

# Grazing alters net ecosystem C fluxes and the global warming potential of a subtropical pasture

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**Abstract.** The impact of grazing on C fluxes from pastures in subtropical and tropical regions and on the environment is uncertain, although these systems account for a substantial portion of global C storage. We investigated how cattle grazing influences net ecosystem CO<sub>2</sub> and CH<sub>4</sub> exchange in subtropical pastures using the eddy covariance technique. Measurements were made over several wet-dry seasonal cycles in a grazed pasture, and in an adjacent pasture during the first three years of grazer exclusion. Grazing increased soil wetness but did not affect soil temperature. By removing aboveground biomass, grazing decreased ecosystem respiration ( $R_{\text{eco}}$ ) and gross primary productivity (GPP). As the decrease in  $R_{\text{eco}}$  was larger than the reduction in GPP, grazing consistently increased the net CO<sub>2</sub> sink strength of subtropical pastures (55, 219 and 187 more C/m<sup>2</sup> in 2013, 2014, and 2015). Enteric ruminant fermentation and increased soil wetness due to grazers, increased total net ecosystem CH<sub>4</sub> emissions in grazed relative to ungrazed pasture (27–80%). Unlike temperate, arid, and semiarid pastures, where differences in CH<sub>4</sub> emissions between grazed and ungrazed pastures are mainly driven by enteric ruminant fermentation, our results showed that the effect of grazing on soil CH<sub>4</sub> emissions can be greater than CH<sub>4</sub> produced by cattle. Thus, our results suggest that the interactions between grazers and soil hydrology affecting soil CH<sub>4</sub> emissions play an important role in determining the environmental impacts of this management practice in a subtropical pasture. Although grazing increased total net ecosystem CH<sub>4</sub> emissions and removed aboveground biomass, it increased the net storage of C and decreased the global warming potential associated with C fluxes of pasture by increasing its net CO<sub>2</sub> sink strength.

**Key words:** cattle; CH<sub>4</sub>; flooded land; grassland; methane; net ecosystem CO<sub>2</sub> exchange; net ecosystem productivity; pasture; subtropical; tropical.

## INTRODUCTION

More than one-fourth of the global land area covered by grasslands is located in the subtropics and tropics (Friedl et al. 2010, Dixon et al. 2014). Soils in these regions store >25% of global organic C, substantially contribute (>35%) to natural sources of CH<sub>4</sub> emitted to the atmosphere, and are important sources of N<sub>2</sub>O (Bouwman 1996, Batjes and Sombroek 1997, Scurlock and Hall 1998, Ringer et al. 2014, Paudel et al. 2016). More than 78% of the land area in these regions is grazed by livestock (Asner et al. 2004, Ramankutty et al. 2008), potentially altering the exchange of CO<sub>2</sub> and CH<sub>4</sub> between grazed systems and the atmosphere. Although grazing pressure is increasing in the subtropics and tropics (Steinfeld et al. 2006) and grazed pastures are a dominant landscape of this region, our understanding of how grazing affects CO<sub>2</sub> and CH<sub>4</sub> fluxes from these systems is limited (Tanentzap and Coomes 2012, McSherry and Ritchie 2013, Gomez-Casanovas et al. 2018).

Grazers alter the global carbon (C) storage in terrestrial ecosystems and the exchange of CO<sub>2</sub> with the atmosphere

(Tanentzap and Coomes 2012). They typically reduce net primary productivity (NPP) as large herbivores forage on plant biomass (Noy-Meir 1993) although, in some cases, plant compensatory mechanisms can alleviate biomass losses (McNaughton 1983, Milchunas and Lauenroth 1993). By changing NPP, grazers alter canopy and root biomass and the distribution of roots through the soil profile (Tanentzap and Coomes 2012). These changes in ecosystem properties can affect soil hydrology and temperature (Day and Detling 1994, Bremer et al. 2009, Zhang et al. 2015). Changes in NPP derived from reduced plant biomass along with changes in soil hydrological and thermal conditions typically affect aerobic decomposition of soil organic matter (heterotrophic respiration,  $R_{\text{het}}$ ; Bond-Lamberty et al. 2004, Gomez-Casanovas et al. 2012). In addition, increased nutrient inputs from dung and urine affect  $R_{\text{het}}$  (Janssens et al. 2010, Brüggemann et al. 2011, Gomez-Casanovas et al. 2016). These grazing-induced changes in C storage and in the emission of C to the atmosphere could potentially affect the global climate system (Tanentzap and Coomes 2012, McSherry and Ritchie 2013).

Enteric ruminant fermentation from grazing animals accounts for >37% of anthropogenic CH<sub>4</sub> emissions globally (FAO 2006). The presence of ruminants has an overriding effect on total CH<sub>4</sub> emissions in temperate, arid, and semiarid ecosystems, dominating variation in soil fluxes (Howden et al. 1994, DeRamus et al. 2003, Ciais et al. 2013).

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CH<sub>4</sub> emitted from flooded ecosystems in the subtropics and tropics accounts for >35% of the natural CH<sub>4</sub> source to the atmosphere (Ringeval et al. 2014, Paudel et al. 2016). Flooded soils emit significant CH<sub>4</sub> as the activity of methanogenic bacteria increases with soil wetness (Updegraff et al. 2001, Turetsky et al. 2014, Blanc-Betes et al. 2016). In addition to soil moisture, the production and oxidation of CH<sub>4</sub> are governed by changes in soil temperature, plant productivity, and manure inputs (Whiting and Chanton 1993, Banger et al. 2012, Bridgham et al. 2013). As grazing can potentially alter these ecosystem properties, it could affect the emission of CH<sub>4</sub> from soils. Grazing could increase net CH<sub>4</sub> emissions from soils by increasing its wetness as a result of biomass removal and decreased transpiration (Bridgham et al. 2013, Turetsky et al. 2014, Shao et al. 2017). The emission of CH<sub>4</sub> from manure deposited in the ecosystem and increased N input due to urine deposition could also increase the net CH<sub>4</sub> source strength of a grazed system (Dong et al. 2006, Banger et al. 2012). However, grazing could decrease soil CH<sub>4</sub> emissions by decreasing the input of C to soil due to biomass removal and by reducing soil wetness as a result of increased evaporation as canopy cover decreases (Shao et al. 2012, 2017, Dangal et al. 2016).

In temperate, arid, and semiarid ecosystems, the effect of grazing on CH<sub>4</sub> emission from soils is in general smaller than CH<sub>4</sub> emissions by enteric ruminant fermentation (Steinfeld et al. 2006, Soussana et al. 2007, 2010, Ciais et al. 2013). However, this may not be the case in grazed grasslands and wetlands in the tropics and subtropics. Many tropical and subtropical ecosystems are temporarily or permanently flooded (Steinfeld et al. 2006) and slight changes in ecosystem properties caused by grazers could substantially affect CH<sub>4</sub> production from soils (Thornton and Herrero 2010). Although subtropical and tropical systems play a crucial role in determining the concentration of CH<sub>4</sub> in the atmosphere and a substantial portion of this region is grazed by livestock (Steinfeld et al. 2006), the effect of grazers on CH<sub>4</sub> emissions from soils under pastures remains unclear (Tanentzap and Coomes 2012, McSherry and Ritchie 2013).

The objective of this study was to determine how cattle grazing affects net ecosystem exchange of CO<sub>2</sub> (NEE) and CH<sub>4</sub> between semi-native subtropical pastures and the atmosphere using the eddy covariance (EC) technique. To determine how the interactions between season and grazing influence GHG exchange, measurements were made over several wet-dry seasonal cycles in a grazed pasture and in an adjacent pasture during the first three years of grazer exclusion. We determined the responses of CO<sub>2</sub> and CH<sub>4</sub> fluxes in these systems grazing, and whether changes in CH<sub>4</sub> emissions from soils due to grazers contributed to differences in ecosystem CH<sub>4</sub> emissions between grazed and ungrazed pastures (i.e., the effect of grazing on soil CH<sub>4</sub> emissions). We also investigated how grazing affects the global warming potential (GWP) associated with C fluxes of these ecosystems. We hypothesize that by removing aboveground biomass grazers will decrease the net CO<sub>2</sub> sink strength of subtropical pastures, especially through decreases in gross primary productivity (GPP). Net ecosystem CH<sub>4</sub> emissions will be higher in grazed than in ungrazed pastures because of enteric ruminant fermentation with changes in CH<sub>4</sub> emissions from soils due to grazers playing a secondary role.

Because grazers remove biomass, we also hypothesize that grazing will increase soil CH<sub>4</sub> emissions as a result of increased soil wetness as well as the emission of CH<sub>4</sub> from manure. By decreasing the net CO<sub>2</sub> sink strength of pasture and increasing its CH<sub>4</sub> source strength, grazers will increase the GWP of this system.

## MATERIALS AND METHODS

### Site description

This study was conducted from 2013 to 2015 at the MacArthur Agro-Ecology Research Center (MAERC), a 4290-ha commercial cattle ranch and ecological field station (27°09' N, 81°11' W; Florida, USA). This area has a subtropical climate with two distinct seasons, a wet and hot season, and a dry and cool season. The wet season goes from mid-May through mid-October, and the dry season from the end of October to the beginning of May. Mean annual precipitation (1980 to 2015) was 1,310 mm, with two-thirds of total annual precipitation falling from June to September (DayMet database; Thornton et al. 2012).

Two 16-ha plots were established in semi-native pastures. Experimental plots were rectangular (500 × 330 m), adjacent to each other, and prior to the grazing exclusion treatment, vegetation, soil, surface elevation, and climate were similar. Vegetation in the plots was a mixture of the introduced C<sub>4</sub> forage grass, bahiagrass (*Paspalum notatum* Flueggé), as well as C<sub>4</sub> native grasses (i.e., *Andropogon* spp. L., *Axonopus* spp. P. Beauv., and *Panicum* spp. Schult). These plots have never been fertilized (Swain et al. 2007, Boughton et al. 2010). Soils under both plots were loamy siliceous hyperthermic Glossarenic Endoqualfs (NRCS, 2010). Both plots contained a network of evenly distributed ditches covering 2.8% of the total area. These pastures have never been irrigated and are fed by rain and groundwater (Swain et al. 2013).

Prior to the experiment, above- and belowground productivity, soil moisture and temperature, soil organic C and bulk density, along with NEE and CH<sub>4</sub> fluxes during the wet and dry seasons between plots were similar (Appendix S1).

Before the experiment, both plots were moderately grazed by cattle (*Bos taurus* L.) at a grazing intensity of 0.625 AU/ha for over 30 yr (AU, animal unit; 1 AU is equivalent to a 1,000 lb cow with a calf; Hersom 2014, Boughton et al. 2010, Swain et al. 2013). In January 2013, one of the experimental plots was fenced (ungrazed pasture, hereafter) to exclude cattle. From 1 January 2013 to 4 March 2013 cattle were not present in the grazed plot; 77 cows were introduced into this plot on 5 March 2013, and remained there until 23 May 2013. In the grazed pasture, cattle were present from 20 September to 11 November in 2013, and from 16 February to 2 May and from 28 July to 18 December in 2014. In 2015, cattle were present in the grazed pasture from 1 April to 8 April, from 21 July to 31 October. Stocking rate was 0.4 AU/ha in 2013, 0.9 AU/ha in 2014 and 0.3 AU/ha (Appendix S1B: Table S1).

### Eddy covariance measurements

The EC towers along with several micrometeorological instruments were established at the center of each plot, and

EC measurements started on 1 July 2013. The EC technique was used to measure the exchange of CO<sub>2</sub>, latent and sensible heat, and CH<sub>4</sub> fluxes between each pasture and the atmosphere. The EC system consisted of a 3D sonic anemometer (81000 V; R. M. Young Company, Traverse City, Michigan, USA), an enclosed CO<sub>2</sub>/H<sub>2</sub>O infrared gas analyzer (LI-7200; LI-COR Biosciences, Lincoln, Nebraska, USA), and an open-path CH<sub>4</sub> analyzer (LI-7700; LI-COR Biosciences) operating at 10 Hz. The height of the EC system was changed over time to keep the sensors close to the vegetation and to minimize occasions when the flux footprint extended beyond the plot's edge. In this study, the minimum height was 2.5 m, and the instruments were always higher than 1.34 times the average plant height (Raupach, 1994).

Auxiliary instrumentation consisted of temperature (Young 43502 with 4347-L probe; R. M. Young Company) and relative humidity sensors (HMP155; Vaisala Oyj, Helsinki, Finland); a barometer (CS105; Campbell Scientific, Logan, Utah, USA); net radiometer for up- and down-welling short- and longwave radiation (CNR4; Kipp&Zonen, Delft, The Netherlands); quantum sensors for up- and down-welling photosynthetic active radiation (PAR; LI-190; LI-COR Biosciences); soil heat flux plates (three per plot; H.FP1, Hukseflux Thermal Sensors, New York, The Netherlands); a rain gauge (tipping bucket rain gauge TR-525M, metric heated; Texas Electronics, Texas, Houston, USA); soil moisture and temperature sensors (TDT SDI-12; Acclima Digital, ID, US and PR2 type profile probe, Delta T Devices, Cambridge, UK). Soil moisture sensors were placed at soil depths of 15, 30, 50, and 70 cm. Soil temperature sensors were placed at soil depths of 10, 15, and 30 cm.

In addition to continuous soil moisture and temperature measurements, these parameters were measured across each pasture with handheld soil moisture and temperature sensors using a HydroSense II attached to a CS658 probe with 20-cm long rods (Campbell Scientific) and a HH-23 Handheld Thermometer attached to a 20-cm K type probe (Omega Engineering, Norwalk, Connecticut, USA). These point measurements were collected at four locations within each pasture every month, and each value was an average of four measurements. Auxiliary measurements were compiled at 30-min intervals and logged to a CR3000 datalogger (Campbell Scientific) synchronized to the Li-7200.

High frequency (10 Hz) CO<sub>2</sub>, H<sub>2</sub>O, and CH<sub>4</sub> data from the EC systems were processed with EddyPro v5.1.1 (LI-COR Biosciences). Fluxes were computed as the covariance of vertical wind velocity and gas concentration over 30-min intervals. A double rotation scheme was used to align the coordinate system to the main wind direction (Wilczak et al. 2001). Cross-wind correction of sonic temperature was implemented by the firmware (81000 V; R. M. Young Company). Lagged covariances between vertical wind velocity and each flux scalar were computed and applied to account for lag times between the sonic anemometer and the flux sensors. Humidity corrections were applied to temperature as well as related fluxes that include sonic temperature (van Dijk et al. 2004). The Webb-Pearman-Leuning density correction and corrections for spectroscopic effects were applied to CH<sub>4</sub> data (Webb et al. 1980). Spectral corrections for high-pass and low-pass filtering were implemented using analytical cospectra formulations as in Moncrieff et al.

(1997, 2004). Spikes in raw flux data were eliminated as in Vickers and Mahrt (1997). The quality of each half-hour average was determined following the 1–9 method in Foken et al. (2004) and low quality data was eliminated (flags >7).

We used several screening criteria to exclude non-representative flux measurements. Diagnostic values for each half-hour scalar flux associated with poor performance of the instrument (e.g., poor laser signal strength for CH<sub>4</sub>; Dengel et al. 2011) were used to filter out poor quality data. We also checked the data for values that were outside a reasonable range. Fluxes of CO<sub>2</sub> were discarded when they were <−70 or >30 μmol·m<sup>−2</sup>·s<sup>−1</sup>, and when the standard deviation of the CO<sub>2</sub> concentration exceeded the mean ± 3.5 standard deviation of a moving window of 200 records following Zeri et al. (2011). For CH<sub>4</sub>, we excluded data following the screening criteria in Baldocchi et al. (2012). Fluxes were discarded when CH<sub>4</sub> concentration was below ambient concentration (<1,740 ppb), when CH<sub>4</sub> fluxes were extreme (>1,500 nmol·m<sup>−2</sup>·s<sup>−1</sup>), and when the standard deviation of the CH<sub>4</sub> concentration exceeded 35 ppb following the criteria in Baldocchi et al. (2012). For latent heat, fluxes below −20 or above 600 W/m<sup>2</sup> were discarded (Zeri et al. 2011). Fluxes of CO<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>O that corresponded to an area outside the edges of each plot were also eliminated using Hsieh cross-wind-integrated flux footprint model (Hsieh et al. 2000). The model was used to calculate the distance where the half-hour fluxes reached 70% of the total. Data were discarded when the distance exceeded the plot's edges. Half-hour CO<sub>2</sub> and CH<sub>4</sub> fluxes were filtered by *u*\* threshold. In this study, the threshold for *u*\* for CO<sub>2</sub> and CH<sub>4</sub> fluxes was 0.1 m/s, consistent with values observed in grasslands (Baldocchi 2003, Zeri et al. 2011). Fluxes of CO<sub>2</sub> and CH<sub>4</sub> with *u*\* below or at 0.1 m/s were excluded. After applying these quality filters, the estimated footprint area was 101,736 m<sup>2</sup> on average for both pastures and all years of study.

Overall, 37–51%, 43–54% and 38–51% of half-hour CO<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>O flux data, respectively, were removed. Missing half-hour NEE data were gap filled and fluxes were partitioned into ecosystem respiration (*R*<sub>eco</sub>) and gross primary production (GPP) using the eddy covariance gap-filling and flux partitioning online tool (Reichstein et al. 2005; tool *available online*).<sup>8</sup> Half-hour CH<sub>4</sub> and H<sub>2</sub>O gaps of <2.5 h were filled using a linear interpolation, and gaps of >2.5 h were filled using the mean diurnal variation method (Dengel et al. 2011, Chamberlain et al. 2015). Missing CH<sub>4</sub> and H<sub>2</sub>O values for any specific half-hour were replaced by the mean of that specific half-hour of four adjacent days (Dengel et al. 2011). Preliminary trials showed that using this method but increasing the time window for adjacent days up to one month did not affect cumulative annual CH<sub>4</sub> and H<sub>2</sub>O sums when climatic conditions remained similar. When cattle were present in the grazed pasture, half-hour CH<sub>4</sub> missing values were filled using values from adjacent days with cattle present. When cows were not present in the grazed pasture, half-hour CH<sub>4</sub> missing values were filled using values from adjacent days without cattle.

Uncertainty in half-hour CO<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>O fluxes generated by random measurement error was estimated as in Finkelstein and Sims (2001). Gap-filling uncertainty was

<sup>8</sup> <http://www.bgc-jena.mpg.de/~MDIwork/eddyproc/index.php>

calculated by adding noise and random artificial gaps to the original data and applying the gap filling algorithm (Reichstein et al. 2005, Zeri et al. 2011). Total uncertainty was estimated by error propagation as in Richardson and Hollinger (2007).

Surface energy balance closure was calculated as in Flanagan et al. (2002). Surface energy measurements at both plots showed that the sum of sensible and latent heat flux was highly correlated ( $r^2 > 0.8$ ) to the sum of net radiation and soil heat flux for all years and experimental plots. The slopes ranged from 0.70 to 0.73 in grazed pasture, and 0.85–0.91 in ungrazed pasture. In 2013, 2014, and 2015, the intercepts of the relationships were  $-25$ ,  $-19$ , and  $-25$   $\text{W/m}^2$  for grazed pasture and  $-9$ ,  $-15$ , and  $-13$   $\text{W/m}^2$  for ungrazed pasture. These values were well within the range reported by most flux sites including grassland ecosystems (Wilson et al. 2002).

The eddy covariance technique has been widely used to measure  $\text{CH}_4$  fluxes from ecosystems around the world including subtropical pastures and wetlands (Dengel et al. 2011, Baldocchi et al. 2012, Chamberlain et al. 2015, 2017, Shoemaker et al. 2015, Kim et al. 2016). In a subtropical grazed pasture near our study site, Chamberlain et al. (2015) showed that the eddy covariance and soil chamber methods yielded similar annual  $\text{CH}_4$  flux estimates. The authors used gap-filling techniques similar to those used here. This implies that our approach is in agreement with others, and that uncertainties on annual scalar estimation associated with the eddy covariance technique are minimal.

#### *Soil and heterotrophic respiration*

In each experimental plot, we measured soil respiration ( $R_{\text{soil}}$ ) in 12  $3\text{-m}^2$  subplots, and  $R_{\text{het}}$  in one-half of these subplots. Measurements were performed at monthly intervals in 2014 between 09:00 and 16:00 over two to three days over the paddocks (e.g., grass patches between ditches). Soil  $\text{CO}_2$  fluxes were measured with an infrared gas analyzer (LI-6400; LI-COR Biosciences) connected to a  $R_{\text{soil}}$  chamber (LI6400-09; LI-COR Biosciences).  $R_{\text{soil}}$  was measured from PVC collars installed to 10 cm depth and  $R_{\text{het}}$  from PVC collars installed to 50-cm depth using the root exclusion collar method (Hanson et al. 2000, Anderson-Teixeira et al. 2013). The deep root exclusion collars were installed 5 months before taking measurements to minimize the influence of old roots in our measurements (Zhou et al. 2007). The shallow collars were installed next to each exclusion collar two months prior to measurements. Measurements of  $\text{CO}_2$  efflux from the exclusions collars represent  $R_{\text{het}}$ , whereas measurements from the shallow collars are derived from plant roots plus soil microorganisms. Root respiration (autotrophic respiration;  $R_{\text{auto}}$ ) was derived by subtracting  $R_{\text{het}}$  from  $R_{\text{soil}}$  from adjacent collars (Anderson-Teixeira et al. 2013). The deep root exclusion collars may have deprived soil microorganisms from substrate (e.g., root exudates), and caused an underestimate of soil microbial respiration rates (Gomez-Casanovas et al. 2012).

#### *Chamber-based $\text{CH}_4$ measurements*

To determine the source of  $\text{CH}_4$  emissions from soils and whether the source was affected by grazing, discrete measurements of  $\text{CH}_4$  flux over the paddocks and ditches were

taken four times during the wet season in 2014. These measurements were not used to obtain annual  $\text{CH}_4$  emission from soil in grazed pasture. At each grassland site, measurements were taken at 12  $3\text{-m}^2$  subplots between 09:00 and 16:00 over two to three days. To minimize potentially confounding effects from daily variability in soil GHG fluxes, measurements at each plot of each land use were randomly alternated for each sampling period. Fluxes were measured with an open path  $\text{CH}_4$  sensor (LI-7700; LI-COR Biosciences) connected to a  $1\text{-m}^3$  static chamber with an aluminum frame supporting transparent Lexan and Propafilm C (ICI Americas, Chicago, Illinois, USA). The chamber, similar to the chamber used by Prater et al. (2006), had a 4-cm aluminum bottom edge to penetrate the soil and form a seal to eliminate leaks. The chamber was equipped with three fans (12V DC) to mix the air inside the chamber, and a lateral lid to allow for ventilation between measurements.

Data were collected at 5 Hz. Measurements were completed in less than 1.5 min. The temperature inside the chamber was not controlled and typically increased by  $<1^\circ\text{C}$  per min. The concentration of  $\text{CH}_4$  inside the chamber increased linearly with time after an initial mixing period (20 s for paddock, 10 s for ditches). The initial mixing period was not included in flux calculations (average of 7% of total measurement period). Initial  $\text{CH}_4$  concentration was typically approximately ambient after the chamber was placed over the canopy. Fluxes of  $\text{CH}_4$  were calculated from the linear increase in  $\text{CH}_4$  concentration over time within the chamber as in Wang et al. (2013). Fluxes with  $r^2 < 0.8$  were discarded (5% of fluxes).

#### *Biomass measurements*

Aboveground standing and litter biomass were collected from eight  $3\text{-m}^2$  subplots in each plot at monthly intervals. Root biomass was collected from six  $3\text{-m}^2$  subplots. Aboveground and litter biomass was collected from  $0.25\text{-m}^2$  quadrants. Standing vegetation was separated into dead and live biomass. Annual fine-root ( $<2$  mm diameter) productivity was estimated using the root-ingrowth-core method (Scurlock et al. 2002). In January 2014, six soil cores (55-cm depth) were retrieved from each grassland site. Root-free soil was then used to fill ingrowth bags (7.5 cm diameter  $\times$  55 cm deep; 2.5 mm mesh) restoring the original location and bulk density of soil in the bags. A year later, the ingrowth bags were retrieved and roots were separated from soil, rinsed with deionized water, and oven dried at  $60^\circ\text{C}$  until reaching constant mass.

#### *Consumed biomass*

Biomass consumed by cattle was estimated using the moveable-cage method (McNaughton 1983). Cattle consumed biomass evenly throughout the pasture and therefore did not favor any wind direction in the tower footprint (data not shown). Eight  $0.25\text{-m}^2$  cages were distributed evenly throughout the grazed plot and were moved every month during the wet and dry seasons. Before each cage was moved, paired  $0.25\text{-m}^2$  plots were clipped within and adjacent to the cage. Aboveground biomass consumed was estimated as the difference in plant biomass within (ungrazed

for a month) and outside (grazed for a month) the cages when biomass between them was different ( $t$  test,  $P < 0.1$ ). In this study, biomass within and outside the cages was different in 14 out of 16 samplings. For samplings in which biomass between them was similar, we calculated consumed biomass by averaging consumed biomass between the previous and adjacent samplings. When cattle were not present in the grazed pasture, no differences in biomass within and outside the cage were found ( $t$  test,  $P < 0.1$ ; data not shown). This indicates that when differences were detected, they were caused by grazing and not cage artifacts. Biomass was oven dried at 60°C until reaching constant mass. Carbon (C) concentrations were measured with a flash combustion chromatographic separation elemental analyzer (Costech 4010 CHNSO Analyzer; Costech Analytical Technologies, Valencia, California, USA).

#### *Partitioning CH<sub>4</sub> fluxes measured by EC method*

When cattle were present, methane fluxes in the grazed pasture were derived from enteric ruminant fermentation and from soils (i.e., paddock and ditches). Because partitioning the sources of CH<sub>4</sub> is challenging and each method is based on different assumptions, we used three approaches to further constrain our estimates of the sources of CH<sub>4</sub> (Dong et al. 2006; Appendix S1). Two approaches were based on the IPCC Tier 2 methodology. We used IPCC Tier 2 because we had feed intake and livestock population data, which could help provide more accurate estimates of CH<sub>4</sub> emissions from enteric ruminant fermentation (Dong et al. 2006). In the first Tier 2 approach, the cumulative CH<sub>4</sub> emission from enteric ruminant fermentation was calculated from the amount of aboveground biomass consumed and estimates of energy density of the forage (Dong et al. 2006, Appendix S1). In the second IPCC Tier 2 approach, CH<sub>4</sub> emissions from cattle were calculated from estimated energy requirements for metabolic activity of grazers (Dong et al. 2006, Chamberlain et al. 2015, Appendix S1). CH<sub>4</sub> emissions by enteric ruminant fermentation were subtracted from CH<sub>4</sub> fluxes measured with the EC method over the same time period to estimate the flux from soil.

In the third approach, CH<sub>4</sub> from enteric fermentation was estimated as the difference between the soil flux and the total flux. Both soil flux and total flux were measured by the EC method. The relationship between soil moisture and temperature and CH<sub>4</sub> fluxes measured by EC when cattle were not present in the grazed pasture was used to estimate CH<sub>4</sub> fluxes from soil when cattle were present in the pasture (Appendix S1). A regression model was developed for each year from the half-hour records of soil moisture, soil temperature, and CH<sub>4</sub> fluxes measured by EC when cattle were not present in the grazed pasture. This method assumed that these abiotic variables were similar in the paddock and in the ditches. In our study, this assumption was reasonable as soil temperature and moisture were similar in the paddock and the ditches for most of the duration of this experiment (data not shown).

In our study, CH<sub>4</sub> emissions from soil in the grazed system also include the emission of CH<sub>4</sub> from manure deposited on the pasture. To estimate the contribution of manure deposition to differences in soil CH<sub>4</sub> fluxes between grazed

and ungrazed pasture, we calculated CH<sub>4</sub> emissions from manure deposition using the IPCC Tier 2 method (Appendix S1).

#### *Cumulative CO<sub>2</sub> and CH<sub>4</sub> fluxes, and statistics*

Cumulative fluxes of each scalar were calculated for each calendar year, to make annual GHG budgets easily comparable with other pastures, as budgets in these systems are usually reported using calendar year (Gilmanov et al. 2010). Because our plots were adjacent to each other and prior to the experiment key ecosystem attributes (soil moisture and temperature, ecosystem productivity, soil organic C and bulk density, NEE and CH<sub>4</sub> fluxes; Appendix S1) were similar between plots, we assume that differences between plots represent the effect of grazing treatment. Cumulative CO<sub>2</sub> fluxes in grazed pasture included animal respiration. Differences in discrete soil CO<sub>2</sub> fluxes, and above- and below-ground biomass between the grazed and ungrazed pastures were tested with complete block repeated measures ANOVA with grazing treatment and sampling period as fixed factors. Differences in discrete CH<sub>4</sub> fluxes from paddocks and ditches between grazed and ungrazed pastures were tested with complete block repeated measures ANOVA with land use and paddock/ditch as fixed factors. The relationship between soil temperature and moisture and CH<sub>4</sub> fluxes was evaluated using a general linear model. Tests were run after transforming the data to ensure normality and homogeneity of variances (Schroeder et al. 1986). All statistical tests were conducted using Matlab (The MathWorks, Inc., Natick, MA, USA).

#### *Net ecosystem C balance and GWP of subtropical grazed and ungrazed pastures*

Net Ecosystem C balance (NECB) represents the net storage or loss of C from an ecosystem. In our study, cumulative CO<sub>2</sub> and CH<sub>4</sub> measured by EC included both respiration and CH<sub>4</sub> emissions from cattle. Therefore, NECB was estimated as the sum of NEE, the C consumed by grazers that was not lost to the atmosphere by either animal respiration or enteric ruminant fermentation, and total ecosystem CH<sub>4</sub> fluxes. NECB was calculated as follows:

$$\text{NECB} = \text{Cumulative C-CO}_2 + \text{Cumulative C-CH}_4 + C_{\text{consumed not lost}}$$

where cumulative C-CO<sub>2</sub> and C-CH<sub>4</sub> is CO<sub>2</sub> and CH<sub>4</sub> measured by EC, respectively;  $C_{\text{consumed not lost}}$  is the C that was consumed by grazers but that was not lost to the atmosphere by either animal respiration or enteric ruminant fermentation.  $C_{\text{consumed not lost}}$  was calculated assuming that 65% of the C consumed by cattle is emitted back to the atmosphere as CO<sub>2</sub> (Thornley 1998, Soussana et al. 2007), and that 6% of the C consumed by cattle is emitted to the atmosphere as CH<sub>4</sub> (Johnson and Johnson 1995). Thus,  $C_{\text{consumed not lost}}$  was 29% of total C consumed by grazers.

Organic and inorganic C leached from pasture typically is small (~25 g C·m<sup>-2</sup>·yr<sup>-1</sup>; Kindler et al. 2011) and was not included in the calculation of NECB. It is likely that grazing did not alter total C leached from pasture, as changes in C

leaching due to grazers occur at time spans longer than considered in this study (Tanentzap and Coomes 2012).

To calculate the GWP, GHG fluxes from each pasture were expressed as CO<sub>2</sub> equivalents (Bridgham et al. 2013, Neubauer and Megegnal 2015). For a 100-yr time horizon, the GWP for CH<sub>4</sub> fluxes was 28 (Myhre et al. 2013). This calculation does not include N<sub>2</sub>O fluxes and therefore refers to the GWP associated with C fluxes.

## RESULTS

Air temperature was highest during each wet season (May–October), declining during the dry season, and was similar among years (Fig. 1). Precipitation varied among years, particularly during the wet season (Fig. 1). Precipitation was greater during the wet season in 2013 than in 2014 and 2015. The dry season was slightly wetter in 2014–2015 than in 2013–2014. At a monthly scale, precipitation was higher in May and June of 2013 (cumulative precipitation of 509 mm), than in May and June of both 2014 and 2015 (cumulative precipitation of 227 and 265 mm, respectively; Fig. 1).

Grazing increased soil wetness but did not affect soil temperature (Fig. 2). Soil wetness at 15-cm depth under the EC tower (Fig. 2a), as well as spatially distributed discrete measurements at 0–20 cm depth (repeated ANOVA,  $P < 0.05$ ; Fig. 2b) was higher in the grazed than in the ungrazed pasture especially during the wet season. During the wet season, the difference in soil moisture between the grazed and the ungrazed pastures was slightly larger in 2013 and 2015 than in 2014 ( $41.6 \pm 0.1$  [mean  $\pm$  standard error] and  $35.1 \pm 0.2$  for grazed and ungrazed pastures in 2013;  $37.0 \pm 0.3$  and  $33.8 \pm 0.4$  for grazed and ungrazed pastures in 2014;  $37.7 \pm 0.2$  and  $32.6 \pm 0.5$  for grazed and ungrazed pastures in 2015; and 16% in 2013, 9% in 2014, and 13% in 2015 higher in grazed than in ungrazed pasture). At the onset of

the wet season (May and June) and for both pastures, soils were wetter in 2013, than in 2014 and 2015 (Fig. 2). Soil moisture at 30 cm depth was slightly higher at the grazed than at the ungrazed pasture, particularly during the wet season (Appendix S2: Fig S1).

The biomass consumed by cattle was greater in 2014 than in 2013 or 2015 ( $182 \pm 37$  g dry mass/m<sup>2</sup> in 2013,  $563 \pm 52$  g dry mass/m<sup>2</sup> in 2014, and  $171 \pm 51$  g dry mass/m<sup>2</sup> in 2015; Appendix S1: Table S1). Grazing intensity was higher in 2014 than in 2013 and 2015, and it was slightly higher in 2013 than in 2015 (Appendix S1).

Grazing consistently decreased green aboveground and dead standing biomass and litter over the course of the study (Appendix S2: Fig. S2 & Table S1). Average green biomass in the ungrazed plot over the course of the experiment was  $460 \pm 143$  g dry mass/m<sup>2</sup>, 3.5-fold higher than in the grazed pasture (Appendix S2: Fig. S2). Grazing reduced standing dead biomass and reductions were larger in 2015 (6.9-fold) than in 2013 (2.9-fold) and 2014 (2.5-fold; Appendix S2: Table S1). Average litter biomass in the ungrazed plots over the course of the experiment was  $683 \pm 151$  g dry mass/m<sup>2</sup> (Appendix S2). Grazing decreased litter biomass, and decreases were larger in 2015 (6.6-fold) than in 2013 (3.8-fold) and 2014 (4.3-fold; Appendix S2, Table S1).

Grazing decreased root biomass (2014) in the top 10 cm by 34%, but caused an increase in biomass (13%) below 10 cm (Appendix S2, Table S2). As most root biomass was in the top 10 cm, the overall effect of grazing was 20% reduction in root biomass.

There were strong diurnal and seasonal patterns to NEE (Fig. 3). Pastures transitioned from strong net CO<sub>2</sub> sources at 07:00–08:00 to sinks of CO<sub>2</sub> with increasing solar radiation and reverted back to sources at sunset. Seasonally, NEE was most negative during the wet season and less negative during the dry season. During the wet season, maximum net CO<sub>2</sub> assimilation rates ranged from  $-3.2$  to

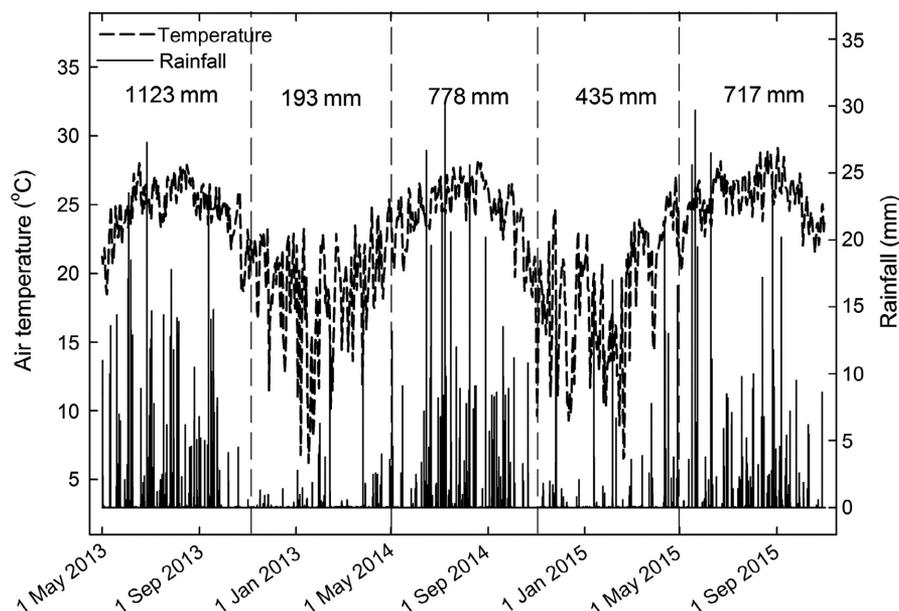


FIG. 1. Daily air temperature and precipitation in 2013, 2014, and 2015. For each year, the vertical dashed lines separate the wet (from May through October) and the dry (from November through April) seasons. Inset values are cumulative precipitation during each season.

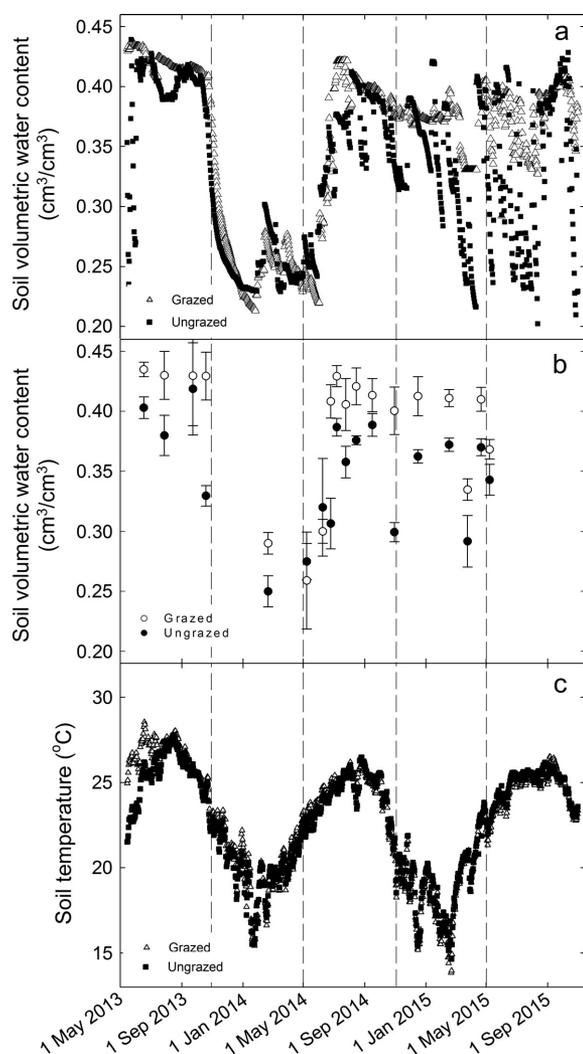


FIG. 2. (A and b) Soil moisture and (c) soil temperature in grazed and ungrazed pastures in 2013, 2014, and 2015. Soil moisture was measured at half-hour intervals (a), and at discrete sampling periods (b). Continuous and discrete soil moisture was measured at 15 cm depth and at 0–20 cm depth, respectively. For each year, dashed lines separate the wet (from May through October) and the dry (from November through April) season.

–4.6 g C·m<sup>-2</sup>·d<sup>-1</sup> in grazed pasture, and from –3.5 to –5.5 g C·m<sup>-2</sup>·d<sup>-1</sup> in ungrazed pasture and were in general lower in grazed than in ungrazed pasture (Fig. 3).

Despite lower net CO<sub>2</sub> assimilation rates during the wet season, grazing consistently increased the net CO<sub>2</sub> sink strength of subtropical pastures (Figs. 3, 4a–c), and over the course of this study, the grazed pasture was a net sink of CO<sub>2</sub> (Fig. 4a). The ungrazed system was CO<sub>2</sub> neutral in 2013 and 2015, and a net CO<sub>2</sub> source in 2014 (Fig. 4). At an annual scale, the grazed pasture accumulated 55, 219, and 187 more g C/m<sup>2</sup> than the ungrazed system in 2013, 2014, and 2015, respectively (Fig. 4).

Grazing decreased GPP by 30% and 8% in 2013 and 2014, respectively (Fig. 4d–f), and it had no effect on productivity in 2015. Over the same time periods, cumulative GPP was lower in 2015 for both grazed and ungrazed pastures compared to 2013 and 2014. For both grazed and ungrazed

pastures, cumulative GPP was larger during the wet season than during the dry season.

Grazing consistently decreased the major elements of ecosystem respiration:  $R_{\text{eco}}$ ,  $R_{\text{soil}}$ , and  $R_{\text{het}}$  (Fig. 4g–i; Appendix S2: Table S3). Decreases in  $R_{\text{eco}}$  caused by grazing were larger in 2013 and 2014 than in 2015 (Fig. 4g–i). For both grazed and ungrazed pastures, cumulative  $R_{\text{eco}}$  was larger during the wet season than during the dry season. In 2014, grazing decreased  $R_{\text{soil}}$  (36%),  $R_{\text{auto}}$  (38%), and  $R_{\text{het}}$  (41%; repeated-measures ANOVA,  $P < 0.05$ ; Appendix S2: Table S3). Decreases in  $R_{\text{soil}}$  and  $R_{\text{het}}$  caused by grazing were consistent during the wet and the dry season. The contribution of  $R_{\text{het}}$  to  $R_{\text{soil}}$  was similar in ungrazed and grazed pastures (44% in grazed pasture and 45% in ungrazed pasture; Appendix S2: Table S3). For both grazed and ungrazed pastures,  $R_{\text{eco}}$  was correlated with GPP (Appendix S2: Table S4).

Grazing slightly increased evapotranspiration in 2013 and 2014, and ecosystem water flux was larger in grazed pasture than ungrazed during the wet season in 2015 and at the annual scale (Appendix S2: Table S5). For both grazed and ungrazed pastures, cumulative evapotranspiration was larger during the wet season than the dry season (Appendix S2: Table S5).

Unlike NEE flux, CH<sub>4</sub> flux did not show substantial diurnal variation; rates tended to be somewhat greater during the day than during the night, but varied little throughout the day (Fig. 5). For both grazed and ungrazed pastures, net CH<sub>4</sub> emissions were larger during the wet season than during the dry season (Fig. 5).

Over the course of this study (i.e., 2013, 2014, and 2015) and during the wet season for each specific year, net CH<sub>4</sub> flux was correlated with soil moisture (Appendix S2, Fig. S3a). For the three years studied, correlations were slightly higher in grazed than ungrazed pasture ( $R^2$  of 0.38 and 0.25 in grazed and ungrazed pasture, respectively). During the wet season, net CH<sub>4</sub> flux was correlated with soil moisture (Appendix S2: Fig. S3). For both grazed and ungrazed pastures, correlations were higher in 2013 compared to 2014 and 2015 ( $R^2$  of 0.74 and 0.87 for grazed and ungrazed pastures in 2013, 0.35 and 0.27 for grazed and ungrazed pastures in 2014, and 0.20 and 0.15 for grazed and ungrazed pastures in 2015).

Grazing consistently increased net ecosystem CH<sub>4</sub> emissions measured by EC, and both grazed and ungrazed pastures were net sources of CH<sub>4</sub> to the atmosphere (Figs. 5, 6a–c). At an annual scale, CH<sub>4</sub> fluxes were 27%, 47%, and 80% larger in the grazed than in the ungrazed pasture in 2013, 2014, and 2015, respectively (Fig. 6). Total annual CH<sub>4</sub> emission from ungrazed pasture was 2.9–3.3 g C-CH<sub>4</sub>/m<sup>2</sup> lower than grazed pasture (Table 2). At a daily scale, and especially during the wet season, CH<sub>4</sub> fluxes were higher in the grazed pasture than in the ungrazed pasture even when cattle were not present at the grazed pasture (e.g., from 1 July 2013 to 19 September 2013; Figs. 5, 6). Compared over the same time periods, cumulative CH<sub>4</sub> fluxes were larger for 2013 than for 2014 and 2015, and they were larger for 2014 than 2015 (Fig. 6).

Point measurements made during the wet season of 2014 confirmed that grazed pasture was a stronger source of CH<sub>4</sub> to the atmosphere than ungrazed pasture ( $P = 0.06$ ; Table 1), and further demonstrated that soil was an

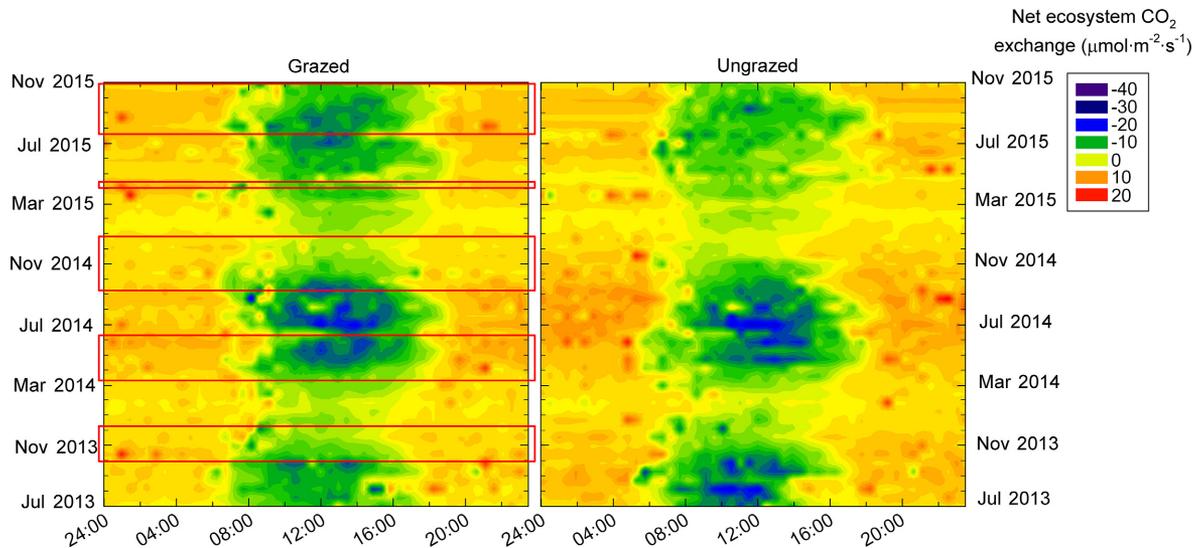


FIG. 3. Gap-filled half-hourly net ecosystem exchange for grazed and ungrazed pastures for 2013, 2014, and 2015. Red rectangles reflect periods of time when cattle were in the eddy covariance (EC) footprint of the grazed pasture. Negative values indicate that the ecosystem is a net sink of  $\text{CO}_2$ , and positive values indicate that it is a net source of  $\text{CO}_2$ . Values shown at local time.

important source of this  $\text{CH}_4$ . Average total  $\text{CH}_4$  flux (i.e., paddocks and ditches) was  $43.7 \pm 9$  and  $26.6 \pm 6$   $\text{mg C-CH}_4\text{-m}^{-2}\text{-d}^{-1}$  in grazed and ungrazed pastures, respectively (Table 1). Differences in total  $\text{CH}_4$  fluxes between grazed and ungrazed pastures were large because of the large difference in fluxes from soils between grazed and ungrazed paddocks rather than ditches (paddock/ditch  $\times$  grazed/ungrazed pasture,  $P < 0.05$ ). Grazing increased  $\text{CH}_4$  fluxes from paddocks and it did not affect  $\text{CH}_4$  fluxes from ditches. Fluxes of  $\text{CH}_4$  were higher from ditches than soils in ungrazed paddocks, but ditches and paddocks were similar in grazed pasture.

All three methods for estimating  $\text{CH}_4$  emissions from enteric ruminant fermentation yielded similar results, and in most cases soil was a larger source of  $\text{CH}_4$  than enteric ruminant fermentation (Table 2; Appendix S2: Fig. S4). The flux of  $\text{CH}_4$  from enteric fermentation was in most cases smaller than the difference in fluxes from soils between treatments, indicating that  $\text{CH}_4$  from soil explained most of the difference between grazed and ungrazed pasture. This was not the case in 2014 when cattle consumed more biomass and their  $\text{CH}_4$  emissions were larger than in other years, and when the difference in soil wetness between grazed and ungrazed pasture was smaller (Appendix S1: Table S1).

In most cases, the contribution of manure to  $\text{CH}_4$  emissions from grazed pasture was small (Table 2; Appendix S2: Table S6). The emission of  $\text{CH}_4$  from manure was lower than differences in soil  $\text{CH}_4$  emissions between treatments in 2013 and 2015, and in 2014 when  $\text{CH}_4$  fluxes from enteric ruminant fermentation were estimated using the multiple regression method. Methane fluxes from manure were lower in 2013 and 2015 than in 2014 (Appendix S2: Table S6). In 2013 and 2015,  $\text{CH}_4$  emissions from manure were  $<0.07$   $\text{g C-CH}_4\text{-m}^{-2}$ , while in 2014 when grazing intensity was greater emissions were higher compared to the other years (Appendix S2, Table S6).

The net storage or loss of C from an ecosystem, NECB, and was calculated as the sum of NEE, the C consumed by grazers that was not lost to the atmosphere by either animal respiration or enteric ruminant fermentation, and total ecosystem  $\text{CH}_4$  fluxes. Grazing consistently increased the net C sink strength of subtropical pastures (i.e., decreased NECB) in 2013, 2014, and 2105 (in grazed system,  $-35 \pm 15$   $\text{g C/m}^2$  in 2013,  $-65 \pm 22$   $\text{g C/m}^2$  in 2014;  $-180 \pm 23$   $\text{g C/m}^2$  in 2015; in ungrazed system,  $-5 \pm 1$   $\text{g C/m}^2$  in 2013,  $+86 \pm 4$   $\text{g C/m}^2$  in 2014;  $-15 \pm 1$   $\text{g C/m}^2$  in 2015). Over the years studied, the grazed pasture was a net C sink, and the ungrazed system was either a net source of C to the atmosphere or a weak C sink.

Though variable among years, grazing consistently decreased the net GWP of subtropical pasture (Fig. 7). Even though the grazed pasture was a net source of  $\text{CH}_4$  to the atmosphere, the stimulation of NEE by grazing more than compensated for the increase in  $\text{CH}_4$  fluxes when calculating GWP.

## DISCUSSION

Contrary to our hypothesis, the grazed subtropical pasture had lower GWP than the ungrazed system during three years of livestock exclusion, and grazing increased the net storage of C (i.e., NECB). Changes in the GWP and C sink strength of pastures were explained by how grazing affected NEE and  $\text{CH}_4$  fluxes. Although grazers removed above-ground biomass, grazing consistently increased the net  $\text{CO}_2$  sink strength of subtropical pastures, and the ungrazed system was either a net source of  $\text{CO}_2$  to the atmosphere or was  $\text{CO}_2$  neutral. In agreement with our hypothesis, grazing increased total net  $\text{CH}_4$  emissions of subtropical pastures through enteric ruminant fermentation and wetter soils due to grazers. However, because the difference in  $\text{CH}_4$  emissions from soils between grazed and ungrazed pastures was greater than ruminant emissions in 2013 and 2015, our

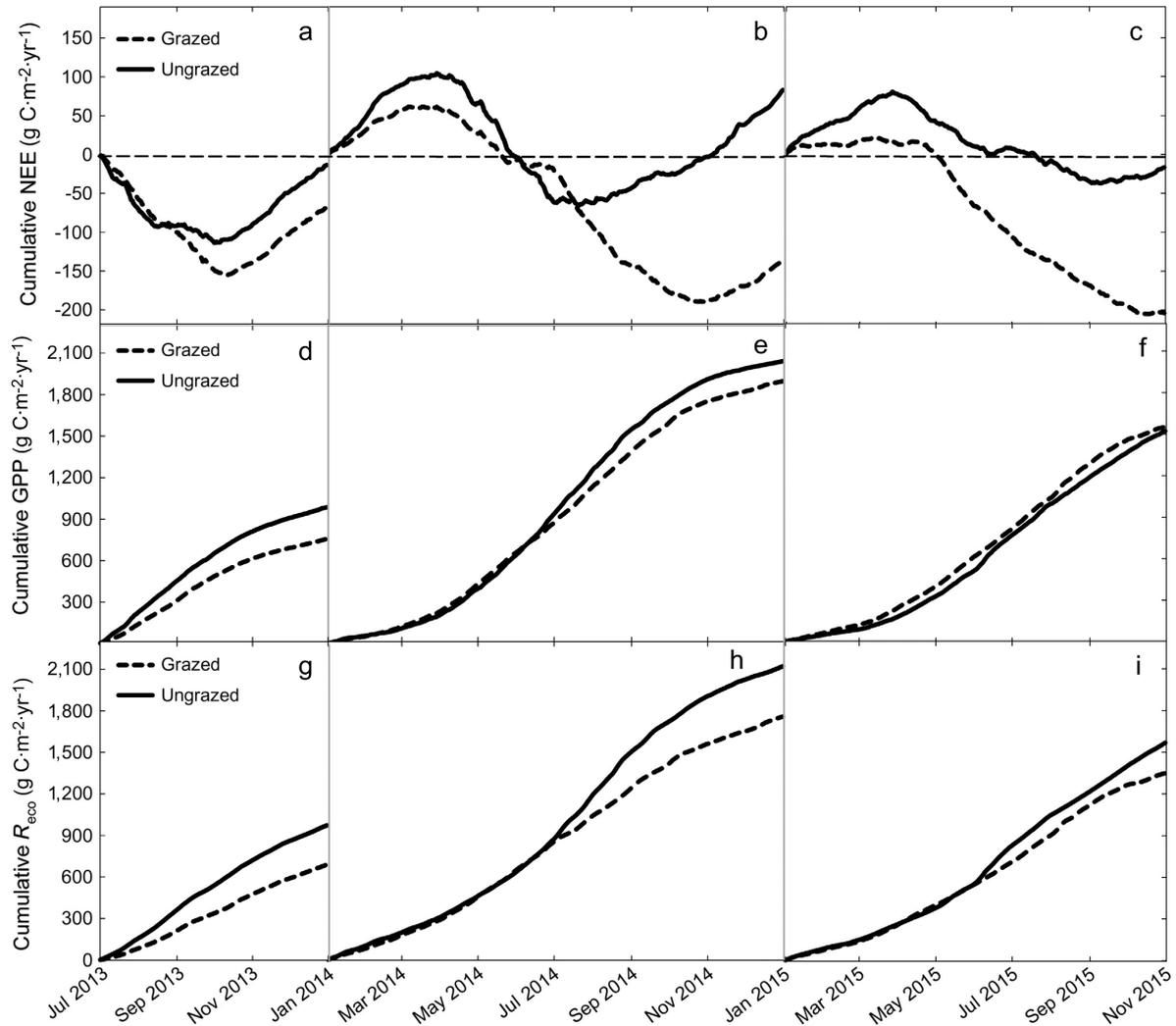


FIG. 4. Cumulative net ecosystem exchange (NEE), gross primary production (GPP), and ecosystem respiration ( $R_{eco}$ ) for grazed and ungrazed pastures in 2013, 2014, and 2015. For cumulative NEE, negative values indicate that the ecosystem is a net sink of  $CO_2$ , and positive values indicate that it is a net source of  $CO_2$ . GPP is positive reflecting gross  $CO_2$  gain from the atmosphere to the ecosystem.  $R_{eco}$  is positive reflecting  $CO_2$  loss from the ecosystem to the atmosphere. For grazed pasture, cumulative NEE was  $-68 \pm 4$  g C- $CO_2/m^2$  in 2013,  $-136 \pm 6$  g C- $CO_2/m^2$  in 2014, and  $-204 \pm 11$  g C- $CO_2/m^2$  in 2015. For ungrazed pasture, cumulative NEE was  $-13 \pm 1$  g C- $CO_2/m^2$  in 2013,  $+83 \pm 4$  g C- $CO_2/m^2$  in 2014, and  $-17 \pm 1$  g C- $CO_2/m^2$  in 2015.

results indicate that the indirect effect of grazing on soil  $CH_4$  emissions can be greater than direct  $CH_4$  produced by enteric ruminant fermentation. Unlike temperate, arid, and semiarid pastures where differences in  $CH_4$  emissions between grazed and ungrazed pastures are mainly driven by enteric ruminant fermentation, enhanced  $CH_4$  production from wetter soils caused by grazers can be a major contributor to greater  $CH_4$  fluxes from subtropical grazed pastures. In relative terms, the reduction of  $CO_2$  flux to the atmosphere caused by grazing was larger than the stimulation of  $CH_4$  production, contributing to the lower GWP of grazed compared to ungrazed pasture.

By increasing the net  $CO_2$  sink strength of pasture (Fig. 7), grazing decreased the GWP of the system, and it is possible that this effect could increase with time or change with greater grazing intensity (Tanentzap and Coomes 2012, McSherry and Ritchie 2013). This study examined the influence of grazing on GWP during the first three years after

exclusion of livestock. Over longer time periods, the accumulation of standing biomass and litter can further limit GPP (Tanentzap and Coomes 2012, McSherry and Ritchie 2013) and potentially increase  $R_{eco}$ , driving a progressive decline in C storage and GWP. However, the responses of GPP and  $R_{eco}$  to changes in the environment are generally non-linear (Burkett et al. 2005, Gomez-Casanovas et al. 2016) so extrapolating our results over longer time periods would be imprudent.

In agreement with Tanentzap and Coomes (2012) and McSherry and Ritchie (2013), we found that grazing can increase the net storage of C (i.e., NECB) of subtropical pasture. Although this study did not include C entering the system through excrement deposition, dung returns little C to soils compared with the aboveground biomass that enters the soil as litter (Pastor et al. 1993, Butler and Kielland 2008, Fornara and Du Toit 2008). In addition, our estimates of net C storage did not include other sources of C loss such

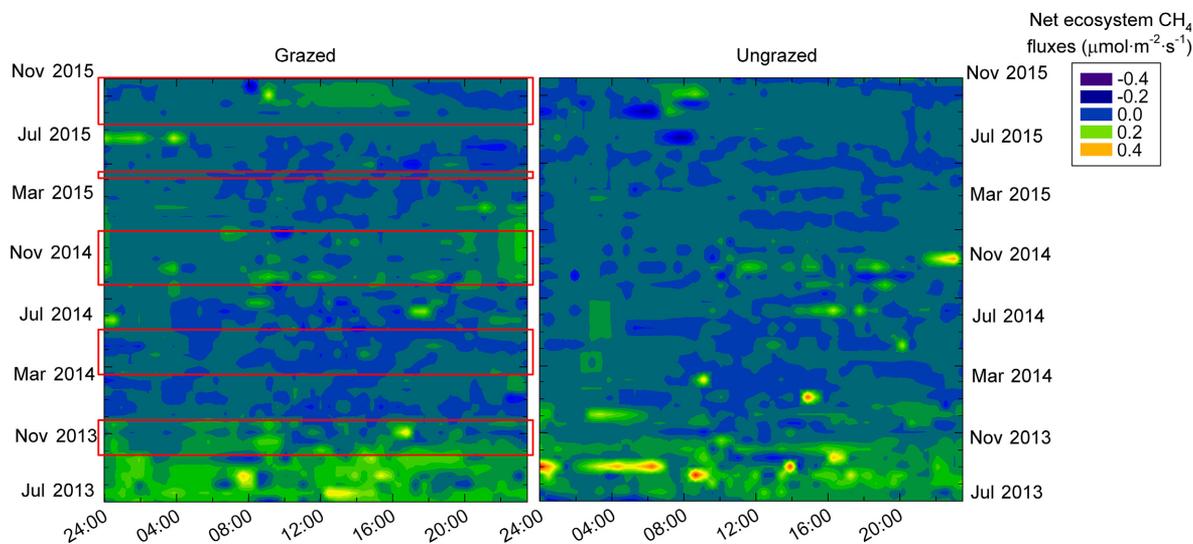


FIG. 5. Contour plots of gap-filled  $\text{CH}_4$  for 2013, 2014, and 2015. Red rectangles reflect periods of time when cattle were in the grazed pasture footprint. Negative values indicate net sink of  $\text{CH}_4$ , and positive values indicate net source of  $\text{CH}_4$ . Values shown at local time.

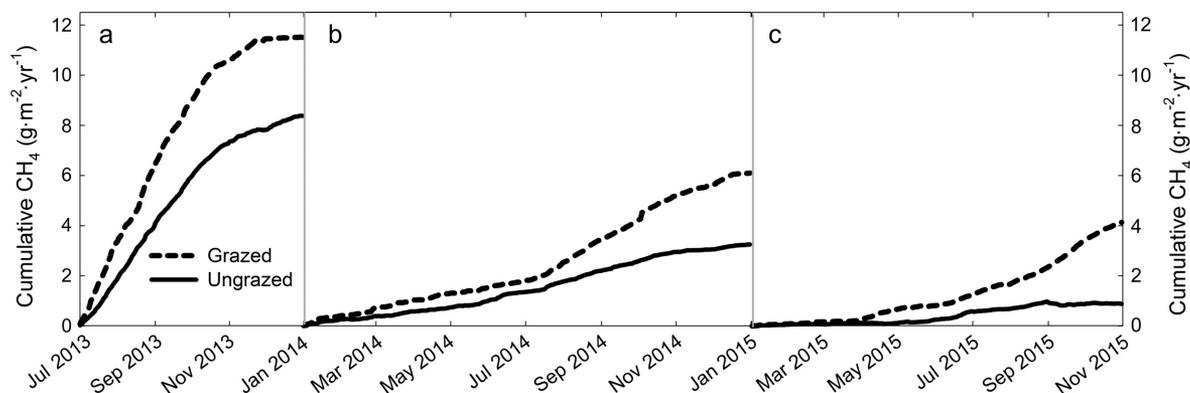


FIG. 6. Cumulative  $\text{CH}_4$  for grazed and ungrazed pastures in (a) 2013, (b) 2014, and (c) 2015. Positive values indicate net source of  $\text{CH}_4$  from the ecosystem to the atmosphere. For grazed pasture, cumulative  $\text{CH}_4$  was  $11.5 \pm 0.6$  g C- $\text{CH}_4/\text{m}^2$  in 2013,  $6.1 \pm 0.3$  g C- $\text{CH}_4/\text{m}^2$  in 2014, and  $4.2 \pm 0.2$  g C- $\text{CH}_4/\text{m}^2$  in 2015. For ungrazed pasture, cumulative  $\text{CH}_4$  was  $8.4 \pm 0.5$  g C- $\text{CH}_4/\text{m}^2$  in 2013,  $3.2 \pm 0.1$  g C- $\text{CH}_4/\text{m}^2$  in 2014, and  $0.9 \pm 0.1$  g C- $\text{CH}_4/\text{m}^2$  in 2015.

TABLE 1. Point  $\text{CH}_4$  measurements ( $\text{mg C-CH}_4\text{m}^{-2}\text{d}^{-1}$ ) from paddocks and ditches for grazed and ungrazed pastures in 2014.

	Paddock		Ditch	
	Grazed pasture	Ungrazed pasture	Grazed pasture	Ungrazed pasture
10 Jun 2014	$-7.4 \pm 0.7$	$-11.7 \pm 0.6$	$-0.07 \pm 0.1$	$-0.4 \pm 0.02$
29 Jul 2014	$15.4 \pm 11.4$	$-13.7 \pm 3.3$	$13.2 \pm 1.1$	$19.1 \pm 13.4$
15 Aug 2014	$41.7 \pm 14.3$	$-3.8 \pm 7.3$	$42.1 \pm 14.5$	$50.1 \pm 20.1$
12 Sep 2014	$33.3 \pm 6.5$	$19.0 \pm 6.1$	$46.5 \pm 17.5$	$48 \pm 24.8$

Notes: Values have been recalculated to account for the area covered by paddocks and ditches relative to the total area of the pasture. Ditches in grazed and ungrazed pastures represented 2.8% of the total area of each pasture. Fluxes of  $\text{CH}_4$  were higher for grazed than ungrazed pasture ( $P = 0.057$ ), they were higher from paddocks under the grazed than the ungrazed pasture, and they were similar from ditches under grazed and ungrazed pastures. The interaction between paddock/ditch and grazed/ungrazed pasture was significant ( $P = 0.006$ ). Fluxes of  $\text{CH}_4$  were higher from ditches than soils in ungrazed pasture, but they were similar in grazed pasture. Positive values indicate net source of  $\text{CH}_4$  from the ecosystem to the atmosphere.

as leaching, and soil erosion that may change as a result of grazing (Allard et al. 2007, Soussana et al. 2007). However, it is likely that grazers did not affect leaching and soil

erosion rates as changes in these processes occur at time spans longer than considered in this study (i.e., decadal scales; Tanentzap and Coomes 2012).

TABLE 2. Cumulative total net ecosystem CH<sub>4</sub> flux from ungrazed and grazed pastures, and estimates of cumulative net CH<sub>4</sub> emission from enteric ruminant fermentation and from soil (i.e., the underlying landscape) for the grazed pasture in 2013, 2014, and 2015.

Year	Ungrazed pasture flux (g C-CH <sub>4</sub> /m <sup>2</sup> )		Grazed pasture flux (g C-CH <sub>4</sub> /m <sup>2</sup> )					
	Total net CH <sub>4</sub> flux	Total net CH <sub>4</sub> flux	Consumed biomass method		Energy requirement method		Multiple regression method	
			Ruminant	Soil	Ruminant	Soil	Ruminant	Soil
2013	8.4	11.5	1.3	10.2 (1.8)	1.5	9.9 (1.5)	0.8	10.7 (2.3)
2014	3.2	6.1	4.1	2.0 (<0)	4.4	1.7 (<0)	2.1	4.0 (0.8)
2015	0.9	4.2	1.2	3.0 (2.1)	1.3	2.9 (2)	1.3	2.9 (2)

Notes: Total net ecosystem CH<sub>4</sub> flux was obtained with the eddy covariance (EC) method. Estimates of CH<sub>4</sub> from enteric fermentation were calculated with two IPCC Tier 2 methods, the “consumed biomass” method and the “energy requirement” method, and also by a multiple regression model method (Appendix S1). Values in parentheses indicate the difference between CH<sub>4</sub> emissions from soils under grazed pasture and ungrazed pasture. Thus, this difference indicates the effect of cattle on CH<sub>4</sub> emissions from soils. Because in 2013 and 2015, this difference was consistently larger than CH<sub>4</sub> emissions from cattle, our results suggest that grazing has a major influence on soil CH<sub>4</sub> emissions. Positive values indicate net source of CH<sub>4</sub> from the ecosystem to the atmosphere.

Net ecosystem exchange is a major determinant of GWP and NECB of grasslands. Grazing consistently increased the net CO<sub>2</sub> sink strength of subtropical pasture because the decrease in R<sub>eco</sub> caused by grazers was larger than the reduction in GPP (Fig. 4). These results agree with studies that indicate that grazing increases the net CO<sub>2</sub> sink strength of grasslands globally, including subtropical grasslands when C consumed by grazers is not accounted for into the budget (Wilsey et al. 2002, Skinner 2008, Shao et al. 2013). Cumulative NEE in grazed and ungrazed grasslands was consistent with published literature values, including those from tropical and subtropical systems (reported range from -366 to 480 g C·m<sup>-2</sup>·yr<sup>-1</sup>; Miranda et al. 1997, San José et al. 2008, Gilmanov et al. 2010, Gomez-Casanovas et al. 2016).

The stimulation of NEE by grazing was the result of its differential effect on GPP and R<sub>eco</sub>. Decreases in GPP were

likely explained by the removal of green aboveground biomass caused by grazing (Fig. 4, Schmitt et al. 2013, Zhang et al. 2015, Appendix S2: Fig. S2). However, in 2015, two years after cattle were excluded from the ungrazed treatment, GPP in grazed and ungrazed pastures was similar. Although grazing consistently decreased standing dead and litter biomass, the difference in non-green biomass between pastures was larger in 2015 than in 2014 and 2013 (Appendix S2: Table S1). Similar GPP in both pastures in 2015 could be explained by standing dead and litter biomass buildup limiting light needed for growth as seen in other studies (Milchunas and Lauenroth 1993). Thus, with longer time after the cessation of grazing than observed in this study, it is likely that the buildup of standing biomass and litter could drive a progressively stronger light limitation of GPP in ungrazed pasture (Milchunas and Lauenroth 1993,

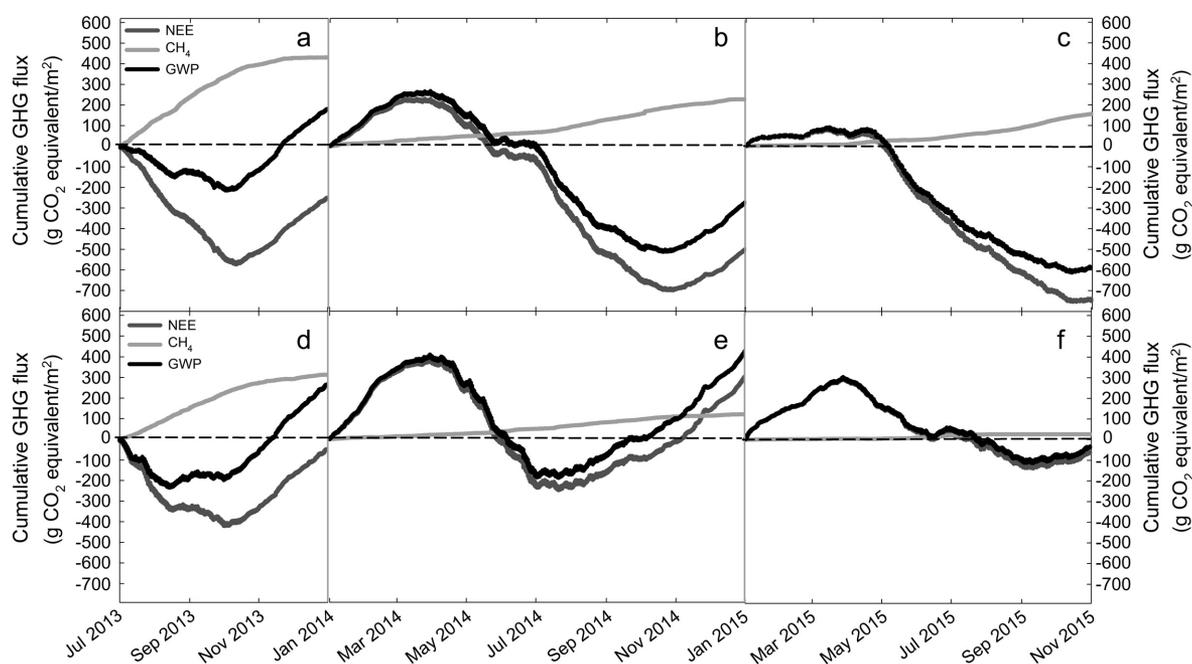


FIG. 7. Cumulative net ecosystem exchange, CH<sub>4</sub>, and Global Warming Potential (GWP, NEE + CH<sub>4</sub>; g CO<sub>2</sub> eq/m<sup>2</sup>) of subtropical (a, b, c) grazed and (d, e, f) ungrazed pasture in 2013, 2014, and 2015. Negative values indicate a net sink for GHG and therefore a net cooling effect on the atmosphere.

Knapp et al. 2012, Tanentzap and Coomes 2012). This progressively stronger light limitation of GPP in ungrazed pasture could reduce GPP in ungrazed pasture relative to grazed pasture, and with time it could further increase the net CO<sub>2</sub> source strength of ungrazed subtropical pastures, especially if grazing decreases  $R_{\text{eco}}$  (Tanentzap and Coomes 2012, McSherry and Ritchie 2013).

Grazing consistently decreased  $R_{\text{eco}}$ , which is composed of  $R_{\text{soil}}$  from plant roots ( $R_{\text{auto}}$ ) and soil microorganisms ( $R_{\text{het}}$ ), and respiration from aboveground biomass. Part of the reduction in  $R_{\text{eco}}$  was simply the removal of respiring aboveground biomass as well as the reduction in root biomass (Appendix S2: Tables S1 & S2). In addition, the removal of leaf area and corresponding reduction of GPP (Fig. 4; Appendix S2: Table S4) would reduce the supply of carbohydrates to fuel respiration by roots and soil microorganisms (Brüggemann et al. 2011, Gomez-Casanovas et al. 2013, DeLucia et al. 2014), further contributing to the decrease in  $R_{\text{eco}}$ . Although root exclusion collars might have deprived soil microorganisms from substrate perhaps decreasing  $R_{\text{het}}$  rates in both grazed and ungrazed pastures (Gomez-Casanovas et al. 2012), in 2014, grazing consistently decreased  $R_{\text{het}}$  and  $R_{\text{auto}}$ , and hence  $R_{\text{soil}}$  (Appendix S2: Table S3). Plant photosynthetic activity (i.e., GPP) and above- and below-ground biomass in particular are major drivers of  $R_{\text{het}}$  and they were lower in grazed than ungrazed pastures (Ekblad and Högberg 2001, Taneva and Gonzalez-Meler 2011, Gomez-Casanovas et al. 2012). Decreases in  $R_{\text{soil}}$  in grazed than in ungrazed pasture could also be explained by reduced O<sub>2</sub> availability in soil under grazed pasture caused by increased soil wetness, especially for soil microorganisms as decomposition of organic matter requires aerobic conditions (Gomez-Casanovas et al. 2012, Blanc-Betes et al. 2016).

Respiration from cattle could have reduced differences in the net CO<sub>2</sub> sink strength of pastures, because in our study, cumulative CO<sub>2</sub> fluxes in grazed pasture included animal respiration. Assuming that 65% of the C consumed by cattle was emitted to the atmosphere (Thornley 1998, Soussana et al. 2007), CO<sub>2</sub> lost by cattle accounted for 3–7% of total  $R_{\text{eco}}$  (47 g C-CO<sub>2</sub>/m<sup>2</sup> in 2013, 146 g C-CO<sub>2</sub>/m<sup>2</sup> in 2014, and 44 g C-CO<sub>2</sub>/m<sup>2</sup> in 2015). These results suggest that the effect of animal respiration on  $R_{\text{eco}}$  was small, and that it did not substantially contribute to decrease differences in the net CO<sub>2</sub> sink strength of pastures.

A major determinant of the GWP of terrestrial ecosystems, CH<sub>4</sub> emissions from grazed temperate and dry grasslands is mainly driven by enteric fermentation by livestock (Saggar et al. 2004, Lassey 2007, Ciais et al. 2013). In agreement with our hypothesis, grazing also increased net ecosystem CH<sub>4</sub> emission in this study (Fig. 6). However, in contrast to temperate, arid, and semiarid grasslands, the effect of grazing on total ecosystem CH<sub>4</sub> emissions was greater in some years than could be explained by enteric ruminant fermentation alone (2013 and 2015; Tables 1, 2).

We hypothesized that grazing would increase soil CH<sub>4</sub> emissions from soils due to increased soil wetness. In addition to emissions from cattle, grazing stimulated CH<sub>4</sub> emission from the soil, and this stimulation was likely explained by the increased activity of soil methanogens, possibly by increasing soil moisture and via CH<sub>4</sub> ebullition caused by cattle trampling (Herbst et al. 2011, Baldocchi et al. 2012, Chamberlain

et al. 2015). The rate of methanogenesis is closely linked to soil wetness (Updegraff et al. 2001, Turetsky et al. 2014, Blanc-Betes et al. 2016) and soil wetness in the grazed paddocks consistently was higher than the ungrazed paddocks (Fig. 2, Bremer et al. 2009, McLain et al. 2002, Gornall et al. 2007, Appendix S2: Fig. S3). The interaction between grazers and soil hydrology substantially contributed to the greater CH<sub>4</sub> efflux from grazed pasture than ungrazed pasture.

Changes in root biomass and distribution due to grazers could explain greater soil wetness in grazed than in ungrazed pasture. Grazing decreased the biomass of roots at shallow soil depth, and increased the relative distribution of roots at deeper soil layers (Milchunas and Lauenroth 1989, 1993, Hild et al. 2001, Nippert et al. 2012, Appendix S2, Table S2). Thus, grazing might have increased the fraction of groundwater vs. surface water transpired to the atmosphere, contributing to increased top soil wetness in grazed than in ungrazed pasture (Canadell et al. 1996, Oliveira et al. 2005).

Evapotranspiration was higher in grazed than in ungrazed pasture (Appendix S2: Table S5), and grazers might have increased evaporation (Dangal et al. 2016, Shao et al. 2017). That grazing increased total evapotranspiration while increasing soil wetness could be explained by an increase in the fraction of groundwater vs. surface water transpired by grazed plants that might have compensated increases in evaporation due to grazers (Jasechko et al. 2013, Schlesinger and Jasechko 2014).

Greater deposits of urine may also have contributed to higher net CH<sub>4</sub> emissions from grazed compared to ungrazed pasture. Urine adds nitrogen to soils and can inhibit methanotrophic activity, decreasing net CH<sub>4</sub> uptake under drier soil conditions and increasing net CH<sub>4</sub> emissions at wetter soil conditions (Abell et al. 2009, Banger et al. 2012, Zheng et al. 2012). However, in our study, the inhibitory effect of nitrogen addition on methanotrophic bacteria activity was probably small because at similar soil moisture levels CH<sub>4</sub> emissions from soils between the grazed and ungrazed pastures were close in magnitude (Appendix S2: Fig. S3).

Methane is also produced by anaerobic fermentation of manure (Ciais et al. 2013). Increased CH<sub>4</sub> emissions from manure could explain differences in soil CH<sub>4</sub> emissions between grazed and ungrazed pasture, because in our study, soil emissions included these emissions. However, the contribution of deposited manure CH<sub>4</sub> emissions to differences in annual CH<sub>4</sub> fluxes from soils between treatments was small, especially in 2013 and 2015. This result is consistent with the small contribution of CH<sub>4</sub> emissions from manure to total CH<sub>4</sub> emitted from grazed soils commonly observed in tropical and subtropical ecosystems even at higher grazing intensities than considered in this study (Dong et al. 2006, Chamberlain et al. 2015).

Increases in above- and belowground biomass production and the corresponding increase in C available as a substrate in the soil often results in greater soil CH<sub>4</sub> emissions (Whiting and Chanton 1993, Matthes et al. 2014, Desai et al. 2015). In our study, grazers increased soil wetness but decreased C input to soils through lower above- and belowground biomass (Fig. 2a, b; Fig. S2). The differential effect of grazing in this study suggests that increases in soil wetness played a larger role in determining differences in soil CH<sub>4</sub> emissions between grazed and ungrazed pasture than variations in

above- and belowground biomass. This finding is in agreement with studies showing that changes in surface soil wetness and water table are major determinants of CH<sub>4</sub> fluxes from soils as they affect the predominant CH<sub>4</sub> metabolic pathway (i.e., methanogenic and methanotrophic pathways; Updegraff et al. 2001, Turetsky et al. 2014, Blanc-Betes et al. 2016) and might indicate that CH<sub>4</sub> fluxes from soils in subtropical pastures are strongly determined by soil wetness on the top soil layers (Chamberlain et al. 2016, 2017).

The influence of grazers on CH<sub>4</sub> emissions from soils (Table 2) varied with year. This influence was greater in years with lower grazing intensity, when grazers consumed less aboveground biomass, and with larger differences in soil wetness between grazed and ungrazed pastures (i.e., 2013 and 2015; Table 2). Climatic models predict that subtropical and tropical regions will experience more frequent floods and extended drought periods (Field et al. 2014). Thus, it is likely that future climatic conditions altering soil wetness will affect the influence of grazers on CH<sub>4</sub> emissions from soils under subtropical and tropical regions.

Both pastures were net sources of CH<sub>4</sub>, and although fluxes were highly variable among years, emissions were within the range reported for temperate flooded and subtropical grasslands (range from  $-0.2$  to  $5$  g C-CH<sub>4</sub>·m<sup>-2</sup>·yr<sup>-1</sup>; Gomez-Casanovas et al. 2016), and grazed and ungrazed temperate and subtropical pastures (range from  $-0.3$  to  $16$  g C-CH<sub>4</sub>·m<sup>-2</sup>·yr<sup>-1</sup>; Couwenberg et al. 2010, Dengel et al. 2011, Teh et al. 2011, Baldocchi et al. 2012, Chamberlain et al. 2015, 2016, 2017). In both pastures, increased net CH<sub>4</sub> emissions in 2013 compared with 2014 and 2015 was likely explained by a wetter wet season (Figs. 2, 4, Table 2, Updegraff et al. 2001). In addition, heavy rainfall occurring in April 2013 right before the onset of the wet season (Fig. 2), and increased soil wetness preceding the wet season likely enhanced soil CH<sub>4</sub> emissions in both pastures (Turetsky et al. 2014).

Although this study focused on how grazing influences the exchange of CO<sub>2</sub> and CH<sub>4</sub> between pastures and the atmosphere, cattle can also stimulate N<sub>2</sub>O emissions by increasing nitrogen-rich urine and dung inputs (Mosier et al. 2004, Davidson 2009). Thus, it is likely that enhanced N<sub>2</sub>O emissions would increase the GWP of grazed compared to ungrazed subtropical pastures.

Grazers could also influence local and regional climate through their influence on net radiation and albedo. By removing biomass, grazing could increase albedo due to decreased net radiation as lower green biomass reflects more incident radiation (Shao et al. 2017). These changes in energy fluxes due to grazers could lead to a cooling effect feedback on local climate in addition to cooling associated with C fluxes (Fig. 7, Miller et al. 2015, Shao et al. 2017).

Considering only CO<sub>2</sub> and CH<sub>4</sub>, the grazed subtropical pasture had lower GWP and higher net C storage than the ungrazed system during three years of cattle exclusion. However, the overall effect of livestock grazing subtropical pastures on the environment will also depend on other factors that affect the emission of GHGs from cattle ranches including the impact of grazers on N<sub>2</sub>O emissions from soils, on-farm operations and transport of livestock products (Steinfeld et al. 2006, 2010).

By increasing the net CO<sub>2</sub> sink of subtropical pasture, our results indicate that grazing decreases its GWP and increases

its net C storage. We showed that increases in the net CO<sub>2</sub> sink strength of grasslands was explained by the removal of aboveground green biomass, and by the impact of grazing on GPP and  $R_{eco}$ . Our results suggest that increased CH<sub>4</sub> emissions from pasture is caused by increased soil wetness due to grazers in addition to enteric ruminant fermentation, and that increased soil wetness is likely caused by variations in above- and belowground biomass and root distribution. Contrary to observations in temperate, arid, or semiarid grazed ecosystems where CH<sub>4</sub> emission by ruminant enteric fermentation drives differences between grazed and ungrazed systems, the effect of grazing on soil CH<sub>4</sub> emissions from subtropical pastures can be greater than CH<sub>4</sub> produced by enteric ruminant fermentation. Future studies need to investigate if the interaction between grazing, C allocation and soil hydrology determining C fluxes from a subtropical pasture might be relevant to other permanently or temporarily flooded ecosystems to improve model accuracy in quantifying the environmental impacts of livestock.

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## SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.1670/full>

## DATA AVAILABILITY

Data available from Figshare: <https://doi.org/10.6084/m9.figshare.5691259>