

Answers to referee 1, comments on 17 January 2013.

We thank the extensive comments by referee 1 and found them very helpful to improve the manuscript. We tried to answer and incorporate almost all suggestions by reviewer 1, however we do not share the reviewer's view regarding that land use change is not a proper framework to present the differences in plant cover and plant composition of the semiarid grassland. Neither that the site named enclosure is not representing a grassland. We exposed our views in the following paragraphs and give answer to other concerns of the reviewer.

Comment. My main concern is about the selection of the sites. It seems that the enclosure does not represent the native vegetation within the area (as it is no longer grazed) and thus cannot really be used to assess land use changes on CO₂ exchange. I think this becomes evident by the fact that the enclosure features a net loss of C. If this would be a truly native grassland, then this would not be sustainable over longer terms. Thus, I do not think that the enclosure can be used as a baseline for the other four ecosystem types that have been converted in one way or the other. A solution to this problem would be to take out all reference to land use change and that the enclosure represents the native short-grass prairie and just talk about these different ecosystem types. But then the authors have to find a new justification for the story.

Regarding this comment, we agree partially with referee 1 in the sense that the enclosure site is a particular case since it does not represent original conditions of the semiarid grassland biome. Important selection factors for the semiarid grassland biome such as grazing by native ungulates and natural fire regimes are currently disrupted. Other factors such as the frequent droughts are probably exacerbated under current land use change conditions. We disagree with reviewer 1 respect to his comment that the enclosure does not represent the native vegetation. Semiarid tropical grasslands are characterized by the almost mono-dominance of the C₄ grass *Bouteloua gracilis* and few subordinated perennial C₄ grasses and C₃ annuals, in contrast to semiarid grasslands in its sub-tropical and temperate distribution, that include an important proportion of C₃ perennial grass species (Hernandez Xolocotzi 1959, Rzedowsky 1975, Aguado Santacruz et al.1998). These vegetation conditions are currently observed in the enclosure site, however semiarid grasslands are considered to require disturbance as a mechanism for self maintenance. Therefore, removal of grazing and fire, two of the most important controlling factors in semiarid grassland ecosystems, in the long term may have important effects on species composition and plant cover type (ex. brush encroachment) and subsequently in ecosystem functioning.

On the other hand, the comment by the reviewer that this site is not currently representing a native grassland because it is performing as a source of C to the atmosphere is not that clear. In this sense, previous studies of C balance in grassland vegetation have reported that grasslands potentially can perform as sources, sinks or neutral in terms of C balance

(Novick et al 2004). Therefore, to consider the net C output as an indicator of native vegetation is not supported by current observations on grasslands. In addition, we think that there was some misunderstanding with the general setting of our study. We were aware that the enclosure site is not a pristine site because of the absence of important controlling factors, so it was considered just as another site. It was not our intention to use this site as our baseline as mentioned by the reviewer. Since, the four sites we monitored in our grassland biome exhibited some degree of disturbance, we decided to contrast day and night CO₂ fluxes based on two criteria, species composition and proportion of plant cover (P17103, P6-20) as was posed in our hypotheses. We were also concerned that the misunderstanding arose from wrongly indicating within the manuscript that the enclosure was considered our baseline. We read carefully the manuscript to found whether this was the case and we could not find any mention in this sense.

Finally, land use change produce pervasive problems in natural ecosystems. Problems such as fragmentation, plant cover loss; species composition changes all have important effects on ecosystem processes. We agree that semiarid grasslands in this region are highly altered however the patchiness and vegetation that is left is consequence of historical and recent use of the land. The reviewer does not provide a convincing argument to make us reconsider that this is not a case of land use change. Even in the case of the enclosure site, the intentional removal of grazers and fire determined a particular use of that site, carrying unknown consequences in ecosystem processes. We are trying to make explicitly clear that the enclosure site does not represent a pristine semiarid grassland neither it is considered a baseline to compare the other sites. In addition we emphasized that our aim was to assess what is the effect of species identity and plant cover status resulting from land use change on day and night CO₂ fluxes and the factors that control them.

C. I was surprised that the authors only had one replicate per ecosystem type. From these results they generalize, which seems a bit weak. I know how time consuming these measurements are and that this can no longer be changed, but at least it should be acknowledged within the manuscript.

For many of the ecological studies, replication is an issue of large criticism. Whether the replicates used in particular study are either truly replicates or they correspond to pseudoreplicates. The reason that we used only one site and six replicates per site it was only matter of logistics. Although one single measurement period took from 120 to 180 seconds, to prepare the dome and instrumentation to measure required around 15 minutes so each measurement cycle needed up to 1.5 hours. In this way we could achieve four measurement campaigns per day. It would have been impossible if we had complete ecosystem as replicates, since this would have implied to load the vehicle and move to the next site. There is also the question of ecosystem replication in ecosystem gas exchange experiments. To really replicate ecosystems gas exchange, we should have recorded a large area (ex. 1 km²) from a site to really characterize its CO₂ footprint. This would be only

possible with eddy covariance technology which is available but inaccessible for most studies because of the costs. We decided instead to characterize five particular grassland sites using six sampling points on each. Thus, our experimental unit was a 12.25 m² plot located around 50 m² of the next plot. Even within a site, plots differed in the proportion of species and plant cover, still maintaining the general features of the vegetation in the site. Since each of these plots responded (CO₂ fluxes) independently to each of the controlling factors we consider these as independent replicates. We will add some comments on this design to alert readers on the characteristics of the study.

The same goes for the fact that the measurements were not conducted simultaneously within the five plots, but one after the other on five consecutive days. There might have been rain events within these 5 days that might skew the measurement taken. Also, were the light conditions the same? Always completely clear sky? Was there a difference in cloud cover between the days? If the conditions were not close to identical then I do not think that a comparison between the five systems is valid. But maybe the authors could account for the fact that conditions were not identical somehow by correcting the data. Of course only if this were true.

Instantaneous NEE measurements were carried out on consecutive days, therefore as reviewer 1 commented, photosynthetic photon flux density (*PPFD*) and air temperature (*Ta*) conditions could vary among days and sites (as shown in figure 2b,d). However, integrated *NEE* was calculated for monthly periods under an ideal *PPFD* daily cycle, in the case of *NEE_{daytime}*, and with an average *Ta* for the night cycle in the case of *NEE_{nighttime}* (as stated in section 2.3). The *PPFD* was derived from the *clear sky calculator* for the quantum sensors website (<http://clearskycalculator.com/quantumsensor.htm>). Air temperature in contrast was obtained from a weather station nearby the *Moderate grazing* site. The same *PPFD* and *Ta* curves were used for modeling *NEE_{daytime}* and *NEE_{nighttime}* in all sites. By using this procedure, we assume *NEE* is normalized among sites regarding *PPFD* and *Ta*, the main drivers for *NEE_{daytime}* and *NEE_{nighttime}*. Soil water content (*SWC*) was not normalized among sites because there existed inter-site *SWC* differences due to site-specific characteristics (e.g. physiological features of plant species, plant cover, soil structure, litter accumulation, etc.; Medina-Roldán et al., 2007). We expected other environmental variables to have negligible effects on *NEE*.

I have never worked with these large chambers the authors used, but it seems that also these chambers would heat up during the time of measurement so that the conditions might be altered during the 120s. Did the authors account for that or use some sort of a cooling devise within the chamber during the measurements?

Similar to Arnone and Obrist (2003) study, temperature increased inside the dome was around or less than 1°C min⁻¹. Measurements were carried out quickly (<3 min), avoiding excessive heating. The small temperature increase we observed had little or no effect on *NEE* estimations (e.g. derived from changes in *VPD* or in leaf stomatal conductance). Moreover, a PRT type temperature sensor (RTD-810, Omega Engineering Inc., Stamford CT) with a linearizer (OM5-IP4-N100-C, Omega Engineering Inc., Stamford CT) was

placed inside the dome and its temperature was used in Eq. 2 to calculate NEE. So, any effect of dome heating was minimized on NEE calculations.

It is not clear how many replicate measurements the authors conducted per time period. They mention that they measured at 8:00, 12:00, 16:00 and 20:00 and took a 120s reading with and without cloth. But only once? Or more often? The authors should clarify this. On page 17109, line 17 it sounds as if the measurement took 3 hours (from 20:00 to 23:00). If this is true what exactly did the authors do during the 3 hours?

We installed six plots per site and all them were measured at each sampling period (8:00, 12:00, 16:00 and 20:00). The time needed for taking measurements was 2-3 min per plot plus 10-15 min for moving the dome and instruments from one plot to another, resulting in an average of 1.5 hours for each sampling cycle. This time was duplicated when the dome cover was used. Also, measurements at night took longer than daytime measurements because fluxes were smaller and therefore they needed longer periods to monitor (1-2 min longer compared to daytime measurements). In addition, measurements at night required additional gear mostly related to illumination that had to be moved on each plot. Finally, time during and between measurement cycles was used to get additional records from soil and vegetation variables.

The authors claim that there were differences in cover and biomass, but do not present this data. I think it would be nice to see the actual data and not just general numbers for these systems as presented in Table 1. Also, it would be nice to have some sense of proportion of C3 to C4 plant species in cover as well as biomass or LAI. Please present these data in a revised version of the manuscript. This would make the results as well as the discussion much stronger.

Information is included in the following table and will be added to the final version.

site	Photosynthetic metabolism	Canopy cover (%) \pm SE	LAI (m ² /m ²) \pm SE	Standing dead biomass (kg/ha) \pm SE
Crop	C3	19.6 \pm 1.33	0.53 \pm 0.07	0.00
	C4			
Exc	C3	5.82 \pm 1.68	0.022 \pm 0.002	1500.33 \pm 116.66
	C4	81.46 \pm 3.07	0.58 \pm 0.05	
Mod	C3	5.15 \pm 1.15	0.063 \pm 0.027	2393.27 \pm 222.7
	C4	81.07 \pm 3.61	0.56 \pm 0.13	
Ovg	C3			82.72 \pm 6.58
	C4	23.23 \pm 1.3	0.17 \pm 0.02	
ShEnc	C3	16.51 \pm 1.46	0.21 \pm 0.05	146.1 \pm 25.41
	C4	9.18 \pm 3.47	0.034 \pm 0.014	

Finally, I was getting somewhat confused throughout the manuscript with NEE. It is not always clear if the author speak of daytime NEE, night time NEE or NEE balance.

Please go through the manuscript and make clear what is meant at each time one of the above is used. Also, I could not find any statistics where the authors compare NEE balance among the plots. In the discussion, page 17116, line 24 they mention that there was no contrasting rates of NEE. Where are these results?

The use of different NEE terms will be clarified throughout the manuscript. Regarding statistics, the ANOVA table presented in Table 2, shows the statistical analysis for integrated $NEE_{daytime}$ and $NEE_{nighttime}$ comparing among sites. The ANOVA table to compare NEE balance among sites is included in Table 2 and will be included in the final version. For accumulated NEE, an uncertainty analysis (ISO, 2008) was performed (error bars in Figure 4b), and no statistical differences were assumed when 95% error bars overlapped.

Table 2. Summary of ANOVA for daily $NEE_{daytime}$, $NEE_{nighttime}$, and $NEE_{balance}$ including the five sites (Crop, Exclosure, Moderate grazing, Overgrazing, and Shrub encroachment)

Source	Daytime					Nighttime					Balance				
	df	SS	MS	F	p	df	SS	MS	F	p	df	SS	MS	F	p
Model	4	2.73	0.68	0.54	0.71	4	3.46	0.87	5.41	<0.01	4	1.95	0.49	0.50	0.74
Error	45	57.06	1.27			45	7.20	0.16			45	44.14	0.98		
Total corrected	49	59.79				49	10.66				49	46.08			

Page 17109, lines 23 ff: the authors explain how they calculated annual NNE rates, but do not present these values at all. There is some mention in the text, but then the values are $g\ C\ m^{-2}\ d^{-1}$? Can the authors clarify this?

As mentioned above, the first method to calculate annual NEE rate (i-iv) provided a rate of NEE in $g\ C\ per\ day$ ($NEE_{daytime} + NEE_{nighttime}$). These rates were reported using the black color within the bars in Figure 4a. The second method consisted on building an annual cumulative NEE. This was carried out assuming invariant daily NEE rates for the whole month, so that, monthly NEE rates were estimated by multiplying daily NEE by the number of days on each month, and were all added afterwards to obtain annual NEE estimates. These estimates can be seen in Figure 4b, and we will make it clear in the text for the next version.

Page 17110, lines 20 ó Page 17111, line 2: I do not quite understand why the bootstrapping was done. Was that due to missing replicates on the individual measurements per time unit?

Eventhough flux measurements were carried out on six plots per site and cycle, observations from a day campaign produced four points per plot (i.e. the four measurement cycles, 8:00, 12:00, 16:00 and 20:00). These data was not enough to describe and model

NEE response curves to different environmental factors. To improve accuracy for curve fitting, all 24 data points from the six plots were pooled together to generate a single curve (or a single model) for each site-month. After modeling for instance, NEE as a function of either PPF or air temperature we had a single integrated NEE value (both daytime and nighttime, $\text{g C m}^{-2} \text{d}^{-1}$), however there were not associated uncertainty values. Thus, bootstrapping procedure was used to estimate uncertainty values for those integrated NEE rates.