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Methane, carbon dioxide and nitrous oxide fluxes from a temperate salt marsh: grazing management does not alter Global Warming Potential

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1 Abstract

Soil greenhouse gas emissions from cattle grazed and un-grazed temperate upper salt marsh were measured using dark static chambers, monthly for one year. Additionally, below-ground gas sampling tubes were used to measure soil methane (CH₄) concentrations. CH₄ efflux from grazed and un-grazed salt marsh did not differ significantly, however grazing did lead to 'hotspots' of underground CH₄ (up to 6 % of total air volume) and CH₄ efflux (peak of 9 mg m⁻² h⁻¹) significantly linked to high soil moisture content, low soil temperatures and the presence of *Juncus gerardii*. Carbon dioxide (CO₂) efflux was greater from the un-grazed marsh (mean of 420 mg m⁻² h⁻¹) than the grazed marsh (mean of 333 mg m⁻² h⁻¹) throughout most of the year and was positively correlated with deeper water table and greater soil temperatures. Grazing was not a significant predictor of nitrous oxide (N₂O) soil emissions. Global Warming Potential (GWP; over 100 years), calculated from mean yearly chamber fluxes for CH₄ and CO₂, did not differ significantly with grazing treatment. Seasonal

variation in the key drivers of soil greenhouse gas efflux; soil temperature, moisture and water table, plus the presence or absence of aerenchymatous plants such as *J. gerardii* were more important to the magnitude of greenhouse gas emissions than grazing management *per se*.

Key words: chamber flux measurements, greenhouse gases, salt marshes, livestock grazing, UK: Ribble estuary

2 Introduction

Methane (CH₄), carbon dioxide (CO₂), and nitrous oxide (N₂O) are all major greenhouse gases. Despite natural wetlands accounting for a third of global CH₄ flux, their contribution to Global Warming Potential (GWP) may be off-set by their carbon sink capabilities and minimal N₂O emissions (Dassonville & Renault, 2002; Denman *et al.*, 2007; Lai, 2009). Managing wetlands to minimise their GWP is therefore crucial. Most previous research focuses on freshwater wetlands such as peatlands (Le Mer & Roger, 2001; Limpens *et al.*, 2008; Lai, 2009) with the GWP of coastal habitats such as tidal flats and salt marshes remaining less well quantified (Pacyna & Manø, 2006). European salt marshes are often managed by livestock grazing to provide a suitable habitat for over-wintering bird species (Adam, 1990; Milsom *et al.*, 2000; Chatters, 2004), however, the impact of this management on the GWP of this habitat is not well known. Grazing management is expected to have clear implications for GWP as soil moisture content, soil temperature and plant community composition, all key drivers of soil greenhouse gas emissions, often differ with grazing intensity (Bakker *et al.*, 1993; Curry, 1994; Lambert, 2000). Despite the fact that salt marshes are by definition inter-tidal wetlands, their upper zones share many characteristics of semi-natural grasslands due to infrequent inundation. Aerated grassland soils are large carbon stores, produce little CH₄ and emit significant amounts of N₂O only under intensive grazing or fertiliser input regimes (Soussana *et al.*, 2007; Allard *et al.*, 2007; Del Grosso, 2010). Upper temperate salt marshes, common throughout Europe and characteristic of the vast area behind summer dykes in the Wadden Sea area of Germany (Bakker *et al.*, 1993), may therefore have

a similar GWP to terrestrial grasslands during the summer months if tidal inundation is rare.

CH₄ is produced by methanogenic archaea from either CO₂ or acetic acid when soil conditions are suitably anoxic (Denman *et al.*, 2007). Where an oxic soil layer exists above an anoxic layer up to 88 % of CH₄ produced can be oxidised by methanotrophs (Calhoun & King, 1997). CH₄ leaves the soil via three pathways, diffusion, ebullition and through the aerenchyma of certain plant species (Van der Nat & Middelburg, 2000). In wetland soils, CH₄ production is increased by standing water or waterlogged soil, high soil temperatures and increased organic matter or substrate availability (Le Mer & Roger, 2001; Ding *et al.*, 2004; Kankaala *et al.*, 2005). In the sulphate rich marine environment sulphate reducing bacteria typically out-compete methanogenic archaea in the anaerobic decomposition of organic matter, a process governed by the redox potential (Piker *et al.*, 1998). As saltmarshes are tidal it is assumed that CH₄ emissions from this habitat are relatively insignificant. Bartlett *et al.* (1985) reported that North American coastal saltmarshes do not contribute significantly to CH₄ emissions. However, in a recent review of CH₄ emissions from temperate tidal marshes Poffenbarger *et al.* (2011) reported that while polyhaline tidal marshes had lower CH₄ emissions than fresh water marshes, oligohaline marshes had the highest and most variable emissions. To our knowledge, CH₄ efflux has not been measured for the upper zone of the salt marsh that may only be tidally inundated a dozen times a year. Cattle grazing may increase soil CH₄ emissions directly via the input of animal dung, a moderate CH₄ source, and indirectly via CH₄ ebullition caused by cattle trampling (IPCC, 1996; Lin *et al.*, 2009; Herbst *et al.*, 2011). In addition, grazed salt marshes may be prone to water-logged ground, have greater plant species richness than un-grazed marshes and are often characterised by *Juncus* species (rushes) that are known to vent CH₄ via their aerenchyma (Adam, 1990; Roslev & King, 1996; Lambert, 2000).

CO₂ efflux is comprised of microbial (soil) and plant respiration. Soil respiration requires aerobic decomposition conditions, intermediate soil moisture and becomes faster with increased soil temperature and ecosystem productivity (Luo & Zhou, 2006). Both European and North American saltmarshes have high levels of primary

productivity (Vernberg, 1993; Mitsch & Gosselink, 2000) but exhibit variable redox potential and soil moisture regimes due to differences in timing and duration of tidal inundation. Regularly inundated salt marshes and mudflats are likely to show very different soil characteristics to upper salt marshes that are less frequently inundated. Studies of grazing and CO₂ efflux have largely concentrated upon grassland systems. In Soussana *et al.* (2007) grasslands under widely differing grazing and fertiliser addition regimes were all net sinks of CO₂. However, livestock grazing may reduce plant respiration directly via removal of above-ground plant biomass by herbivores and also reduce soil respiration indirectly via decreased supply of readily available carbon to roots and microbes (Luo & Zhou, 2006). Despite this effect, 'hotspots' of CO₂ emissions from livestock dung, up to 50 % higher than control plots have been recorded (Lin *et al.*, 2009) and should also be taken into account. Grazing intensity also influences soil carbon storage. Light, moderate or heavy grazing can all increase soil carbon, depending on grassland type (Kemp & Michalk, 2007). Conversely, extensively grazed or un-grazed grasslands may store more carbon than intensively managed grassland (Campbell *et al.*, 1997; Soussana *et al.*, 2004).

N₂O soil emissions occur where an aerobic soil surface layer coupled with an anaerobic layer immediately beneath provides suitable conditions for aerobic nitrifying bacteria to produce nitrate, from which anaerobic denitrifying bacteria produce N₂O (Mitsch & Gosselink, 2000). Nitrification may also occur in the oxidised root zone of plants. N₂O soil emissions are increased by high nitrate availability and compacted, waterlogged, warm soil (Van Groenigen *et al.*, 2005; Lin *et al.*, 2009; Del Grosso, 2010). In general, N₂O emissions are considered detrimental as they increase atmospheric pollution. However, in coastal systems, denitrification leading to increased N₂O efflux may be seen as environmentally beneficial, when this prevents the release of nitrate into the marine environment that can lead to eutrophication (Brin *et al.*, 2010). There are very few studies concerning N₂O emissions from freshwater or saltwater wetland soils (Poffenbarger *et al.*, 2011). Most studies from grasslands record greater soil N₂O emissions with increased livestock grazing intensities. Animal trampling leads to compact, warm, waterlogged soils, and the addition of animal waste a nitrate source, providing ideal conditions for soil N₂O

efflux (Van Groenigen *et al.*, 2005; Saggar *et al.*, 2007; Lin *et al.*, 2009; Del Grosso, 2010). However, Wolf *et al.* (2010) provides conflicting evidence, soil N₂O emissions were highest in un-grazed steppe grasslands and lowest in grasslands with highest stocking densities.

In this study we examine the effect of cattle grazing on greenhouse gas efflux and estimated GWP of a temperate upper saltmarsh. The following three hypotheses were examined: 1) Soil CH₄ efflux will be greater in the cattle grazed than the un-grazed salt marsh due to differences in compaction and soil moisture content; 2) Combined soil and plant CO₂ efflux will be greater in the un-grazed (aerobic, free draining soil with a large above-ground plant biomass) than the grazed marsh; 3) Soil N₂O efflux will be greater in the grazed than the un-grazed marsh due to differences in compaction, waterlogging and nitrate supply.

3 Study area, materials and methods

3.1 Crossens marsh

The salt marshes of the Ribble estuary cover 2000 ha in total. The study area, Crossens Marsh (53° 41' 15" N, 2° 57' 4" W), is located on the southern edge of the Ribble estuary in north-west England and is part of the Sefton Coast Special Protection Area managed by Natural England. The marsh has been arbitrarily split into two management types by a fence line that has been *in situ* for at least forty years, running more-or-less perpendicular to the shore. The grazed marsh is characterised by predominantly *Festuca rubra* saltmarsh National Vegetation Community (NVC; SM16d) and the un-grazed marsh by *Elytrigia repens* saltmarsh (SM28; Rodwell, 2000). The grazed part of the marsh covers 517 ha and is grazed uniformly by around 100 bullocks from late May to early October (0.2 cattle per hectare) to provide an overwintering feeding habitat for pink-footed geese (*Anser brachyrhynchus*). All experimental units were selected within the oligohaline (salinity = 0.5 – 5 PSU (practical salinity units)) high marsh zone where numerous creeks are present but tidal inundations are relatively rare, limited to around eight events a year on high equinox tides. A paired experimental design was used with six experimental units of approximately 10 m x 10 m set up on each side of a 600 m long section of the

fence line, 100-150 m apart, in a 'mirror image' formation, giving six grazed (G1-G6) and six un-grazed (U1-U6) units (Figure 1). Each experimental unit was located between 20 m and 50 m from the fence line to ensure an adequate buffer zone and checked for comparable elevation within ± 10 cm. All measurements were carried out within these experimental units.

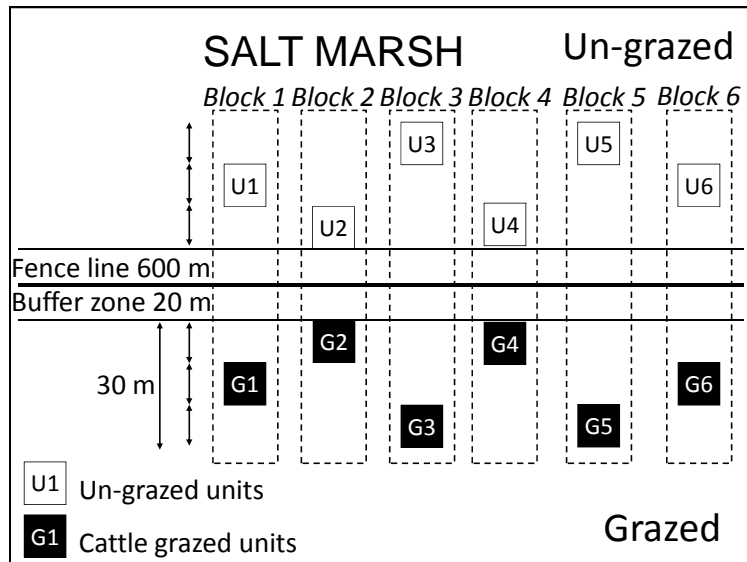


Figure 1 Experimental design at Crossens Marsh. All units are 10 m x 10 m square at 20 - 30 m, 30 - 40 m or 40 - 50 m from the fence line. Not to scale.

3.2 Marsh characterisation

The following measurements were taken for saltmarsh characterisation in 2009. Soil samples were collected during September from the top 15 cm of soil to measure salinity and pH. Soil was sieved to 2 mm and a sub sample of 10 g was taken from each sample and shaken with 25 ml of deionised water (1:2.5 dilution factor). A *Hanna* pH209 pH meter was used to measure pH and a *Jenway* 4520 Conductivity meter to measure electrical conductivity (mS cm^{-1}) as a proxy for salinity. Samples to determine bulk density and soil organic matter content were also collected using intact soil cores of 3.8 cm diameter and 15 cm depth. Cores were dried at 105 °C for 72 hours and the dry mass divided by the volume of the core to calculate bulk density. Loss-on-ignition was used to estimate organic matter content (Ball, 1964). Soil carbon stock in kg C m^{-2} was calculated from bulk density and the conversion factor of soil carbon as 0.55 of soil organic matter (Emmett *et al.*, 2010). Soil moisture content and

temperature were recorded at six locations within each experimental unit during September. Soil conductivity was measured in direct volts using a *Delta T* Theta Meter HH1 (four probes of 6 cm) and converted to percentage soil moisture content using a calibration suitable for organic soils. Soil temperature was measured using a digital thermometer (single 11 cm probe). Vertical water infiltration rate, inversely related to waterlogged soil conditions, was measured using three single ring infiltrometers (Carroll *et al.*, 2004) per experimental unit.

The potential for nutrient cycling by microbes was assessed using a measure of mineralisable N (Rowe *et al.*, 2011). Three N mineralisation cores, 3.8 cm diameter and 15 cm depth, were taken from each experimental unit, during September. Soil cores were taken using plastic corers, capped at both ends to minimise soil disruption, and stored intact at 4 °C. Accumulated inorganic N was flushed from the cores by spraying with a solution of similar ionic concentration to UK rain over 7 days until 150 ml of leachate had been collected. Cores were incubated at 10 °C for 28 days, homogenised and a sub-sample extracted using 1 M KCl for the analysis of ammonium and nitrate content (Rowe *et al.*, 2011). N mineralization rate was calculated over these 28 days assuming that all previous inorganic N had been removed during the 7 day flushing period. Mineralisable N was expressed as $\mu\text{g N g}^{-1} \text{ day}^{-1}$ on both a dry soil weight and organic matter basis. The biological activity of soil meso-faunal decomposers, a proxy for aerobic soil activity, was measured using custom made bait lamina (Terra Protecta GmbH, Germany), 15 cm long with 16 holes filled with cellulose, bran and charcoal. Ten bait lamina per experimental unit were set up in two lines of five, 50 cm apart, pushed vertically into the ground so the top hole was 1 cm below the soil surface. Strips were placed in the ground in late June for 44 days and again in mid September for 34 days until 10 – 40 % of the bait had been degraded. Strips were removed, washed and each hole assessed for biological activity or 'feeding rate'. Feeding rates were standardised to percentage bait removed per 7 days.

Above-ground net primary productivity (ANPP), peak biomass from three grazer excluded areas per experimental unit, was recorded as a direct measure of primary productivity. At the beginning of March, vegetation was cut to ground level in three

50 cm x 50 cm areas per experimental unit. Each cut area was protected from cattle by an 8 cm mesh gabion (50 x 50 x 50 cm) and vegetation allowed to re-grow until peak biomass at the end of August when areas were re-cut within a central 25 cm x 25 cm area. Vegetation was dried at 80 °C for 72 hours then weighed and converted to kg dry wt m⁻² yr⁻¹ to provide a measure of ANPP. Above-ground living plant material and plant litter were collected for five 25 cm x 50 cm quadrats per experimental unit in July, one root core of 5 cm diameter and 10 cm depth was also taken per quadrat and washed to remove all soil. Above-ground vegetation, litter and roots were all dried at 80 °C for 72 hours and weighed to give indicators of above-ground live plant biomass, litter biomass and below-ground root biomass respectively. Above-ground biomass can be linked to dark chamber respiration rates.

3.3 CH₄, CO₂ and N₂O chamber fluxes

Above-ground greenhouse gas fluxes were measured by a closed dark static chamber method. Each chamber consisted of a polyvinyl chloride (PVC) pipe of 15 cm height (30 cm internal diameter) with a rubber Septa sampling point located half way up, sealed to a 2 mm acrylonitrile butadiene styrene (ABS) lid, painted silver to reflect heat. These chambers were of similar diameter to those commonly used, but lower in height, and were chosen to increase the likelihood of measuring minor fluxes of CH₄ and N₂O by increasing the surface area to volume ratio. During measurement periods each static chamber was attached by a rubber seal to an in situ PVC pipe base (0.71 m²). The base was placed firmly in the soil to a depth of 5 cm with 10 cm visible above-ground in June 2010, to give a combined chamber and base volume of 0.018 m³. Vegetation and plant litter were not removed from within the chambers.

Two chambers per experimental unit, with bases 3 m apart, were used to measure daytime (between 11am and 3pm) greenhouse gas fluxes once a month for twelve months from September 2010 to August 2011. Gas samples were taken with a 30 ml syringe at 0, 30, 60, 90 and 120 minutes after chamber placement and immediately transferred to a 22 ml vial, over pressurised in the field but returned to lab pressure prior to analysis. Internal chamber temperature was recorded in two grazed and two un-grazed chambers per month using Tinytag data loggers (TGP-4017 -40 to +85°C;

Gemini Data Loggers). Gas analysis was carried out over the following three days using a Perkin Elmer Clarus 500 Gas Chromatograph (GC) with a Porapaq QS (80 – 100 mesh) analytical column and Turbomatrix 40 headspace autoanalyser. CO₂ and CH₄ were detected by FID, N₂O by ECD (at 375 °C, sample oven at 40 °C) to give ppm and peak area (mV) measurements for the three gases. The following standard gases were used to calibrate the GC, A: N₂O 1 ppm, CH₄ 3.9 ppm, CO₂ 995.5 ppm; B: N₂O 5.1 ppm, CH₄ 50.4 ppm, CO₂ 510.8 ppm; and C: N₂O 2 ppm, CH₄ 20.3 ppm, CO₂ 257.7 ppm. Samples were run after a calibration was achieved with an r^2 of > 0.99 for all three gases.

3.4 Flux calculation

Greenhouse gas fluxes were calculated from ppm and peak area measurements for each chamber using a GCflux model (Levy *et al.*, 2011) run on Genstat 13.1 (Payne *et al.*, 2011). For CO₂ and N₂O, fluxes were calculated for the full 0 to 120 minutes time scale (5 time points). For CH₄ fluxes the first time point (time 0) was excluded as it was often high compared with ambient air concentration (~1.8 ppm) due to probable ebullition from disturbance in placing the chamber as in Alm *et al.* (2007). The GCflux model calculated fluxes, with chamber volume and temperature accounted for, by five methods: 1) Simple averaging; 2) Linear regression; 3) Intercept method; 4) Negative exponential regression; 5) Asymptotic regression. Final model selection, and therefore flux output for further analysis, was based on the highest r^2 value. Within this study method 2) 'Linear regression' was consistently the best model fit for each data set. Regression values of $r^2 < 0.7$ were excluded unless they were indicative of low level or zero fluxes as in Waddington *et al.* (2010). Flux output in nmol m⁻² s⁻¹ was converted to mg m⁻² h⁻¹ for statistical analysis.

3.5 CH₄ soil concentration

Underground soil CH₄ concentrations were measured using plastic gas sampling tubes, 10 cm long, with an internal diameter of 16.5 mm, with 8 small holes (2.5 mm diameter) drilled at either 2.5 cm, 5 cm or 7.5 cm along the tube, depth when inserted in soil (Figure 2), to allow soil air at this depth to enter the tube. A silicone bung was used to seal the base of the tube, a 17.5 mm Septa suba seal was attached to the top

of each tube to allow gas sampling via syringe. The gas sampling tubes were installed in the field in July 2010, flush with the soil surface, three per experimental unit, one 2.5 cm, 5 cm and 7.5 cm, 5 cm apart in a triangle formation. Each set of tubes was protected by a rain hat and wire basket to prevent interference by cattle. The gas sampling tubes were allowed to equilibrate for one month to allow measurement of gas from the tubes to accurately reflect the soil concentration of CH₄ at each soil depth. Gas was sampled from each tube once in October 2010, January and July 2011 using a 30 ml syringe. Samples were immediately transferred to a pre-evacuated 22 ml glass vial, over pressurised in the field but returned to lab pressure prior to analysis. Gas analysis was carried out using the GC. CH₄ was detected using FID to give ppm and peak area measurements. As many measurements were higher than the lab standard CH₄ concentrations of A (3.9 ppm), B (50.4 ppm) and C (20.3 ppm), additional standards of 0.1 % (1000 ppm), 1 % (10,000 ppm) and 10 % CH₄ (100,000 ppm) were used to calculate ppm from area measurements more accurately. Soil CH₄ percentages for underground gas samples were calculated directly from soil CH₄ concentrations in ppm.

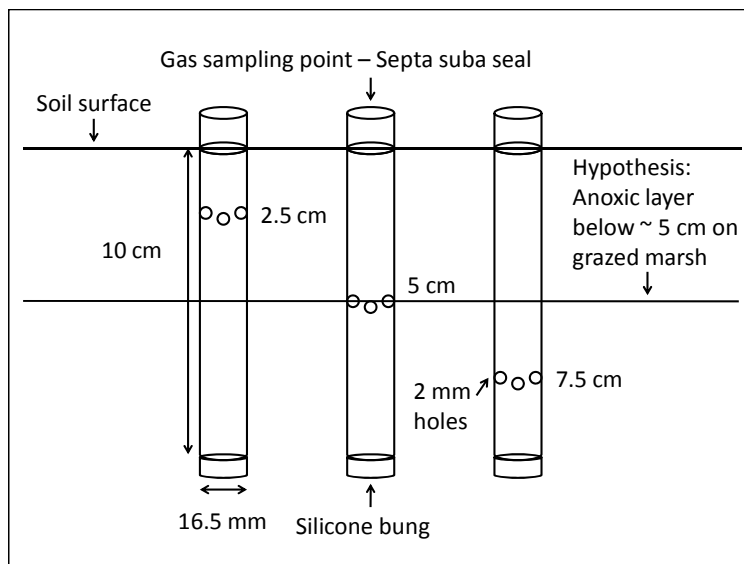


Figure 2 Schematic representation of the underground gas sampling tubes.

3.6 Environmental measurements as predictors of chamber gas fluxes

As soil temperature, soil moisture content and water table level are common drivers of soil greenhouse gas emissions these were measured monthly for twelve months alongside gas chamber measurements to provide possible predictive data for the size of greenhouse gas efflux. In addition plant community composition, particularly the presence of *Juncus* species, can influence the production of CH₄ via aerenchyma; this was therefore recorded in the middle of the twelve month sampling period and used in later predictive models (2.7). Soil temperature and soil moisture content were recorded adjacent to each chamber immediately prior to monthly gas measurements (with the same equipment as in section 2.2). It was also recorded if chambers or experimental units appeared waterlogged. Water table measurements were taken monthly at the same time as gas measurements from dip wells, one per experimental unit, located between the two chambers. The dip wells, 1 m depth, 35 mm internal diameter, PVC slotted screen (Stuart Well Services Ltd., Norfolk, UK), were installed in October 2010. Water table measurements were therefore not available for September or October. Plant species percentage cover was assessed by eye for each experimental unit during May 2011 for three 1 m x 1 m quadrats and within the two gas measurement collar areas.

3.7 Statistical analysis

Differences between grazing treatments for all environmental variables and flux measurements were analysed using ANOVAs on linear mixed effects (lme) models using R (R Development Core Team, 2011) taking into account the effect of sampling month 'lme (CO₂flux ~ grazing*month, random = ~1|block/grazing/month'. This approach was used to enable the raw data to be analysed accounting for replication at the level of the experimental unit (n=6; Crawley, 2007). In addition, lme models were used to assess the influence of water table level, soil moisture and soil temperature, all recorded alongside chamber measurements; percentage cover of *Agrostis stolonifera*, *Aster tripolium*, *E. repens*, *F. rubra*, *Glaux maritima*, *Juncus gerardii*, *Puccinellia maritima* and *Triglochin maritima* recorded from chambers and experimental units; and salinity, bulk density, organic matter content, above ground biomass, litter biomass, all measured prior to chamber measurements; on underground soil CH₄ concentration and above-ground CO₂ and CH₄ fluxes. Results

of best model fit are presented here based on lowest Akaike information criterion (AIC) and quantile probability plot (qqnorm) with most normal distribution.

3.8 Up-scaling conceptual diagram

A conceptual diagram was produced to compare the GWP of greenhouse gas fluxes from the grazed and un-grazed salt marsh over the Crossens marsh study site. Mean greenhouse gas fluxes from chamber measurements over the study year, for grazed versus un-grazed marsh ($n = 6$), were converted to CO₂ equivalents ($\text{g CO}_2\text{e m}^{-2} \text{yr}^{-1}$) for a 100 year GWP ($\text{CO}_2 = 1$, $\text{CH}_4 = 25$, $\text{N}_2\text{O} = 298$; Denman *et al.*, 2007) and expressed as a comparative flux estimate for grazing type. Carbon stored in plant biomass for the grazed and un-grazed salt marsh, over one year, was calculated from mean ANPP for each treatment using a shoot carbon value of 42 % (unpublished data) to give a value in $\text{g C m}^{-2} \text{yr}^{-1}$. This was then converted to CO₂ equivalents in $\text{g CO}_2 \text{m}^{-2} \text{yr}^{-1}$ using a conversion factor of $\times 3.67$ (molar mass of CO₂ = 44, molar mass of C = 12, $44/12 = 3.67$). CO₂ allocated to roots was not calculated. In addition to variables directly measured within this study, CH₄ efflux via cattle, enteric (i.e. microbial fermentation within rumen and large intestine), from waste and via trampling were also estimated to provide a more realistic comparison between grazing regimes. CH₄ efflux via cattle was calculated based on 100 bullocks on Crossens Marsh over 517 ha for 1/3 of the year (beef cattle emit $48 \text{ kg CH}_4 \text{hd}^{-1} \text{yr}^{-1}$ enteric CH₄ & $6 \text{ kg CH}_4 \text{hd}^{-1} \text{yr}^{-1}$ CH₄ from waste; IPCC 1996). CH₄ efflux via ebullition caused by cattle trampling was not directly measured but was included in the diagram as an additional factor that may influence greenhouse gas emissions as in (Herbst *et al.*, 2011). The effect of grazing on GWP ($\text{CH}_4 \text{ soil efflux} + \text{CH}_4 \text{ cattle efflux} + \text{CO}_2 \text{ efflux} - \text{ANPP} = \text{GWP} (\text{g CO}_2\text{e m}^{-2} \text{yr}^{-1})$) was analysed by ANOVA on an lme model using R.

4 Results

4.1 Marsh characterisation

Bulk density, soil carbon stock, soil moisture content, soil temperature and below-ground plant biomass were all significantly higher on the grazed marsh in comparison to the un-grazed marsh. Soil pH, water infiltration rate, ANPP, above-ground plant

biomass, litter biomass and vegetation height were all significantly greater on the un-grazed marsh (Table 1). Soil salinity and organic matter content were not significantly different between treatments although salinity showed greater spatial variability (between experimental units) on the grazed marsh. Nitrate mineralisation rate was significantly greater for the un-grazed marsh but ammonium mineralisation rate was significantly greater on the grazed marsh. Total nitrogen mineralisation was not significantly different between grazing treatments (Table 1). Below-ground meso-faunal feeding activity (Figure 3), a proxy for aerobic soil conditions, was significantly greater in un-grazed than grazed marsh (ANOVA; $F = 37.37$, d.f. = 5, $p < 0.01$). Within each marsh type feeding activity was faster in summer than autumn (ANOVA; $F = 18.89$, d.f. = 10, $p < 0.01$). On the grazed marsh no feeding activity was recorded below 4.5 cm in summer and 3 cm in the autumn, indicating possible anaerobic conditions.

4.2 CH₄ chamber fluxes

CH₄ fluxes were recorded monthly from September 2010 to August 2011 (Figure 4). Mean CH₄ fluxes fell within the range of 0.01 to 1.27 mg m⁻² h⁻¹ (GCflux model 2: linear flux, mean $r^2 = 0.68$). Peak CH₄ fluxes of up to 9.82 mg m⁻² h⁻¹ on the grazed marsh and 0.28 mg m⁻² h⁻¹ on the un-grazed marsh were recorded in February, one of the most water-logged months. CH₄ production showed high spatial heterogeneity between experimental units with chambers within G1 and G2 exhibiting consistently larger fluxes than other grazed units. There were no significant differences in CH₄ fluxes with grazing treatment, either for one year's data (Figure 4) or for each month separately but there were significant differences between months (ANOVA; $F = 3.22$, d.f. = 98, $p < 0.01$). For the grazed marsh soil moisture content and the presence of *J. gerardii* both positively increased CH₄ soil flux whereas increased temperature significantly decreased CH₄ flux (moisture ANOVA; $F = 6.73$, d.f. = 50, $p < 0.05$, *Juncus* ANOVA; $F = 14.34$, d.f. = 4, $p < 0.05$, temp ANOVA; $F = 8.10$, d.f. = 50, $p < 0.01$). For the un-grazed marsh no soil or vegetation factors were significant for CH₄ flux, indicative of the negligible flux recorded for this treatment.

Table 1 Soil properties and vegetation characteristics measured from the grazed and un-grazed marsh. Sampling depths are presented alongside treatment means \pm 95% confidence intervals,

ANOVA results (n = 6) and number of replicate samples per experimental unit. For vegetation height, for each of the 6 replicates per treatment the mean of 10 measurements was used in the analysis. Org. mt indicates organic matter. This table includes some results previously published in Ford et al. (2012).

	Depth (cm)	Grazed	Un-grazed	F statistic		Rep
<i>Soil</i>						
Salinity (PSU)	0-15	2.5 ± 1.0	2.0 ± 0.7	1.78	<i>ns</i>	3
pH	0-15	7.6 ± 0.2	7.9 ± 0.2	7.49	*	3
Bulk density (g cm ⁻³)	0-15	0.8 ± 0.1	0.7 ± 0.0	11.56	*	3
Organic matter content (%)	0-15	7.4 ± 1.5	6.3 ± 0.8	0.48	<i>ns</i>	3
Carbon stock (kg C m ⁻²)	0-15	4.74 ± 0.7	3.69 ± 0.3	7.51	*	3
Moisture content (%)	0-6	52.6 ± 0.2	44.5 ± 2.5	10.32	*	6
Water infiltration rate (mm min ⁻¹)	n/a	0.02 ± 0.01	8.52 ± 2.06	182.98	*	2
Temperature (°C)	0-11	14.9 ± 0.1	14.2 ± 0.1	37.52	*	6
Nitrogen mineralisation rates						
NO ₃ ⁻ (µg N g ⁻¹ dry wt day ⁻¹)	0-15	0.04 ± 0.03	0.25 ± 0.15	32.87	*	3
NH ₄ ⁺ (µg N g ⁻¹ dry wt day ⁻¹)	0-15	0.08 ± 0.03	0.02 ± 0.02	24.59	*	3
NO ₃ ⁻ & NH ₄ ⁺ (µg N g ⁻¹ dry wt day ⁻¹)	0-15	0.12 ± 0.05	0.28 ± 0.15	2.50	<i>ns</i>	3
NO ₃ ⁻ (µg N g ⁻¹ org. mt day ⁻¹)	0-15	0.54 ± 0.48	3.75 ± 2.16	52.37	*	3
NH ₄ ⁺ (µg N g ⁻¹ org. mt day ⁻¹)	0-15	1.19 ± 0.57	0.34 ± 0.28	18.34	*	3
NO ₃ ⁻ & NH ₄ ⁺ (µg N g ⁻¹ org. mt day ⁻¹)	0-15	1.73 ± 0.76	4.10 ± 2.21	5.56	<i>ns</i>	3
<i>Vegetation</i>						
Above-ground net primary productivity (kg dry wt m ⁻² yr ⁻¹)	n/a	0.58 ± 0.10	1.20 ± 0.16	9.09	*	3
Above-ground biomass (kg dry wt m ⁻²)	n/a	0.3 ± 0.1	0.7 ± 0.1	24.15	*	5
Litter biomass (kg dry wt m ⁻²)	n/a	0.0 ± 0.0	0.3 ± 0.1	24.68	*	5
Below-ground biomass (kg dry wt m ⁻²)	0-10	3.4 ± 0.4	1.0 ± 0.2	73.86	*	5
Vegetation height (cm)	n/a	8.2 ± 0.8	19.2 ± 1.4	103.28	*	6

Significant differences between grazing treatments indicated by *(P < 0.05), ***(P < 0.01) and ****(P < 0.001). Non significant results recorded as *ns* (P > 0.05).

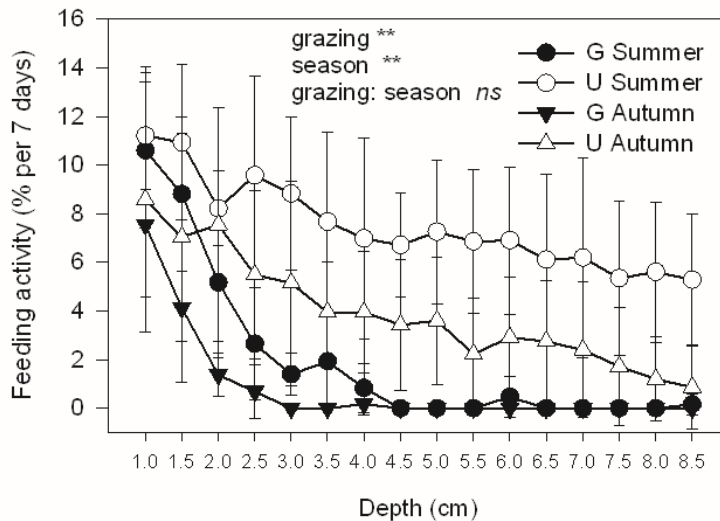


Figure 3 Below-ground meso-faunal bait lamina feeding activity for both grazing treatments (G = grazed; U = un-grazed) in summer and autumn 2009 as a function of soil depth. Values represent means \pm 95% confidence intervals. Significant differences denoted by ** ($P < 0.01$), non-significant by *ns*.

4.3 CO₂ chamber fluxes

Mean monthly CO₂ fluxes of 74 to 949 mg m⁻² h⁻¹ were recorded, up to a peak of 2570 mg m⁻² h⁻¹ on the grazed and 1811 mg m⁻² h⁻¹ on the un-grazed marsh in June, one of the warmest months (GCflux model 2: linear flux, mean $r^2 = 0.83$). Grazing, month and grazing: month interaction all had significant effects on CO₂ fluxes (Figure 4) with warmer weather linked to larger fluxes, more often on the un-grazed than the grazed marsh (grazing ANOVA; $F = 16.572$, d.f. = 5, $p < 0.05$, month ANOVA; $F = 22.68$, d.f. = 110, $p < 0.001$, month:grazing ANOVA; $F = 2.406$, d.f. = 110, $p < 0.05$). High soil temperatures and deeper water table led to greater CO₂ fluxes for both grazing management treatments (temp ANOVA; $F = 127.19$, d.f. = 117, $p < 0.001$, water ANOVA; $F = 14.885$, d.f. 105, $p < 0.001$). For both the grazed and un-grazed salt marsh CO₂ fluxes were most spatially variable (between experimental units) over the summer months.

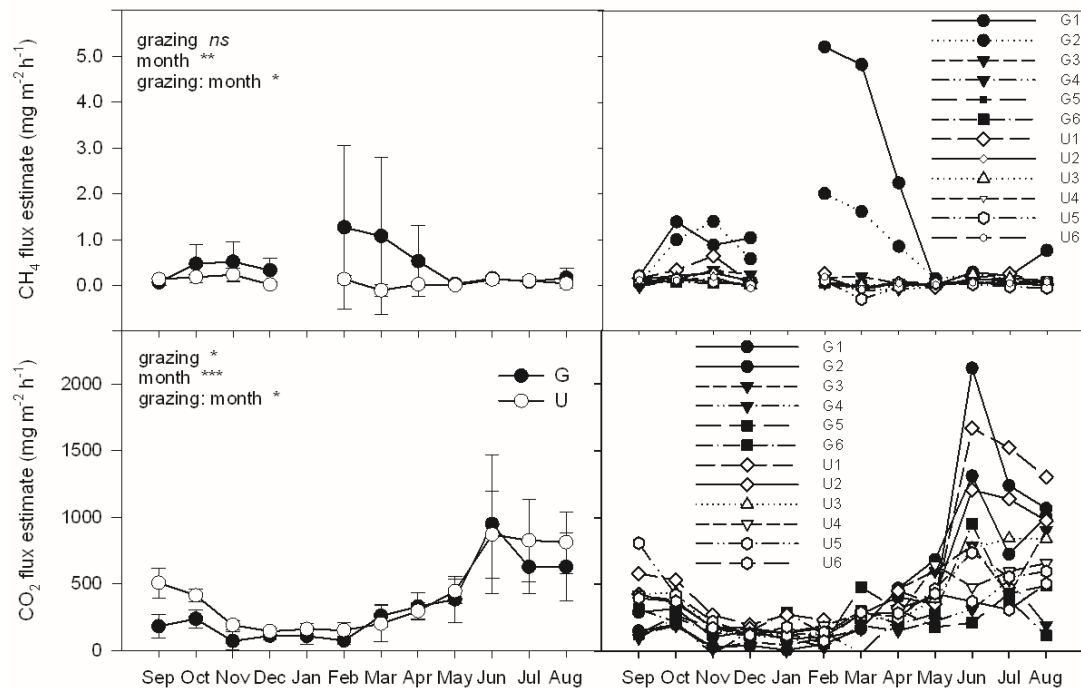


Figure 4 Monthly methane and carbon dioxide fluxes, comparison with grazing treatment (G = grazed; U = un-grazed) for September 10 to August 11. Values in left hand panels represent means \pm 95% confidence intervals. Values in right hand panels represent mean of 2 gas chambers for grazed (G1-G6) and un-grazed (U1-U6) experimental units. Significant differences between grazing treatments, month or grazing: month interaction indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$). Non significant results recorded as *ns* ($P > 0.05$).

4.4 N₂O chamber fluxes

N₂O fluxes of between -0.003 and 0.050 mg m⁻² h⁻¹ for the grazed and -0.040 and 0.005 mg m⁻² h⁻¹ for the un-grazed marsh were recorded over four months; September, November, December and January (model 2: linear flux, mean $r^2 = 0.47$); other months were excluded due to accidental moisture collection within vials leading to false peak area output on the GC. There were no significant differences in flux with grazing (ANOVA; $F = 4.47$, d.f. = 5, *ns*) and recorded fluxes were very low with a mean of 0.003 mg m⁻² h⁻¹.

4.5. CH₄ soil concentration

During the waterlogged months of October and January, underground soil CH₄ concentrations were spatially variable (between experimental units), particularly on the grazed marsh, representing between 0.002 % and 6.29 % of the total soil air volume (Figure 5). In line with the results from the static chamber fluxes, the highest percentage of CH₄ was recorded from experimental units G1 and G2. The un-grazed marsh did not accumulate high levels of CH₄, 0.001 % to 0.081 %. CH₄ was only detectable in very low concentrations in July due to the very dry conditions. Underground soil CH₄ concentration was significantly different between time periods (month ANOVA; $F = 24.24$, d.f. = 22, $p < 0.001$) but not significantly different between grazing treatments. For the grazed salt marsh, lower soil temperatures and the presence of *J. gerardii* (saltmarsh rush) within units correlated significantly with soil CH₄ concentration (temp ANOVA; $F = 4.56$, d.f. = 35, $p < 0.05$, Juncus ANOVA; $F = 7.64$, d.f. = 4, $p < 0.05$). For the un-grazed salt marsh low soil temperatures and high soil moisture content correlated with high soil CH₄ concentration (temp ANOVA; $F = 89.210$, d.f. = 22, $p < 0.001$, Moisture ANOVA; $F = 4.618$, d.f. = 22, $p < 0.05$).

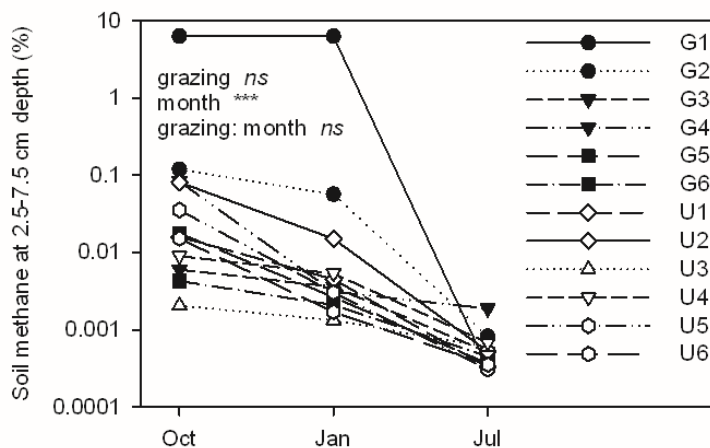


Figure 5 Influence of grazing on underground soil methane concentrations, expressed as percentage on a volumetric basis for October 2010, January and July 2011 on a log scale. Values shown for all grazed (G1-G6) and un-grazed (U1-U6) experimental units. Significant differences between grazing treatments, month or grazing: month interaction indicated by *** ($P < 0.001$). Non significant results recorded as ns ($P > 0.05$).

4.6 Environmental measurements as predictors of chamber gas fluxes

Soil temperature, measured adjacent to chambers, was significantly affected by grazing (ANOVA; $F = 8.08$, d.f. = 5, $p < 0.05$) and month (ANOVA; $F = 3962.765$, d.f. = 110, $p < 0.001$). As the interaction between grazing and month was also significant (ANOVA; $F = 19.931$, d.f. = 110, $p < 0.001$) this indicates that daytime soil temperature was higher on the grazed marsh during the spring and summer and higher on the un-grazed marsh in winter (Figure 6). Soil moisture adjacent to chambers, within the top 6 cm of soil, did not significantly alter with grazing (ANOVA; $F = 1.75$, d.f. = 5, *ns*) despite a trend towards higher moisture content in grazed soils in most months (Figure 6). The effect of month was significant (ANOVA; $F = 1.87$, d.f. = 98, $p < 0.05$). Water table level, measured within dipwells, was not significantly different between grazing treatments (ANOVA; $F = 0.02$, d.f. = 5, *ns*) but effect of sampling month was highly significant (ANOVA; $F = 57.99$, d.f. = 97, $p < 0.001$). Temperature, soil moisture, water table level and the presence of *J. gerardii* were all significant indicators of either CH₄ efflux, CO₂ efflux or soil CH₄ concentration.

4.7 Up-scaling conceptual diagram

The up-scaling conceptual diagram (Figure 7) shows no significant difference in GWP over 100 years for the grazed and un-grazed salt marsh (ANOVA; $F = 0.41$, d.f. = 5, *ns*). The GWP of the upper saltmarsh was estimated to be $\sim 2000\text{g CO}_2\text{e m}^{-2}\text{ yr}^{-1}$, regardless of grazing management.

5 Discussion

5.1 Marsh characterisation

The grazed marsh was characterised, in 2009, by compact, moist soil, anaerobic below ~ 5 cm with high available ammonium characteristic of reduced conditions, probably caused by cattle trampling. Below ground root biomass and soil carbon stock were also greater on the grazed marsh. The un-grazed marsh had more aerobic,

free draining soil, experienced a smaller range in temperature, with greater available nitrate and faster below-ground meso-faunal feeding rate than the grazed

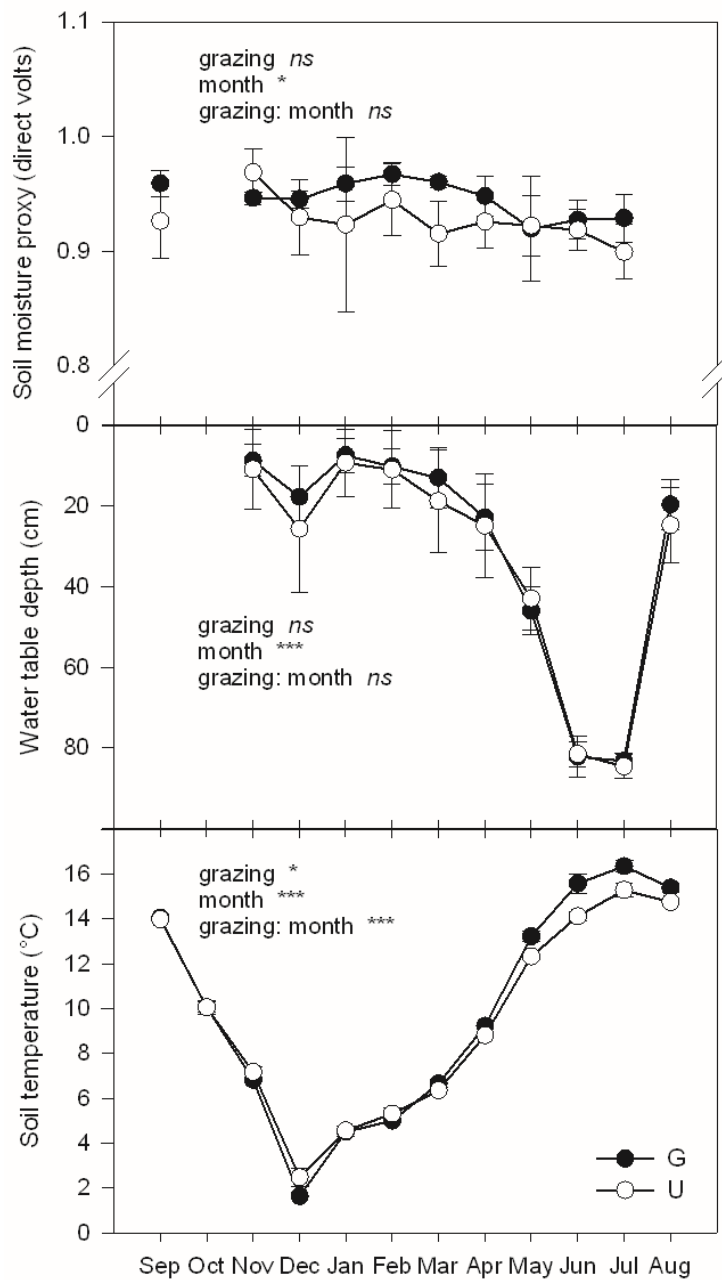


Figure 6 Influence of grazing on soil moisture, water table depth and soil temperature, measured monthly alongside gas measurements. Values represent means \pm 95% confidence intervals. Significant differences between grazing treatments, month or grazing:month interaction indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$). Non significant results recorded as *ns* ($P > 0.05$).

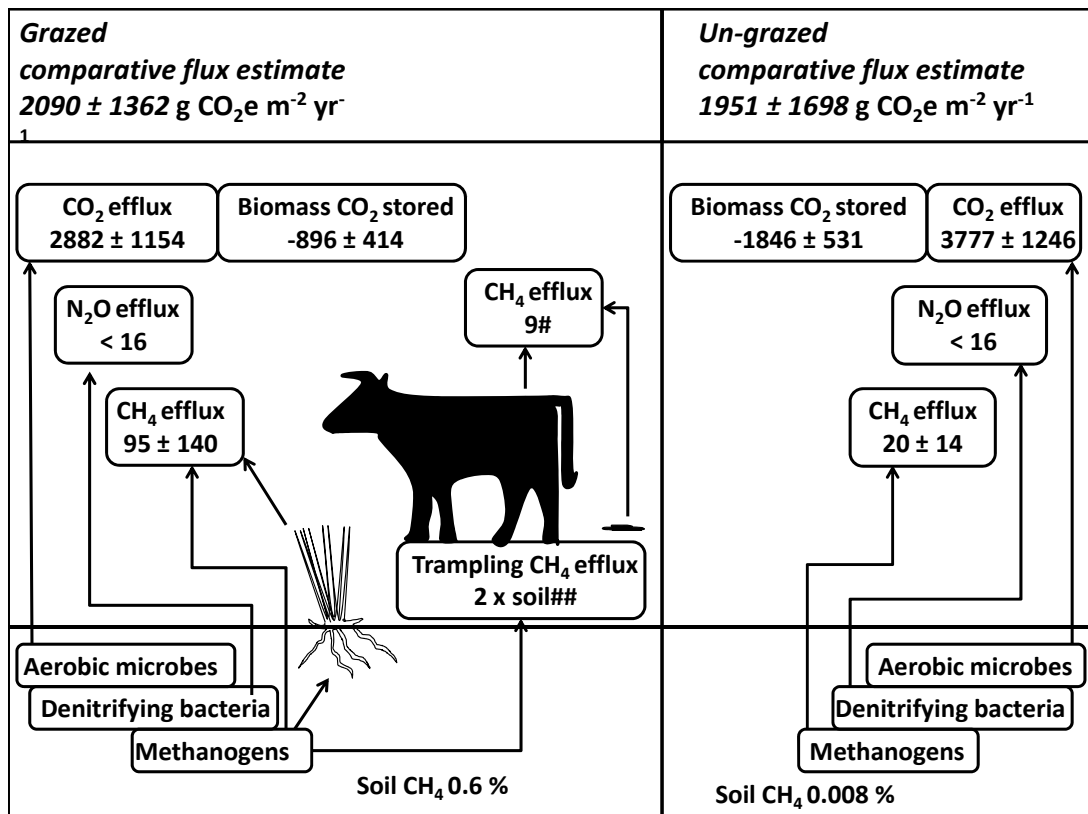


Figure 7 Up-scaled conceptual diagram for total greenhouse gas efflux from grazed and un-grazed salt marsh using yearly means (calculated from monthly means; Fig. 6.), \pm 95% confidence intervals, to give a flux estimate per year for Crossens Marsh. All gas fluxes are expressed in $\text{g CO}_2\text{e m}^{-2} \text{ yr}^{-1}$ for Global Warming Potential (GWP) of 100 years ($\text{CO}_2 = 1$, $\text{CH}_4 = 25$, $\text{N}_2\text{O} = 298$; Denman *et al.*, 2007). No significant difference in GWP between grazed and un-grazed salt marsh (ANOVA; $F = 0.41$, d.f. = 5, *ns*). # CH₄ efflux via cattle (calculated for Crossens Marsh; IPCC, 1996); ## CH₄ efflux via ebullition caused by cattle trampling (Herbst *et al.*, 2011).

marsh. Grazing intensity also clearly affected vegetation characteristics, with the grazed marsh characterised by short vegetation and greater plant diversity. Fast growing dominant species such as *E. repens* were not present on the grazed marsh allowing rushes such as *J. gerardii* to develop, in comparison the un-grazed salt marsh was mainly a monoculture of *E. repens* with no *Juncus* species present. Above-ground plant and litter biomass were greater on the un-grazed marsh.

In addition to preliminary marsh characterisation the key known drivers of greenhouse gas emissions: soil temperature, soil moisture content, water table level and plant community were measured alongside chamber measurements for the

2010-2011 experimental period. This allowed us to measure the effect of grazing and season on both environmental characteristics and greenhouse gas efflux. Results indicated that despite differences in temperature and soil moisture content between grazing treatments, the effect of month of measurement was a more important determinant of environmental characteristics than grazing treatment *per se*. For example, soil temperature spanned a range of ~15 °C over the measurement year but differences between grazing treatments were rarely greater than ~2 °C except in the summer months. Also, water table depth ranged from <30 cm over winter and spring to >80 cm in summer but remained constant across grazing treatments.

5.2 CH₄

Grazing intensity was not a significant predictor of either soil CH₄ concentration or CH₄ soil efflux. However, for the grazed salt marsh both under-ground and soil efflux CH₄ were spatially variable, with 'hotspots' occurring in conditions of high soil moisture content, low soil temperature and presence of *J. gerardii*. During the waterlogged autumn and winter months, underground soil CH₄ concentrations of up to 6 % and a peak CH₄ flux of 9.82 mg m⁻² h⁻¹ were recorded from the grazed marsh. In contrast, the highest recorded soil CH₄ level from the un-grazed marsh was 0.08 %, with a peak flux of 0.28 mg m⁻² h⁻¹. Over the one year study period, mean monthly CH₄ fluxes across both grazing treatments varied from 0.01 to 1.27 mg m⁻² h⁻¹ in line with fluxes recorded from North American and Australian salt marshes, a temperate tidal lagoon and a European flooded coastal meadow (Priemé, 1994; Deborde *et al.*, 2010; Chmura *et al.*, 2011; Livesley & Andrusiak, 2012) but greater than those recorded from a UK salt marsh (Dausse *et al.*, 2012). Our variable soil CH₄ fluxes support the recent review by Poffenbarger *et al.* (2011), that oligohaline marshes have more temporally and spatially variable emissions than previously thought.

It is well known that CH₄ efflux is increased by anaerobic waterlogged soils (Ding *et al.*, 2004; Kankaala *et al.*, 2005). It is more unusual for soil CH₄ flux to be correlated with low temperatures, as in this study. In fact, high soil temperatures are usually indicative of high CH₄ efflux (Le Mer & Roger, 2001). This unexpected result can be explained by high temperatures being correlated to drier summer months, where

CH₄ flux was minimal or absent. The positive relationship between *Juncus* within plots and CH₄ flux may be due to *Juncus* itself, or the conditions it needs to grow. Roslev and King (1996) found that *Juncus effusus* stems, growing in a freshwater marsh, act as a conduit for CH₄ release from soil. In addition, the largest CH₄ efflux from a North American salt marsh was recorded from a mixed *Juncus* – *Carex* plant community (Magenheimer *et al.*, 1996). Plant mediated CH₄ transport, via aerenchyma, may account for up to 90 % of total soil CH₄ efflux in vegetated marshes (Livingston & Hutchinson, 1995; Van der Nat & Middelburg, 1998; Van der Nat & Middelburg, 2000). Methanogens are usually most active at neutral or slightly alkaline conditions (Le Mer & Roger, 2001), as provided by both grazing treatments in this study. Soil organic matter content was not found to be indicative of CH₄ efflux in the study salt marsh. As peak CH₄ soil efflux was relatively low compared to the high CH₄ concentration found under the soil on the grazed upper salt marsh, it is likely that the majority of CH₄ produced is subsequently oxidised by methanotrophs at the soil surface or rhizosphere where oxic conditions exist (Ma & Lu, 2011). As livestock grazed salt marshes are often characterised by compact soil, prone to waterlogging, and the presence of *Juncus* species (Bakker *et al.*, 1993; Lambert, 2000; Bos *et al.*, 2002) it is possible that this management may increase soil CH₄ efflux.

5.3 CO₂

Grazing was a significant predictor of CO₂ efflux, with greater fluxes recorded from the un-grazed marsh throughout summer, autumn and winter but from the grazed marsh during spring. CO₂ efflux was of greater magnitude than the CH₄ efflux. With mean annual fluxes of 420 mg m⁻² h⁻¹ for un-grazed and 333 mg m⁻² h⁻¹ for the grazed marsh, up to a peak of 2570 mg m⁻² h⁻¹ in summer, these values are broadly comparable to both UK and North American salt marshes (Chmura *et al.*, 2011; Dausse *et al.*, 2012), and illustrate the importance of season to the magnitude of CO₂ flux. We can infer from biomass and ANPP measurements that above-ground carbon storage was greater in un-grazed salt marsh. In contrast, root and soil carbon stocks were greater in the grazed marsh as in Allard *et al.* (2007). High rates of CO₂ efflux, from both grazing treatments, were positively predicted by a deeper water table, indicative of aerobic soil and higher soil temperatures as in Luo & Zhou (2006).

Studies from grasslands, tidal flats and saltmarshes show that soil CO₂ efflux is consistently amplified by increasing soil or air temperature (Raich, 1992; Klassen & Spilmont, 2012). We therefore suggest that temperature fluctuation due to seasonal trends and future climate change are more important to the carbon budget of temperate salt marshes than grazing management.

5.4 N₂O

Grazing was not a significant predictor of N₂O soil emissions throughout the winter months. The grazed marsh had higher ammonium mineralisation rates but lower nitrate mineralisation rates than the un-grazed salt marsh. As nitrates are more readily converted to N₂O than ammonium it might be expected that the un-grazed marsh would produce more N₂O than the grazed marsh but this was not the case. Higher fluxes of N₂O, up to 0.05 mg m⁻² h⁻¹ were recorded from the grazed marsh, compared to a maximum of 0.005 mg m⁻² h⁻¹ from the un-grazed marsh. These results are comparable to cattle grazed and un-grazed New Zealand grassland (Saggar *et al.*, 2007) but lower than values recorded for a UK saltmarsh (Blackwell *et al.*, 2010). As the recorded fluxes were very low and not predicted by any measured soil or vegetation characteristics we regard the upper saltmarsh as neither a source nor sink of N₂O.

5.5 Validity of up-scaling

Static chambers are perhaps not the best way to measure overall greenhouse gas budgets for a habitat such as a saltmarsh due to temporal and spatial flux variations (Denman *et al.*, 2007). However, they are an essential tool in the measurement of treatment differences such as grazing intensity that would be largely impossible with the eddy covariance technique (Sullivan *et al.*, 2010), which is more applicable to catchment scale measurements. Within this study we provided a conceptual diagram (Figure 7) of mean comparative yearly flux estimates of GWP for grazed and un-grazed saltmarsh and found that grazing management does not significantly alter GWP. This comparative approach was justified as sampling monthly for one year provided a fuller picture of soil greenhouse gas efflux than sampling just once or twice as was common in previous saltmarsh studies (Lindau & Delaune, 1991; Wang

et al., 2007; Dausse *et al.*, 2012). In order to make this up-scaling exercise more realistic, in addition to directly measured soil greenhouse gas emissions and ANPP, CH₄ efflux via cattle (enteric and waste) was also estimated based on cattle intensity at the study site. Cattle may also increase soil CH₄ efflux via trampling. Herbst *et al.* (2011) found that CH₄ flux doubled from background 'soil' levels when cows grazed in the vicinity of an eddy covariance tower, although part of this effect may be due to CH₄ released directly from the cows, it is also likely that part of this effect is CH₄ ebullition via trampling. This potential CH₄ source would be greatest when livestock were present on the salt marsh during waterlogged times of year. In this study CO₂ soil efflux was responsible for a much larger proportion of GWP than CH₄ efflux, only partially offset by the CO₂ 'locked up' in plant biomass (Figure 7). The conditions needed for high rates of soil respiration, a low water table and warm soil, were the opposite of the cooler waterlogged soil conditions that stimulated soil CH₄ efflux. Where N₂O soil efflux was measured it did not contribute markedly to GWP. As GWPs for grazed and un-grazed saltmarsh were estimated from a combination of chamber measurements and ANPP it is not possible to directly compare the estimated GWP of ~2000 g CO₂e m⁻² yr⁻¹, regardless of grazing intensity, to the GWP of other habitats. However this flux was of comparative magnitude to a North American peat land (Strack & Waddington, 2007).

6 Conclusion

In this study it was hypothesised that livestock grazing management would influence soil physical characteristics (e.g. soil temperature and moisture content) and plant community composition (e.g. presence of *Juncus* species) that would in turn regulate the CH₄, CO₂ and N₂O fluxes of saltmarsh habitats. Our results showed that soil temperature, soil moisture content, water table depth and the presence of *J. gerardii* were the most significant predictors of saltmarsh greenhouse gas flux. However, the effect of grazing intensity on these variables was small compared to the much greater impact of seasonal variability. Our first hypothesis was refuted, as soil methane efflux was not consistently greater in the cattle grazed than the un-grazed salt marsh. However, 'hot spots' of both underground soil methane concentration and soil methane efflux were only present on the grazed marsh, occurring under conditions

of high soil moisture content, low soil temperatures and the presence of *J. gerardii*. Our second hypothesis, that combined soil respiration and plant carbon dioxide efflux would be greater in the un-grazed marsh, was partially substantiated as CO₂ efflux was greater from the un-grazed marsh throughout the majority of the year and was positively correlated with deeper water table and higher soil temperatures. Our third hypothesis, that soil nitrous oxide efflux would be consistently greater from the grazed salt marsh was refuted due to lack of evidence. Grazing was not a significant predictor of N₂O soil emissions. The GWP (100 years) of a temperate upper salt marsh, calculated from mean yearly chamber fluxes of greenhouse gases (CH₄ and CO₂) and offset by ANPP, was not significantly altered by livestock grazing management.

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