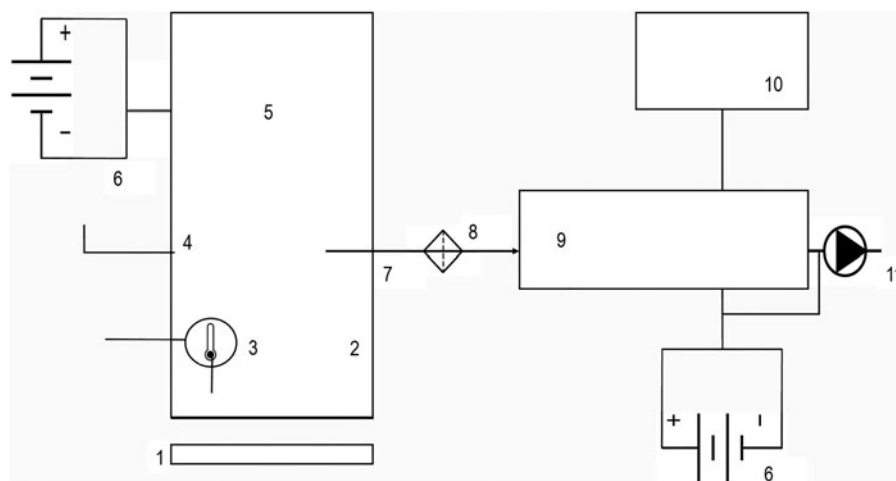


Fig. 3. Scheme of the gas-exchange measurement system set-up. (1) metal collars (height = 8 cm, inner diameter = 25 cm), hammered into the soil around 3 weeks before to let the system recover from the disturbance; (2) methacrylate chamber (height = 38.5 cm, inner diameter = 25 cm), rubber joint at its base to provide sealing at the chamber-ring junction; (3) multi-logger thermometer (TMD-56, Amprobe, USA); (4) vent to avoid under pressure inside the chamber (Davidson *et al.*, 2002); (5) fan to homogenize the air in the headspace; (6) batteries; (7) polyethylene liner with ethyl vinyl acetate shell tube (Bev a Line IV, longitude = 15.3 m, inner diameter = 3.175 mm); (8) air filter (pore size = 0.1 μm); (9) infrared gas analyser (LI-840, LI-COR, USA); (10) laptop and (11) air pump, output flow set at $1.67 \cdot 10^{-5} \text{ m}^3/\text{s}$, which is 1 l/min.

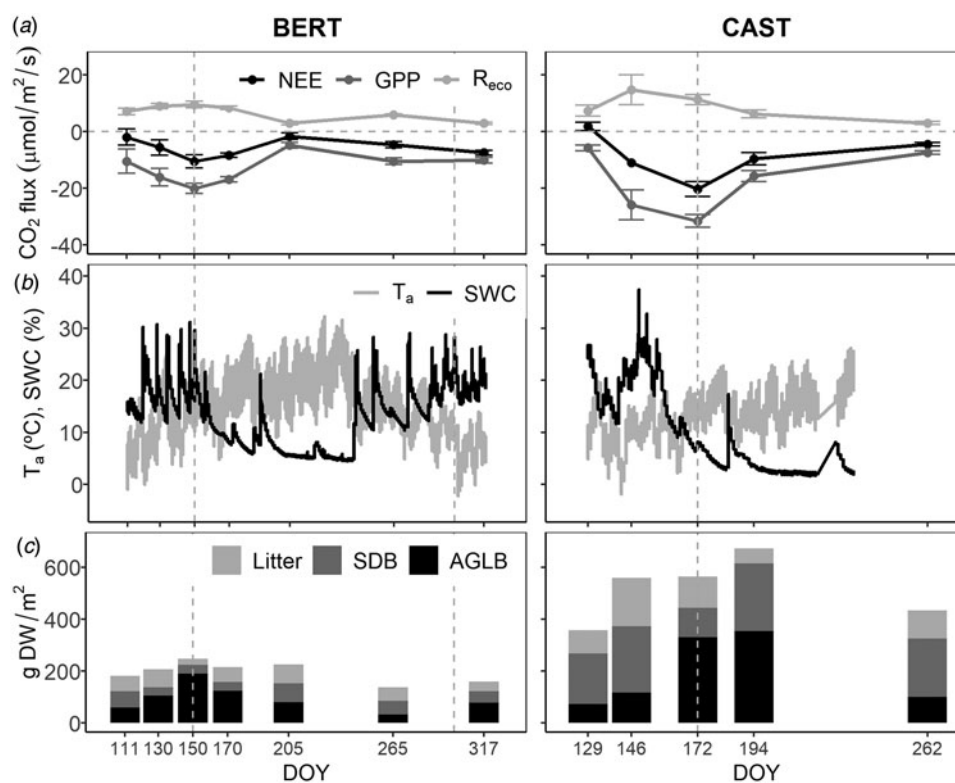


Fig. 4. Seasonal dynamics (DOY): (a) Mean daytime CO_2 exchange fluxes: net ecosystem exchange (NEE), gross primary production (GPP) and ecosystem respiration (R_{eco}) \pm standard error; (b) 30 min averaged air temperature (T_a) and volumetric soil water content (SWC) at 5 cm depth, source: eddy covariance stations. A system failure of the eddy covariance flux station at CAST caused missing meteorological data from DOY 219 up to the end of the study period and (c) mean litter, SDB and AGLB. Grey dashed vertical lines indicate the beginning and end of the grazing period.

including PFT dominance as covariates were compared by an analysis of variance (ANOVA).

Results

Seasonal carbon dioxide flux dynamics in montane and subalpine grasslands

Mean daytime NEE was mostly dominated by assimilation at both sites, ranging from -2 ± 1 to $-10 \pm 2 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ at BERT,

and from 2 ± 1 to $-20 \pm 3 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ at CAST. Mean daytime GPP showed the strongest seasonal pattern and the highest differences between sites, ranging from -5 ± 1 to $-20 \pm 2 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ at BERT and from -6 ± 1 to $-32 \pm 2 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ at CAST. Finally, mean daytime R_{eco} ranged from 3.0 ± 0.4 to $10 \pm 1 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ at BERT and from 3.1 ± 0.5 to $15 \pm 5 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ at CAST (Fig. 4(a)).

Carbon dioxide exchange seasonal patterns (Fig. 4(a)), evolved according to environmental conditions (Fig. 4(b)) and phenology (Fig. 4(c)). The modelling showed that NEE was

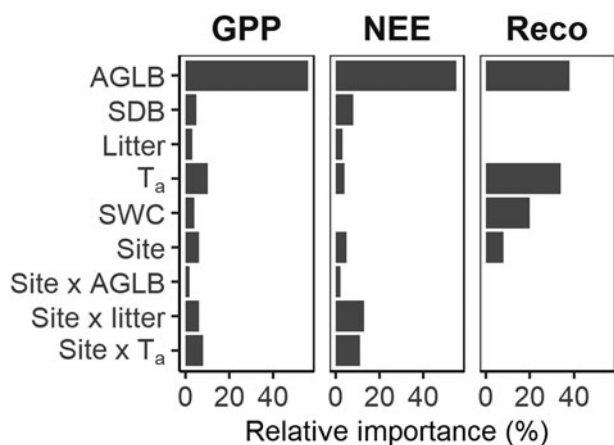


Fig. 5. Relative importance of explicative variables linear modelling (Table 1): AGLB, SDB, litter, air temperature (T_a), soil water content (SWC) and site, with BERT as the reference level. 'Site x' indicates interactions between the site and the given variable.

mainly driven by AGLB (Fig. 5), increasing net CO_2 uptake – more negative NEE – with increasing AGLB (Table 1); while net CO_2 uptake decreased with increasing SDB and litter (Table 1).

Moreover, there were some interactions between site and environmental conditions (Table 1 and Fig. 5). Net CO_2 uptake was a priori lower at CAST than at BERT (less negative NEE, site effect, Table 1), and the AGLB was proportionally taking-up CO_2 at lower rates at CAST than at BERT (site \times AGLB, Table 1). However, net CO_2 uptake increased with temperature at a higher rate at CAST than at BERT (site \times T_a effect, Table 1).

GPP behaved similarly to NEE: GPP was mainly driven by AGLB (Fig. 5), increasing the gross uptake – more negative GPP – with increasing AGLB, and decreasing the gross uptake with SDB (Table 1). Gross uptake in addition increased with increasing temperature and SWC (Table 1). GPP presented the same interactions between site, environmental variables and vegetation as NEE did (Table 1). Finally, R_{eco} was also mainly driven by AGLB (Fig. 5), increasing emissions with AGLB, followed by temperature, and SWC (Table 1).

Plant functional type dominance on net ecosystem exchange light response

CO_2 exchange fluxes recorded during the intensive diel campaign confirmed that NEE was mainly driven by PAR at a diel timescale (Fig. 6). The logistic sigmoid light response function (Eqn (3)) explained 69% of the variability, when assessing NEE per grassland ground area (Model 1.1, Table 2).

The inclusion of PFT dominance as covariates of the light response function parameters (α , GPP_{sat} and $R_{\text{eco,day}}$), was not significant when assessing NEE per grassland ground area (Model 1.2, Table 2). However, the logistic sigmoid adjustment per site and per PFT dominance suggested that there were differences between PFT when assessing the NEE per unit of AGLB (NEE_{AGLB} , Fig. 6(b)). Accordingly, when assessing the $\text{NEE}_{\text{AGLB}} \sim \text{PAR}$ relationship, there were significant differences between the null model and the model that included PFT dominance as covariate of the parameters (ANOVA Model 2.1 v. Model 2.2, Table 2), which also increased the explained variability, from 0.66 to 0.72 (R^2 Model 2.1 v. Model 2.2, Table 2). Differences among PFT in the NEE_{AGLB} were mainly driven by differences in the GPP_{sat} . G-dominated plots having significantly lower GPP_{sat} than L-dominated plots (Model 2.2, Table 2).

Table 1. Carbon dioxide (CO_2) exchange linear model results

	NEE ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)				GPP ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)				R_{eco} ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)			
	Est.	s.e.	t	P	Est.	s.e.	t	P	Est.	s.e.	t	P
Intercept	-7	3.0	-2.46	0.02	4	6.0	0.60	0.6	-10	2.4	-4.01	<0.001
AGLB	-0.05	0.010	-4.70	<0.001	-0.06	0.012	-5.39	<0.001	0.015	0.0035	4.37	<0.001
SDB	0.019	0.0090	2.16	0.03	0.02	0.010	1.88	0.06				
Litter	0.05	0.020	2.30	0.02	0.04	0.022	1.63	0.1				
T_a	0.2	0.18	0.95	0.3	-0.5	0.25	-1.92	0.06	0.6	0.11	5.76	<0.001
SWC					-36	17.3	-2.08	0.04	34	5.9	5.77	<0.001
Site	27	8.3	3.23	0.002	29	9.9	2.92	0.005	1.5	0.88	1.75	0.08
Site \times AGLB	0.03	0.016	1.83	0.07	0.04	0.018	2.01	0.05				
Site \times litter	-0.08	0.027	-3.08	0.003	-0.07	0.030	-2.43	0.02				
Site \times T_a	-1.7	0.62	-2.81	0.006	-2.1	0.71	-2.97	0.004				
R^2_{Adj}	0.53				0.6532				0.50			
DF	71				70				75			
F -Statistic	12.05				<0.001				17.53			
									<0.001			

Net ecosystem exchange (NEE), gross primary production (GPP) and ecosystem respiration (R_{eco}), as function of aboveground living biomass (AGLB), standing dead biomass (SDB), litter, air temperature (T_a), soil water content (SWC) and site, with BERT as the reference level. 'Site x' indicates interactions between site and the given variable. Estimates of the explanatory variables (Est.), standard error (s.e.), t and P value. Model adjusted R^2 , degrees of freedom (DF) and F -statistic.

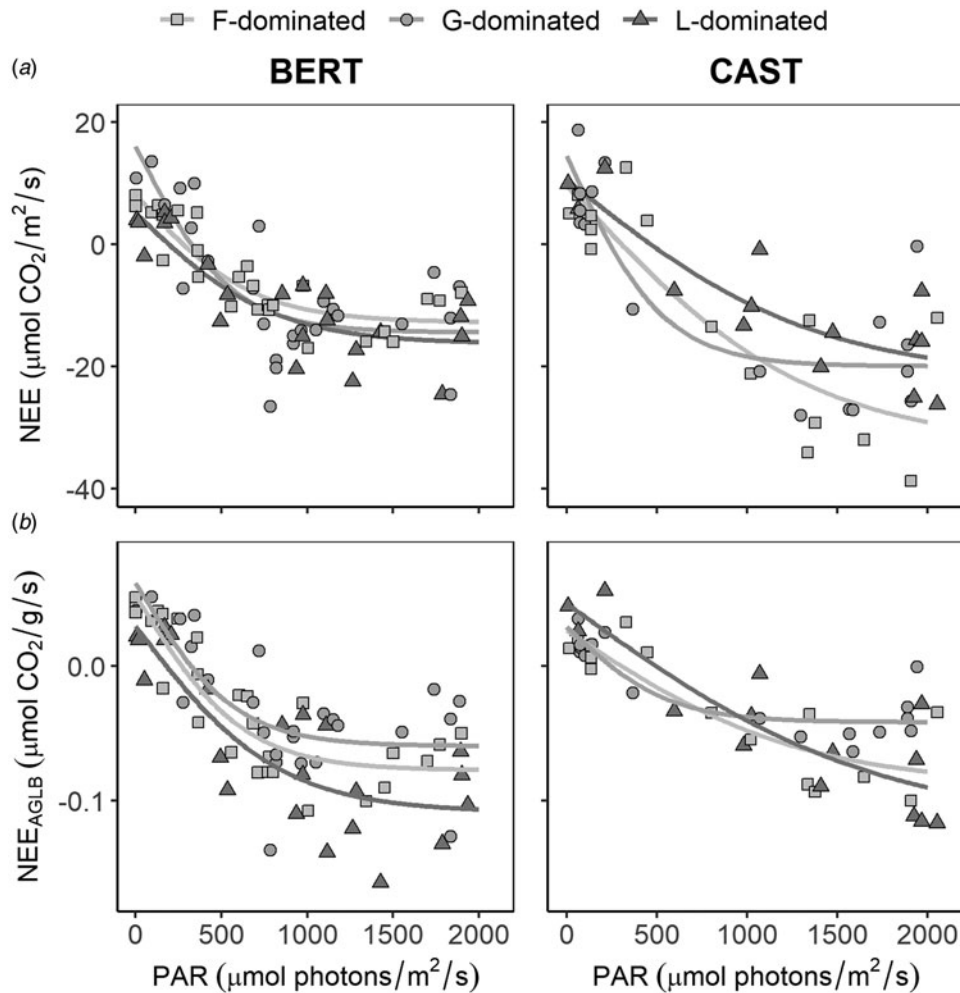


Fig. 6. Observed NEE (points) v. predicted NEE (line) by the logistic sigmoid light response function (Eqn (3)) per site and per PFT dominance –forbs dominated (F-dominated), grasses dominated (G-dominated) and legumes dominated (L-dominated) – based on (a) NEE per unit of grassland ground area ($\text{NEE } \mu\text{mol CO}_2/\text{m}^2/\text{s}$) and (b) NEE per unit of AGLB ($\text{NEE}_{\text{AGLB}} \mu\text{mol CO}_2/\text{g}/\text{s}$).

Discussion

Differential contributions of phenology and environmental variables on carbon dioxide seasonal dynamics between elevation belts

Contextualizing recorded CO_2 exchange fluxes (Fig. 4(a)), they were higher than in other seminatural grasslands in the Pyrenees previously reported (Gilmanov *et al.*, 2007, 2010; Wohlfahrt *et al.*, 2008a; Sjögersten *et al.*, 2012).

For instance, Gilmanov *et al.* (2007) reported in Alinyà, a montane grassland (1770 m a.s.l.) that might be climatically comparable to BERT, maximum daily aggregated GPP of $-25.7 \text{ g CO}_2/\text{m}^2/\text{day}$. Whereas in BERT, considering the light response function (Eqn (3)), the estimates of the parameters subtracted from the $\text{NEE}_{\text{AGLB}} \sim \text{PAR}$ relationship (Table 2, Model 2.1), and the AGLB sampled during the peak biomass ($190 \pm 21 \text{ g DW}/\text{m}^2$, DOY 150, Fig. 4(c)), maximum daily aggregated GPP can be estimated $\approx -31 \text{ g CO}_2/\text{m}^2/\text{day}$. Such difference may well be because there are important vegetation differences between both sites, with a maximum productivity at Alinyà around $131 \pm 12 \text{ g DW}/\text{m}^2$ (unpublished data), while at BERT it is roughly a 45% higher ($190 \pm 21 \text{ g DW}/\text{m}^2$), although other factors, as for

instance soil differences – soil at Alinyà is a lithic cryrendoll (Gilmanov *et al.*, 2007), while the soil at BERT is a udic calciscept – may also be influencing.

Another example is the CO_2 exchange fluxes reported by Sjögersten *et al.* (2012) in a subalpine grassland of the south-easter Pyrenees, very close to our subalpine site CAST. They reported maximum NEE values of $-0.7 \pm 0.8 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ in June, while our NEE in a similar date (DOY 172, $-20 \pm 3 \mu\text{mol CO}_2/\text{m}^2/\text{s}$, Fig. 4(a)) amply exceed this value. Such a huge difference is only realistic if it is the result of a large difference in AGLB between both grasslands, possibly in combination with different phenological development stages and grazing pressure. Sjögersten *et al.* (2012) reported in June an AGLB of $107 \pm 15 \text{ g DW}/\text{m}^2$, while in our site CAST we had $330 \pm 40 \text{ g DW}/\text{m}^2$ in late June (+210%, DOY 172, Fig. 4(c)), reaching the peak biomass around that date. Indeed, the AGLB reported by Sjögersten *et al.* (2012) in June is more similar to our value in late May (DOY 146, $116 \pm 33 \text{ g DW}/\text{m}^2$, Fig. 4(c)). These differences reveal how dynamic those grasslands are, and exemplify the need for a better understanding of CO_2 drivers in mountain ecosystems to perform accurate predictions and upscaling.

Table 2. Nonlinear mixed-effects models results, by the logistic sigmoid light response function (Eqn (3))

		Parameter	Est.	s.e.	<i>t</i>	<i>P</i>	D.F.	<i>R</i> ²
Model 1.1 NEE ~ PAR	α	Intercept	0.035	0.0060	5.83	<0.001	117	0.69
	GPP _{sat}	Intercept	28	3.5	7.92	<0.001		
	R _{eco,day}	Intercept	10	1.6	6.12	<0.001		
Model 1.2 NEE ~ PAR + PFT	α	Intercept	0.025	0.0085	3.00	0.003	111	0.68
		F-dominated	0.00	0.011	0.20	0.8		
		G-dominated	0.02	0.016	1.44	0.2		
	GPP _{sat}	Intercept	25	3.7	6.76	<0.001		
		F-dominated	6	5.6	1.03	0.3		
		G-dominated	6	4.8	1.31	0.2		
	R _{eco,day}	Intercept	7	2.8	2.45	0.02		
		F-dominated	1	3.6	0.41	0.7		
		G-dominated	7	4.1	1.73	0.1		
ANOVA model 1.1 v. 1.2						0.97		
Model 2.1 NEE _{AGLB} ~ PAR	α	Intercept	0.00013	0.000038	3.47	0.0007	117	0.66
	GPP _{sat}	Intercept	0.12	0.011	10.26	<0.001		
	R _{eco,day}	Intercept	0.037	0.0085	4.34	<0.001		
Model 2.2 NEE _{AGLB} ~ PAR + PFT	α	Intercept	0.00012	0.000041	2.97	0.004	111	0.72
		F-dominated	0.00003	0.000042	0.64	0.5		
		G-dominated	0.00002	0.000067	0.35	0.7		
	GPP _{sat}	Intercept	0.14	0.019	7.43	<0.001		
		F-dominated	-0.02	0.022	-1.10	0.3		
		G-dominated	-0.05	0.022	-2.29	0.02		
	R _{eco,day}	Intercept	0.03	0.012	2.58	0.01		
		F-dominated	0.01	0.014	0.68	0.5		
		G-dominated	0.01	0.016	0.64	0.5		
ANOVA model 2.1 v. 2.2						0.001		

Net ecosystem exchange (NEE) as a function of photosynthetically active radiation (PAR): (1) NEE ~ PAR per grassland ground area (NEE, $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) and (2) NEE normalized by living biomass (NEE_{AGLB}, $\mu\text{mol CO}_2/\text{g}/\text{s}$). Models 1.1 and 2.1 (null models), parameters as fixed effects: quantum yield (α), asymptotic gross primary production (GPP_{sat}) and daytime ecosystem respiration (R_{eco,day}). Models 1.2 and 2.2 plant functional type (PFT) dominance as covariates. PFT dominance with L-dominated as the reference level. Estimates (Est.), standard error (s.e.), *t* and *P* value. Model *R*², degrees of freedom (DF) and ANOVAs comparing models.

In line with this dynamism, the current results emphasize the role that phenology plays as an important factor influencing CO₂ exchange fluxes. The well-known effect of AGLB as a CO₂ exchange driver was clear, but the relevance of other vegetation fractions, including SDB and litter, which lowered the gross and net CO₂ uptake capacity of the ecosystem (Table 1 and Fig. 5) was important.

Moreover, there were interesting interactions between site, phenology and environmental variables. On one hand, the AGLB at the subalpine grassland, CAST, was proportionally taking-up CO₂ at lower rates than at the montane grassland BERT; resulting in proportionally lower rates of NEE per unit of AGLB (site \times AGLB effect on NEE, Table 1). This suggests that environmental conditions were more constraining in CAST than at BERT, and vegetation at CAST could proportionally photosynthesize at lower rates than at BERT. However, although CAST was probably more temperature limited, the gross and net CO₂ uptake capacity increased more markedly in CAST than at BERT as soon as temperatures increased (site \times T_a effect on NEE and GPP, Table 1).

Accordingly, some ecosystem functions, including biomass production and CO₂ exchange, in high elevation mountain grasslands have been reported to be more temperature-limited than water-limited (Sebastià, 2007), being mostly constrained to the warm months. Thus, the pronounced gross and net CO₂ uptake with vegetation development at CAST (Fig. 4), is in line with the fact that in the Mediterranean region high-elevation grasslands are generally highly productive during the summer, while montane grasslands have a longer growing season but less productive (García-González, 2008). In fact, these phenological differences describe their managing use.

On the other hand, there were important site differences in the way that SWC drove GPP and R_{eco} (Fig. 4), partly related to phenological differences between both elevations and vegetation development strategies. SWC enhanced both gross CO₂ uptake and release fluxes (Table 1), in agreement with earlier works (Law *et al.*, 2002; Flanagan and Johnson, 2005; Davidson and Janssens, 2006; Bahn *et al.*, 2008; Imer *et al.*, 2013). However, when the SWC dropped, CO₂ exchange fluxes diminished

especially at BERT, while that diminishment at CAST was not so pronounced. Hence, although the SWC during the peak-biomass was clearly lower at CAST than at BERT (Fig. 4(b), SWC below 10% indicates a dry period), the low SWC did not cause an immediate decrease of the CO₂ exchange fluxes at CAST (Fig. 4(a)).

This may well be because CAST had high SWC during the spring, which allowed the development of the vegetation once the temperature increased (Fig. 4). The well-developed AGLB was able to cope with the SWC deficit during the summer drought, and GPP and R_{eco} did not decrease at CAST as much as at BERT. This suggests that BERT was probably more water-limited than CAST, in agreement with some studies that have highlighted that summer drought effects on productivity (Gilgen and Buchmann, 2009) and CO₂ assimilation (Bollig and Feller, 2014) may be more intense at sites with lower annual precipitation, as is the case of BERT in comparison with CAST.

Accordingly, vegetation may be adopting different development strategies between sites. Plants at CAST may be taking a 'water spending strategy' (Leitinger *et al.*, 2015), meaning that some of the typical grassland species may not regulate the stomatal conductance until SWC approaches the wilting point under occasional droughts (Brilli *et al.*, 2011). However, it must be considered that long term changes in water availability would finally lead to shifts in vegetation composition towards more opportunistic species in perennial alpine and subalpine grasslands (Sebastià, 2007; Debouk *et al.*, 2015).

Also, CAST has a less stony soil, which allows the development of a more complex radicular system (mean belowground biomass in the first 20 cm at the peak biomass stage in 2012: BERT, 730 and CAST, 3158 g DW/m², unpublished data), which could be offsetting the superficial SWC deficit.

Ultimately, the inclusion of site could be acting as a proxy of the intrinsic characteristics of each altitudinal belt (montane *v.* subalpine), including information of complex interactions between biotic and abiotic variables, as well as current and past management practices (Leifeld *et al.*, 2015).

Finally, AGLB was also an important driver of R_{eco} (Table 1 and Fig. 5), indicating that CO₂ release was most likely dominated by the autotrophic than by the heterotrophic component of R_{eco}. In agreement, it has been reported that the magnitude of R_{eco} components changes considerably over the year in grassland ecosystems, and the autotrophic respiration reaches its maximum during the growing season (Gomez-Casanovas *et al.*, 2012).

Plant functional type dominance on net ecosystem exchange light response

PFT dominance influenced on the NEE light response, when accounting for NEE_{AGLB} (Model 2.2, Table 2). Grass dominated (G-dominated) plots had lower GPP_{sat} than plots dominated by legumes. This is in agreement with previous studies that have reported that legumes yield higher CO₂ exchange rates than forbs and grasses, per unit of biomass (Reich *et al.*, 2003). Such differences in CO₂ exchange rates between PFT dominance groups are most likely related to identity effects regarding the eco-physiological characteristics of each PFT. Legumes have the ability to fix atmospheric nitrogen (e.g. Reich *et al.*, 2003, 1997) and have higher leaf nitrogen content, which results in higher photosynthetic capacity and CO₂ uptake (Reich *et al.*, 1997, 1998; Lee *et al.*, 2003; Busch *et al.*, 2018). In addition, legumes have higher specific leaf area than grasses, a trait that has been related to increased photosynthesis rates (Reich *et al.*, 1998).

However, L-dominated plots tended to have lower AGLB than G-dominated and F-dominated plots (Fig. S2), and although G-dominated plots had lower GPP_{sat} resulting in lower NEE_{AGLB} than L-dominated plots (Fig. 6(b)), their higher biomass offset this difference at the grassland ground scale (Model 1.2, Table 2). In this regard, previous studies showed that different PFT have different strategies to produce and maintain their biomass and access resources (Craine *et al.*, 2002). Legumes access nitrogen to avoid nutrient limitation and produce high-nitrogen biomass, while grasses and forbs produce low-nitrogen biomass. Low-nitrogen species, especially grasses, have lower rates of physiological activity but generate dense and long-lived tissues that result in more biomass in the long term compared to high-nitrogen species, as is the case of legumes (Craine *et al.*, 2002). Moreover, symbiotic fixation of atmospheric nitrogen by legumes requires additional energy in comparison with nitrogen acquisition from the soil (Postgate, 1998; Minchin and Witty, 2005), causing more investment of photosynthates in the nitrogen fixation processes.

In addition, apart from the effects referable to the identity effects of each PFT, possible interactions between PFT must be considered. L-dominated plots had higher evenness than G-dominated plots (Fig. S1), meaning that L-dominated plots had higher functional diversity. Hence, functional diversity and PFT interactions may be producing an enhancement of the CO₂ exchange per unit of biomass in addition to the rates of each single PFT. This would be in agreement with the 'complementarity hypothesis', which postulates that trait dissimilarity among species or PFT maximizes resource use strategies and ecosystem functioning (Tilman *et al.*, 1997). Several studies have reported diversity and compositional effects, mainly due to grass-legume interactions on several ecosystem functions, including CO₂ exchange, yield and/or nitrogen availability (Nyfeler *et al.*, 2009, 2011; Finn *et al.*, 2013; Ribas *et al.*, 2015). For instance, Ribas *et al.* (2015) found the highest CO₂ respiration rates in plots dominated by legumes with a certain proportion of grasses, and a positive effect of evenness on respiration, verifying and disaggregating a coupled effect of the dominant PFT from PFT interaction (evenness) effects.

In our study case, dominance and interaction effects cannot be disentangled, but certainly PFT composition was influencing NEE_{AGLB} (Model 2.2, Table 2), via PFT-specific light response differences, in seminatural mountain grasslands.

Conclusions

Phenology plays an important role as a CO₂ exchange driver at the seasonal scale, driving differences between elevation belts (montane *v.* subalpine). Although the subalpine grassland (CAST) had a later vegetation development, CAST was clearly more productive (AGLB ~ +74%) than the montane grassland (BERT) during the peak biomass stage, and yielded higher NEE values (NEE ~ +48%). Thus, at least in mountain environments, detailed information on phenology is key to understand the *a priori* counterintuitive finding that a high-elevation grassland (CAST) is more productive than a comparable grassland at the montane elevation (BERT), with a longer growing season and warmer summer temperature. There were elevation differences in the way that environmental variables and phenology mediated CO₂ exchange fluxes. Although CAST was more temperature constrained, temperature enhanced gross and net CO₂ uptake at higher rates at CAST than at BERT. Also, both grasslands

experienced a pronounced summer dry period, which substantially reduced productivity at the lower elevation, from which only a minor recovery could be observed in autumn. However, the delayed phenology at the subalpine grassland reduced vegetation's sensitivity to summer dryness, which did not experience a reduction in CO₂ exchange, even though the low SWC.

Moreover, vegetation composition, in terms of PFT, influenced on the CO₂ exchange. Legume dominated plots presented higher NEE rates than grass dominated plots per unit of AGLB; with higher GPP_{sat} than grass dominated plots. Overall, a deeper knowledge of phenology and vegetation ecophysiological responses under different climatic conditions is key to upscale CO₂ exchange fluxes in a seasonal and inter-annual scale in semi-natural mountain grasslands.

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