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Cattle grazing drives nitrogen and carbon cycling in a temperate salt marsh

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ABSTRACT

We examined the impact of long-term cattle grazing on soil processes and microbial activity in a temperate salt marsh. Soil conditions, microbial biomass and respiration, mineralization and denitrification rates were measured in upper salt marsh that had been ungrazed or cattle grazed for several decades. Increased microbial biomass and soil respiration were observed in grazed marsh, most likely stimulated by enhanced rates of root turnover and root exudation. We found a significant positive effect of grazing on potential N mineralization rates measured in the laboratory, but this difference did not translate to *in situ* net mineralization measured monthly from May to September. Rates of denitrification were lowest in the grazed marsh and appeared to be limited by nitrate availability, possibly due to more anoxic conditions and lower rates of nitrification. The major effect of grazing on N cycling therefore appeared to be in limiting losses of N through denitrification, which may lead to enhanced nutrient availability to saltmarsh plants, but a reduced ability of the marsh to act as a buffer for land-derived nutrients to adjacent coastal areas. Additionally, we investigated if grazing influences the rates of turnover of labile and refractory C in saltmarsh soils by adding ^{14}C-labelled leaf litter or root exudates to soil samples and monitoring the evolution of $^{14}CO_2$. Grazing had little effect on the rates of mineralization of ^{14}C used as a respiratory substrate, but a larger proportion of ^{14}C was partitioned into microbial biomass and immobilized in long- and medium-term storage pools in the grazed treatment. Grazing slowed down the turnover of the microbial biomass, which resulted in longer turnover times for both leaf litter and root exudates. Grazing may therefore affect the longevity of C in the soil and alter C storage and utilization pathways in the microbial community.

1. Introduction

Grazing can have a profound effect on microbial communities, breakdown of organic matter and rates of nutrient cycling in terrestrial habitats (Holland and Detling, 1990; Pastor and Cohen, 1997; Frank and Groffman, 1998b; Belovsky and Slade, 2000; Baron et al., 2002). In salt marshes, the effects of grazing on plant communities (Bakker, 1985; Andresen et al., 1990; Bouchard et al., 2003; Kleyer et al., 2003), nitrogen mineralization (Wilson and Jefferies, 1996; Van Wijnen et al., 1999; Kiehl et al., 2001) and microbial activity (Buckridge and Jefferies, 2007) have been investigated, but we still have limited understanding of how herbivory impacts cycling of nitrogen (N) and carbon (C). Salt marshes differ from other terrestrial systems since they are inundated by tides that saturate the soil and limit oxygen penetration. A well-defined vertical biogeochemical zonation tends to develop (Sørensen et al., 1979; Herbert, 1999), but the anaerobic microbial component in saltmarsh soils is dominated by sulfate reducers that control energy flow and greatly influence biogeochemical cycles (Howarth and Teal, 1980). The unique properties of salt marshes mean that grazing may have different consequences for biogeochemical cycling compared to in other terrestrial environments. It is critical to understand the effect of grazing on cycling of C and N in salt marshes to be able to predict how their roles as major C sinks (Chmura et al., 2003; IUCN, 2009) and as sinks or sources of N (Herbert, 1999) are affected.

European salt marshes are widely used for sheep and cattle grazing. In addition to providing farming revenue, grazing is used as a management tool for conservation of plant diversity and to enhance habitat diversity for birdlife (Bouchard et al., 2003; Kleyer et al., 2003). Herbivores have been found to increase (Holland and Detling, 1990; Frank and Groffman, 1998b; Tracy and Frank, 1998;

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Rossignol et al., 2006) or decrease (Van Wijnen et al., 1999; Bakker et al., 2004) rates of nutrient cycling in grasslands through changes to the plant community and the abiotic environment. Three major mechanisms are thought to affect the rates of cycling of C and N: 1) changes in the quantity and 2) quality of resources available to microbes, and 3) alteration of soil abiotic properties (Van Wijnen et al., 1999; Bardgett and Wardle, 2003).

Moderate grazing can lead to increased net primary production (Milchunas and Lauenroth, 1993; Singer and Schoenecker, 2003), but both productivity and plant diversity are typically reduced under heavy grazing pressure (Semmartin and Oesterheld, 2001; Baron et al., 2002). Generally, grazing tends to reduce the standing crop of vegetation (Rusch and Oesterheld, 1997; Pucheta et al., 1998; Fahnestock and Detling, 2002) and prevent litter accumulation, which in turn may reduce mineralization and nutrient cycling rates (Bakker et al., 1983; Bazely and Jeffries, 1985; Van Wijnen et al., 1999). Herbivory can also regulate the magnitude and quality of C and N inputs to soil indirectly by changing root turnover and exudation patterns (Bardgett et al., 1998; Hamilton and Frank, 2001; Frank et al., 2002). Root exudation influences the size and activity of the soil microbial community, which is typically limited by the availability of dissolved organic C, and is a key driver of soil respiration rates and C cycling (Raich and Tufekcioglu, 2000; Toal et al., 2000; Nguyen and Henry, 2002).

Herbivory also regulates the quality of organic matter in two main ways: First, grazing can change litter decomposition rates by decreasing (Hobbie, 1992; Pastor et al., 1993) or enhancing (Pastor et al., 1993) the nutritional quality of plant tissue (Wedin, 1995; Pastor and Cohen, 1997). Second, herbivores can return highly decomposable organic matter as dung and urine that is rich in labile nutrients, but patchy in distribution both spatially and temporally (Bardgett et al., 1998; Frank et al., 2000; Bakker et al., 2004).

Grazing can also alter abiotic soil properties such as bulk density, water content, salinity, aeration, and temperature, which influence microbial activity and nutrient cycling processes (Bardgett et al., 1998; Van Wijnen et al., 1999; Bakker et al., 2005). In addition to compacting the soil, which alters water infiltration rates and drainage, changes to vegetation and litter induced by grazers change the amount of shading and evapotranspiration (Naeth and Chanasyk, 1995; Krümmelbein et al., 2009). Grazing can thereby indirectly affect soil moisture and temperature, two key factors controlling decomposition and mineralization rates (Sierra, 1997; Kiehl et al., 2001; Theodose and Martin, 2003). Moisture regulates oxygen diffusion into the soil and therefore modifies anaerobic processes, such as denitrification. The impact of grazing on denitrification has been described for grasslands, where increased amounts of labile C made available by grazers increased rates of denitrification (Frank and Groffman, 1998a; Frank et al., 2000; Le Roux et al., 2003). There is, to our knowledge, no published investigation of how grazing affects denitrification in salt marshes, but the same herbivore-induced changes that are described above are likely to influence rates.

The purpose of this study was to examine the impact of long-term cattle grazing on soil processes and microbial activity in a temperate salt marsh and the implications for C and N turnover. We expected cattle grazing to improve the quality of organic matter available to microbes by improving the nutritional quality of the plant biomass, stimulating root turnover and exudation of labile C from roots, and by deposition of N-rich dung and urine. We therefore hypothesized first that rates of mineralization of C and N would be higher in the grazed marsh and second that the increased availability of high-quality organic matter would increase microbial biomass and activity. We expected trampling and compaction by the cattle to increase soil moisture and limit oxygen penetration making soil conditions more anoxic. Our third hypothesis was therefore that rates of denitrification would be higher under grazed conditions. To test these hypotheses we first examined how grazing influenced N cycling by measuring mineralization and denitrification rates. Second, to investigate the role of grazing in regulating microbial activity and C cycling, we measured microbial biomass and respiration and the rates of turnover of plant litter and root exudates.

2. Materials and methods

2.1. Site description

This study was carried out from May to September 2009 in the Ribble estuary, NW England (53° 41′ 0″ N, 2° 58′ 0″ W). The Ribble is a coastal plain estuary with an intertidal area of around 10,000 ha and a tidal range of 7.9 m. The soil is classified as Saltmarsh Solonchak (sulphidic supratidal hydrosoil). Salt marshes cover over 2000 ha of the estuary and constitute one of the largest areas of unbroken salt marsh in Britain.

The area of marsh included in this study is located in the southern part of the estuary and is managed to enhance habitat diversity for birds. The marsh has been split into two management types for over four decades; ungrazed and cattle grazed, with a fence line restricting cattle grazing. The grazed marsh within the study area covers an area of 515 ha where an average of 100 cattle (0.2 cattle ha^{-1}) feed between May and early October.

Sampling was carried out within the high marsh zone where numerous creeks are present but tidal inundations are limited to between 6 and 10 events a year on the highest equinoxial tides. Six experimental units or plots of approximately 10 m × 10 m were set up in each of the grazed and ungrazed areas. All plots were within the same elevation zone (±10 cm), located 100–150 m apart and between 20 and 50 m from the fence line to ensure an adequate buffer zone adjacent to the fence. Sampling times and numbers of replicates are indicated in Table 1.

Table 1
Soil properties, microbial biomass and soil respiration (means ± SE) measured in the top 10 cm of the grazed and ungrazed marsh. Means and standard errors were calculated using monthly means of the cores collected within each plot. Significant differences between grazed and ungrazed treatments are indicated by *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$), while *ns* indicates no significant difference ($p > 0.05$). The number of samples per treatment (n) and the months the samples were collected are given.

	Grazed	Ungrazed		n	Month sampled
pH	7.4 ± 0.1	8.2 ± 0.2	*	6	Sept[a]
Bulk density (g cm^{-3})	0.8 ± 0.0	0.6 ± 0.1	*	6	Sept[a]
Moisture content (%)	52 ± 0.2	44 ± 1	***	6	July–Sept[b]
Soil conductivity (volts)	1.0 ± 0.0	0.8 ± 0.0	**	6	July–Sept[b]
Soil temperature (°C)	16 ± 0.3	14 ± 0.3	***	6	July–Sept[b]
Soil organic matter content (%)	15 ± 0.4	12 ± 0.4	*	3	May–Sept[c]
Soil nutrients (μmol kg^{-1} dry wt)					
NO_2^-	3 ± 1	9 ± 2	*ns*	3	May–Sept[c]
NO_3^-	18 ± 4	85 ± 14	*	3	May–Sept[c]
NH_4^+	278 ± 17	296 ± 20	*ns*	3	May–Sept[c]
Total inorganic N	300 ± 17	392 ± 15	***	3	May–Sept[c]
Respiration (pmol CO_2 s^{-1} cm^{-3} soil)	100 ± 6	59 ± 6	**	6	Sept[a]
Microbial biomass (mg g^{-1} dry soil)					
C	2.5 ± 0.5	1.1 ± 0.1	*	6	Sept[a]
N	0.3 ± 0.0	0.2 ± 0.0	*	6	Sept[a]
C:N	7.6 ± 0.7	5.8 ± 0.8	*ns*	6	Sept[a]

[a] Samples collected only once and data analyzed using *t*-tests.
[b] Samples collected bi-weekly and data analyzed using repeated measures ANOVA.
[c] Samples collected once a month and data analyzed using repeated measures ANOVA.

2.2. Soil and vegetation characteristics

Moisture content, soil conductivity, and temperature were measured at six locations in each plot bi-weekly from June to September 2009. Temperature measurements were taken using a Whatman thermometer single probe inserted vertically into the soil to a depth of 11 cm and left for 30 s to equilibrate before the temperature was recorded. Soil conductivity was measured using a ML2x ThetaProbe (Delta-T Devices Ltd, Cambridge, UK) inserted into the soil to a depth of 6 cm. Soil conductivity measurements were recorded directly in volts and converted to percentage moisture content using a calibration suitable for organic soils (>10% organic matter).

Bulk density was measured on a 5 cm diameter core collected from the top 10 cm of soil in each of the grazed and ungrazed plots in September. Whole cores were dried for at least 48 h at 80 °C until constant weight and their dry mass divided by the volume of the core.

Organic matter content was measured monthly in the top 10 cm of soil in five samples from three plots within each treatment from May to September 2009. Approximately 5 g of soil was weighed out and organic matter content was determined as the percent weight loss after ignition at 550 °C for 5 h.

Soil pH was determined on soil collected in each of the six ungrazed and grazed plots in September. Approximately 5 g of moist soil was shaken with 12.5 ml deionized water (1:2.5 dilution by weight) before measuring the pH with a Hanna pH209 pH meter.

Plant diversity and percentage cover were estimated by eye in five 1 m × 1 m quadrats randomly placed within each grazed and ungrazed plot in July 2009. Plant cover of up to 120% was recorded to allow for layering effects. Plants were identified to species level according to nomenclature by Stace (1997). To estimate above-ground biomass, surface vegetation was collected from an area of 25 cm × 50 cm within each quadrat. Belowground biomass was estimated by taking one soil core of 5 cm diameter by 10 cm depth in each quadrat. Root cores were rinsed under running water above a 250 μm sieve to remove adhering soil. Dry weights of vegetation and root samples were determined after drying the samples at 80 °C for 24 h.

2.3. Microbial biomass and soil respiration

Soil microbial biomass was estimated in September 2009 using the $CHCl_3$-fumigation–extraction procedure of Brookes et al. (1985). Briefly, two aliquots of 5 g of soil from the top 10 cm of each plot in the grazed and ungrazed marsh were weighed out. One of each pair of aliquots was immediately extracted in 25 ml of 0.5 M K_2SO_4. The second aliquot was fumigated with $CHCl_3$ for 24 h at room temperature to lyse any microbial cells and release intracellular N and C before extraction in 25 ml of 0.5 M K_2SO_4. The extracts were centrifuged and filtered before analysis of C and N on a Shimadzu TOC-V–TN analyzer (Shimadzu Corporation, Kyoto, Japan). Microbial C and N were calculated using the equations.

$$\text{Soil microbial biomass C} = \left(C_f - C_{uf}\right) \Big/ K_{EC} \quad (1)$$

$$\text{Soil microbial biomass N} = \left(N_f - N_{uf}\right) \Big/ K_{EN} \quad (2)$$

where C_f and N_f are the C and N extracted from the fumigated soil and C_{uf} and N_{uf} are the C and N extracted from the unfumigated soil. Corrections for the extraction efficiency were made using $K_{EC} = 0.45$ for C (Beck et al., 1997) and $K_{EN} = 0.54$ for N (Brookes et al., 1985).

To measure microbial and root respiration in the soil, a 2.5 cm diameter core was collected from the top 10 cm of marsh in each plot in September. Basal soil respiration of each field-moist core was measured on an SR1 Automated Multichannel Respirometer (PP Systems Ltd, Hitchin, UK) at 20 °C over a 1.5 h period once the rate of respiration had stabilized.

2.4. Extractable inorganic N and net nitrogen mineralization

Soil inorganic N content and *in situ* net N mineralization were measured monthly in three of the grazed and ungrazed plots from May to September 2009 according to methods by Hazelden and Boorman (1999). On each sampling date, five pairs of soil cores (6 cm diameter) were collected in each plot from the upper 10 cm of soil using a metal corer. One of each pair of cores was kept cool and brought to the laboratory. The other core was wrapped in a gas-permeable plastic bag, re-buried in the soil and incubated *in situ* for a month. Each month the incubated cores were removed from the site and the sampling procedure repeated.

In the laboratory, soil cores were homogenized and large roots removed. From each of the initial and the incubated cores, 25 g of moist soil were extracted with 60 ml of 1 M KCl for 1 h, centrifuged, and the supernatant filtered through GF/F filters and frozen for subsequent analysis. A further 30 g of each soil sample was dried at 80 °C for at least 48 h to determine moisture content. The concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) in soil extracts were determined by standard colorimetric methods (Grasshoff et al., 1983) adapted for flow injection analysis on a LACHAT Instruments Quick-Chem 8000 autoanalyzer (Hales et al., 2004). The concentration of ammonium (NH_4^+) was determined with the fluorometric method of Holmes et al. (1999) using a HITACHI F2000 fluorescence spectrophotometer. Nutrient concentrations were expressed per soil dry weight. The daily net mineralization rates were considered to be the difference in N between the initial and incubated soil cores divided by the number of days of incubation and were expressed per unit area of salt marsh (kg N ha^{-1} day^{-1}). To compare the production of N across the whole sampling period, we calculated the average daily production of NO_3^- and NH_4^+ from the monthly mean mineralization rates.

2.5. Potential N mineralization

Cores for potential mineralization measurements were collected in September 2009 from the top 10 cm of soil in each grazed and ungrazed plot ($n = 6$). The cores were kept cool and brought to the laboratory, where they were homogenized and two 5 g aliquots of each core weighed out. One of these was incubated with 30 ml deionized water in a sealed 70 ml vial at 40 °C for 21 d (Russell et al., 2006). The other aliquot was immediately extracted in 40 ml of 2 M KCl for 1 h. Upon completion, the incubated samples were extracted in the same manner. Extracts were centrifuged, filtered through GF/F filters and frozen for subsequent analysis. Nutrient analyses were performed using the methods described above. Potential net N mineralization was calculated as the difference in the concentration of N per dry weight of soil between the incubated and the initial.

2.6. Denitrification

Basal and potential denitrification rates were measured using the method of Drury et al. (2006). Briefly, cores were collected in September 2009 from the top 10 cm of soil in each of the six grazed and ungrazed plots. The cores were kept cool and brought to the laboratory, where they were homogenized. Two 30 g aliquots of field-moist soil from each core were placed in two 250 ml bottles, one for basal and one for potential denitrification measurements.

For basal denitrification, 30 ml of deionized water was added to each soil sample. For potential denitrification, 30 ml of deionized water containing 9 mg of glucose and 1.5 mg of NO_3^- was added. Each bottle was sealed with a cap fitted with a silicone septum to enable collection of gas samples. Soil slurries were shaken and the headspace in the flask flushed with N_2 gas and 10% acetylene. Gas samples were taken with a syringe after 1, 2, 3, and 5 h, injected into evacuated gas chromatograph vials and stored for analysis. After collection of each sample, a mixture of N_2 gas and 10% acetylene of the same volume as the sample was injected back into the incubation bottle to restore the internal pressure. Gas analysis was carried out using a Perkin Elmer Clarus 500 Gas Chromatograph (GC) equipped with a Porapaq QS (80–100 mesh) analytical column and turbomatrix 40 headspace autoanalyzer. N_2O was detected using ECD (at 400 °C, sample oven at 40 °C). The carrier gas pressure was 138 kPa, and the injection pressure 160 kPa, with all other controls as defined by the Perkin Elmer standard setup. The volume of N_2O evolved was adjusted via the Bunsen absorption coefficient (0.632 ml N_2O ml^{-1} water at 20 °C; Tiedje, 1982) to account for dissolution in the soil solution. Denitrification rates were calculated per unit mass of dry soil.

2.7. Carbon turnover

To investigate how grazing might influence C turnover and microbial yield, degradation rates of two contrasting ^{14}C-labelled substrates were compared. To examine turnover of a complex C source we used ^{14}C-labelled leaf litter from ryegrass (*Lolium perenne* L.) with an activity of 12.3 kBq g^{-1}. The leaf litter was pulse-labelled in an atmosphere of $^{14}CO_2$ as described in Hill et al. (2007) and the plant material subsequently harvested after 7 d. The plant material was then dried, ground to pass 1 mm and its ^{14}C distribution into water, alcohol and acid soluble and acid insoluble components determined by chemical fractionation using the method of Jones and Darrah (1994). 50 mg of finely ground plant material was extracted sequentially in 8 ml deionized water for 30 min at 85 °C, 8 ml 20% ethanol for 30 min at 80 °C, 5 ml 0.3% HCl for 3 h at 95 °C, and 5 ml 1 M NaOH for 1 h at 95 °C. After each extraction, the sample was centrifuged at 5000 g for 15 min and the supernatant removed for ^{14}C analysis by liquid scintillation counting. Subsequent analysis revealed 32.9 ± 1.5% of the ^{14}C label was water soluble, 4.2 ± 0.2% was ethanol soluble, 16.8 ± 0.6% could be extracted by HCl, 27.5 ± 0.4% by NaOH and 18.5 ± 2.2% remained insoluble.

To examine the turnover rate of low molecular weight (LMW) compounds, we used a mixture composed to mimic plant root exudates. The mixture contained the dominant root exudate components and was made up of ^{14}C-labelled glucose (50 mM), fructose (5 mM), sucrose (5 mM), citrate (10 mM), malate (5 mM) and succinate (2 mM) with a total activity of 8.4 Bq μmol^{-1} C (Jones et al., 2004). Two cores (2.5 cm diameter) were collected from surface soil (0–10 cm) in September 2009 in each grazed and ungrazed plot. The cores were cut into 2 cm pieces, placed in glass jars and 100 mg of ^{14}C-labelled leaf litter (0.02 kBq g^{-1} soil) or 0.5 ml of ^{14}C-labelled root exudates (0.03 kBq g^{-1} soil) were added to the field-moist soil. To capture $^{14}CO_2$ evolved from the soil, a vial containing 1 ml of 1 M NaOH was placed inside each jar before sealing the jars. The soils were incubated at 20 °C. After known incubation times (0.5, 1, 3, 6, 9, 24, 48, 96, 192, 336, 504, 672, 864, 1176, and 1512 h) the NaOH traps were changed and the amount of trapped $^{14}CO_2$ determined using a Wallac 1409 liquid scintillation counter (EG&G Ltd, Milton Keynes, UK) and Wallac Optiphase 3 scintillation fluid (EG&G Ltd).

Substrate mineralization in most soils has a biphasic pattern of $^{14}CO_2$ production with an initial rapid phase followed by a slower secondary phase of production (Nguyen and Guckert, 2001; Boddy et al., 2007; Oburger and Jones, 2009). The first phase approximates the depletion of the ^{14}C-labelled substrate from the soil solution. The slower second phase is attributable to the turnover of the substrate after incorporation into the microbial biomass in the form of secondary metabolites and their subsequent microbial turnover. For leaf litter, the breakdown is likely more complex, but we can assume that there is an initial pool of low molecular weight C that is readily available (e.g. sugars, organic acids, amino acids, soluble protein) (Vaieretti et al., 2005). This pool was estimated to be around 37% based on the fractions of the ^{14}C in the plant material that were extractable by water and ethanol. This LMW carbon can be rapidly leached into the soil solution and is expected to reflect the turnover of labile C. During the slow second phase of processing, the more recalcitrant C in the litter (e.g. cellulose, hemicelluloses, lignin) will continue being broken down and incorporated into the microbial biomass at the same time as the microbes themselves will turn over. For both the leaf litter and the root exudate mixture, a double exponential decay model was used to describe the mineralization kinetics. This commonly used model can only separate substrates into two unconnected pools, however, we acknowledge that this is a simplification, when in fact we are dealing with many pools with different levels of connectivity. The model has to be considered as a summation for all the different medium- and long-term C pathways. Despite this limitation, the model results provide a valuable estimation for the dominating processes (Boddy et al., 2007; Oburger and Jones, 2009). We therefore fitted a double first-order exponential decay model to the data using Sigmaplot.

$$S = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \quad (3)$$

where S is the proportion of ^{14}C remaining in the soil (expressed as % of the activity added at the beginning of the experiment) at time t. Variables a_1 and a_2 describe the sizes of the C pools and the rate constants k_1 and k_2 the degradation rates of the rapid and slow phases respectively.

The half-life ($t_{1/2}$) of the first pool can be calculated using a first-order kinetic model defined by the equation.

$$t_{1/2} = \ln(2)/K_1 \quad (4)$$

where k_1 is the rate constant for the degradation rate of the pool. The half-life for pool a_2 cannot be calculated because we do not know enough about its connectivity to a_1 (Boddy et al., 2007).

Equation (3) cannot be solved explicitly for the half-life of the substrate (the half-life for the slow and rapid pools combined). We therefore calculated the half-life for the substrate in two steps following methods by Boddy et al. (2007, 2008). First, we expressed the proportion of ^{14}C added remaining in the soil (S) at $t_{1/2}$ (the half-life of the substrate) in terms of the sizes of the two pools, a_1 and a_2 using the relationship.

$$S_{t_{1/2}} = \frac{(a_1 + a_2)}{2} \quad (5)$$

Second, after substitution of equation (5) into equation (3), the half-life was computed numerically by applying the Newton–Rhapson method. The microbial yield (Y), also termed C use efficiency, was calculated as the amount of C partitioned into microbial biomass as a proportion of the total C added.

$$Y = \frac{a_2}{(a_1 + a_2)} \quad (6)$$

2.8. Statistical analysis

Before calculating means and standard errors for the grazed and ungrazed treatments, data from measurements taken within each

plot were pooled and averaged. Statistical analysis was then performed at the plot level. Differences in soil and vegetation properties, microbial biomass and soil respiration, average daily rates of *in situ* mineralization, potential mineralization and denitrification rates between grazed and ungrazed salt marsh were evaluated by paired *t*-tests after using the Levene's test to check for unequal variances. Data that were collected monthly or bi-weekly (temperature, field moisture, soil nutrients, soil organic matter content, and *in situ* mineralization rates) were evaluated using repeated measure ANOVAs. The assumption of sphericity was evaluated and a Huynh–Feldt epsilon correction was applied to the *p*-value when required. A post-hoc test with a Bonferoni correction was used to test for within-subject effects.

Parameters of the double exponential equations fitted to the ^{14}C mineralization data were subject to a two-way ANOVA to enable comparison across grazing treatments and substrate type (leaf litter or root exudates). To ensure the data complied with the assumptions of analysis of variance (ANOVA), normality was tested using Kolmogorov–Smirnov test and homogeneity of variances tested using Levene's test.

To illustrate differences in abiotic properties between treatments and among plots, we used data of abiotic properties (moisture, organic matter content, bulk density, temperature and inorganic N) and compared these in a principal components analysis (PCA). To illustrate differences among and variation within plots we graphed data from each individual core (five per plot) collected monthly from May to September.

3. Results

3.1. Soil and vegetation characteristics

There were significant differences in several of the soil properties between the grazed and ungrazed marsh (Table 1). Bulk density, moisture, soil conductivity, temperature and organic matter content were higher in the grazed marsh, whereas soil pH was lower. There was a black anoxic layer below 4–5 cm depth in the grazed marsh, whereas no evidence of anoxia was found within the top 10 cm of the ungrazed marsh. The dominant form of inorganic N in both grazed and ungrazed marsh was NH_4^+, which made up over 75% of the total extractable inorganic N (Table 1). The grazing regime did not have an impact on the concentrations of NH_4^+ or NO_2^-, but the ungrazed marsh had significantly higher concentrations of NO_3^- and total extractable inorganic N (Fig. 1, Table 1).

The clustering of samples based on the PCA of soil abiotic conditions agreed well with observed differences in means of the soil properties (Table 1). Representative PCA results are shown for May and July in Fig. 2. Samples from the ungrazed marsh had higher concentrations of NO_3^- and samples from the grazed marsh had higher bulk density, temperature, and organic matter content. Interestingly, one of the three plots sampled in the ungrazed marsh (UG1) clustered away from the other two ungrazed plots. This site had a higher moisture and NH_4^+ contents, but lower bulk density.

The ungrazed salt marsh was dominated by the grasses *Elymus repens* (L.) and *Festuca rubra* (L.), which made up over 90% of the total cover (Table 2). *Triglochin maritima* (L.) and *Sonchus arvensis* (L.) were also common whereas all other species combined made up <2% of the ground cover. The grazed marsh was characterized by higher species richness compared to the ungrazed (Table 2). In the grazed marsh, the grasses *F. rubra* (L.), *Puccinellia maritima* (Parl.), *Agrostis stolonifera* (L.) made up the largest portion of cover (26.8%, 28.9% and 20.4% respectively). Forbs and sedges and rushes were also abundant in the grazed marsh and each made up around 12% cover.

Above-ground biomass was approximately 3 times higher in the ungrazed marsh compared to the grazed marsh (Table 2). There was also a significant difference in the belowground biomass between treatments: The ungrazed marsh had a lower biomass of roots and the root network consisted of few but coarse roots. In contrast, roots in the grazed marsh had around three times more biomass and were made up of a highly branched dense network of fine roots (Table 2).

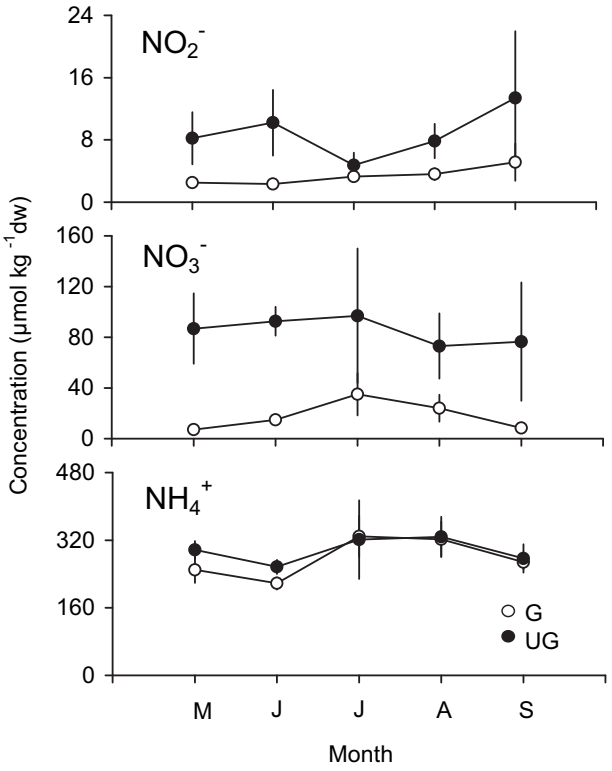

Fig. 1. Soil nutrient concentrations of NO_2^-, NO_3^- and NH_4^+ (means ± SE) measured monthly in the top 10 cm of grazed (G) and ungrazed (UG) salt marsh ($n = 3$). Treatment effects were evaluated using one-way repeated measures ANOVAs. Nitrate concentrations were significantly higher in ungrazed marsh ($p < 0.05$), but concentrations of NO_2^- ($p = 0.186$) and NH_4^+ ($p = 0.609$) did not differ between treatments.

3.2. Microbial biomass and respiration

There was a significant difference in the soil microbial biomass between the grazed and ungrazed marsh. Higher microbial C and N (Table 1; $p < 0.05$) and rates of soil respiration (Table 1; $p < 0.01$) were measured in the grazed marsh. Microbial C-to-N ratios were not affected by grazing regime (Table 1; $p = 0.09$).

3.3. Nitrogen mineralization

Grazing did not have a significant impact on net N mineralization rate (repeated measures ANOVA; $p = 0.619$). There was a significant effect of month with rates of mineralization being lower in September compared to in July ($p < 0.01$). The repeated measures ANOVA also revealed a significant interaction between treatment and time ($p < 0.05$) suggesting that the effect of grazing on mineralization depends on which month was tested (Fig. 3). In July the average rate of mineralization was more than twice as high in the grazed treatment whereas in August the highest rate was measured in the ungrazed. It is also notable that plot 1 in the ungrazed marsh (UG1) typically had higher rates of mineralization in each month compared to the other two plots sampled in the same treatment (Fig. 3, top). In August, the highest N mineralization

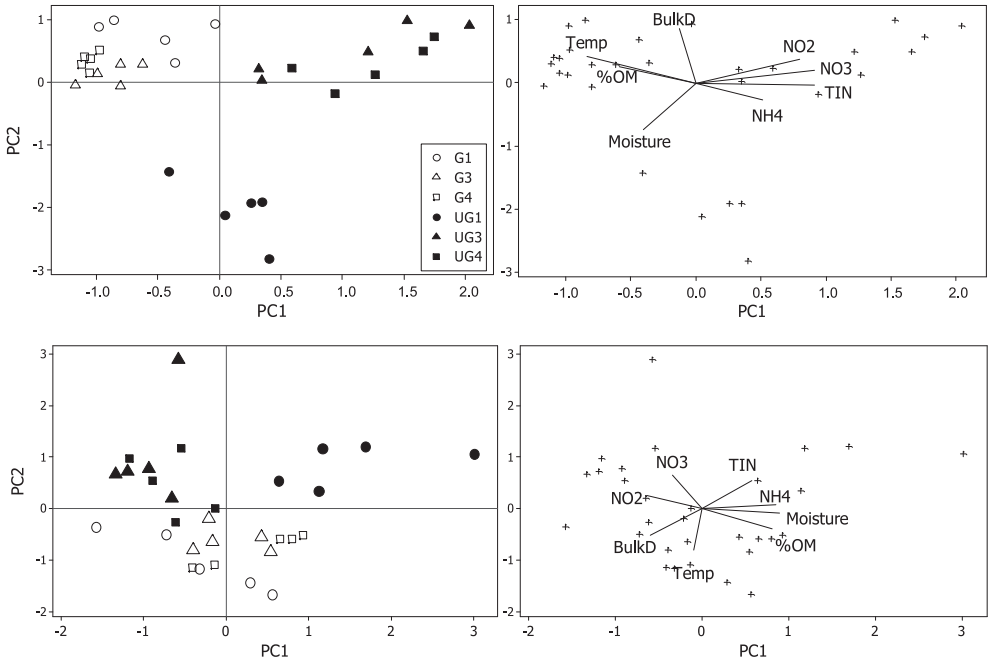

Fig. 2. Ordination by PCA of sampling plots according to soil characteristics in May (top two panels) and July (bottom two panels) for grazed (G) and ungrazed (UG) salt marsh. Measurements were taken from five cores within each plot. Environmental parameters analyzed are as follows: BulkD = bulk density, TIN = total inorganic nitrogen, NO_3 = nitrate, NO_2 = nitrite, NH_4 = ammonium, %OM = organic matter content, Temp = soil temperature.

rate was measured in plot UG1 in the ungrazed treatment at 0.47 kg N ha^{-1} day^{-1}. Rates in the other two ungrazed plots tested were much lower (0.03 and 0.22 kg N ha^{-1} day^{-1}).

Net N mineralization rates measured *in situ* from May to September averaged 0.74 and 0.59 kg N ha^{-1} day^{-1} in the grazed and ungrazed marsh respectively (Fig. 4). The results of a *t*-test revealed no significant effect of grazing on the average rate of N mineralization ($p = 0.569$), but the relative amounts of NO_3^- and NH_4^+ differed between treatments (Fig. 4a). More NO_3^- was produced from net mineralization in the ungrazed compared to the grazed treatment ($p < 0.01$; Fig. 4). In contrast, more NH_4^+ was mineralized in the grazed treatment, but the difference between treatments was not statistically significant ($p = 0.214$; Fig. 4a).

Potential N mineralization was significantly higher in soil from the grazed marsh (14.10 kg N ha^{-1} day^{-1}) compared to ungrazed marsh (8.37 kg N ha^{-1} day^{-1}) (*t*-test; $p < 0.01$; Fig. 4b). These rates were over one order of magnitude higher than the rates measured *in situ*.

3.4. Denitrification

The basal denitrification rate in soil from the ungrazed marsh (0.46 ± 0.11 kg N ha^{-1} d^{-1}) was 17 times higher than that of the

Table 2
Vegetation characteristics (means ± SE) in grazed and ungrazed salt marsh measured in July 2009 ($n = 6$). Treatment effects were evaluated using *t*-tests. Significant differences between grazed and ungrazed treatments are indicated by *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$), while *ns* indicates no significant difference ($p > 0.05$).

	Grazed	Ungrazed	
% Cover			
Agrostis stolonifera L.	20 ± 12	0.2 ± 0.2	*ns*
Elymus repens L.	0.8 ± 0.8	60 ± 13	**
Festuca rubra L.	27 ± 9	31 ± 10	*ns*
Puccinellia maritima Parl.	29 ± 12	0 ± 0	*
Triglochin maritima L.	11 ± 4	8 ± 8	*ns*
Glaux maritima L.	6 ± 2	0 ± 0	*
Sonchus arvensis L.	0 ± 0	3 ± 2	*ns*
Trifolium repens L.	4 ± 3	0 ± 0	*ns*
Other	7 ± 2	2 ± 1	*
Species richness (species m^{-2})	6.6 ± 0.5	3.7 ± 0.2	***
Above-ground biomass (kg dry wt m^{-2})	0.3 ± 0.1	1.0 ± 0.1	***
Belowground biomass (kg dry wt m^{-2})	3.4 ± 0.3	1.0 ± 0.1	***

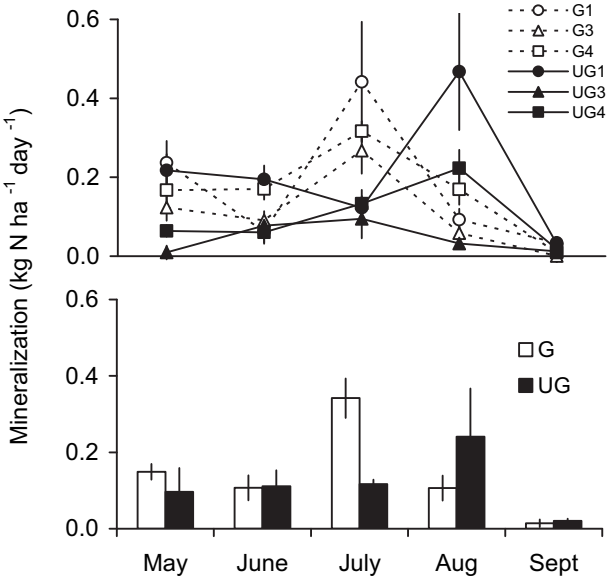

Fig. 3. Net nitrogen mineralization rates measured *in situ* in grazed (G) and ungrazed (UG) salt marsh. Top: Mineralization rates per plot calculated from five soil cores (mean ± SE). Bottom: Mineralization rates per treatment (mean ± SE; $n = 3$). There was no significant effect of grazing on mineralization rate ($p = 0.619$), but a significant effect of month ($p < 0.01$) and a significant interaction between treatment and time ($p < 0.05$) (bottom panel; repeated measures ANOVA).

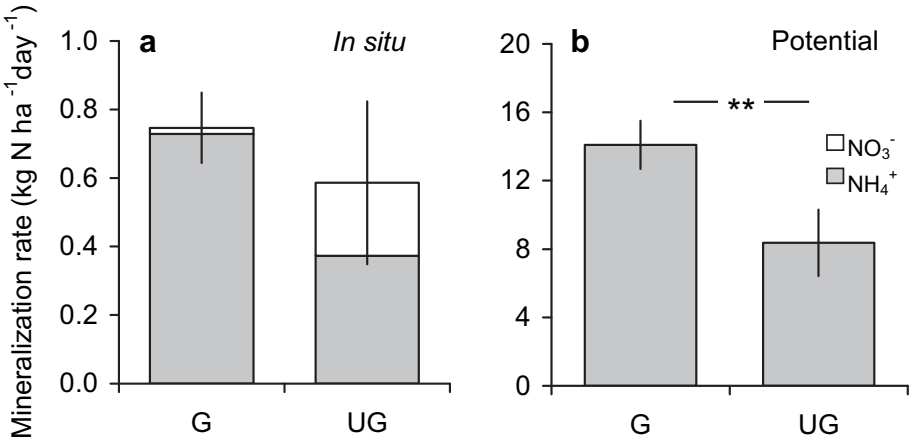

Fig. 4. *In situ* (a) and potential (b) nitrogen mineralization rates (means ± SE) in the top 10 cm of soil of grazed (G) and ungrazed (UG) salt marsh. *In situ* rates were measured between May and September ($n = 3$) and potential rates in September ($n = 6$). Relative amounts of nitrate (NO_3^-) and ammonium (NH_4^+) mineralized are shown in white and gray respectively. (Potential rates of nitrate production were too small to be shown (<0.05 kg N ha^{-1} day^{-1})). The average daily production of N produced *in situ* did not differ between treatments (*t*-test; $p = 0.569$). More NO_3^- was mineralized in the ungrazed marsh (*t*-test; $p < 0.01$), but the amount of NH_4^+ did not differ between treatments (*t*-test; $p = 0.214$). The rate of potential mineralization was significantly higher in the grazed marsh than in the ungrazed as indicated by **(*t*-test; $p < 0.01$).

grazed marsh (0.03 ± 0.01 kg N ha^{-1} d^{-1}) (Fig. 5; *t*-test, $p < 0.01$). The addition of glucose and NO_3^- did not stimulate denitrification in the ungrazed marsh (0.46 ± 0.12 kg N ha^{-1} d^{-1}), but increased rates in the grazed marsh (0.76 ± 0.13 kg N ha^{-1} d^{-1}). The potential rates of denitrification were not significantly different between the two grazing regimes (Fig. 5; *t*-test; $p = 0.118$).

3.5. Carbon mineralization

Processing of radiolabeled substrates in saltmarsh soil had a typical biphasic pattern of an initial rapid phase of $^{14}CO_2$ evolution followed by a secondary slower mineralization phase for both leaf litter and root exudates (Fig. 6). The proportion of ^{14}C remaining in the soil over time (expressed as % of the total ^{14}C-substrate added to the soil) conformed to a double exponential decay model ($r^2 > 0.995$ for both grazing treatments). The constants of the decay models, substrate half-lives and the microbial yields are presented in Tables 3 and 4.

The amount of ^{14}C taken up by the microbial community and initially partitioned into catabolic and anabolic processes is described by the size of pools a_1 and a_2 and by the microbial yield value. Grazing significantly affected the relative size of a_1 and a_2 by increasing the relative amount of ^{14}C partitioned into microbial biomass and subsequent turnover of necromass, metabolites and storage compounds ($p < 0.001$). A greater proportion of the root exudate was immediately used in catabolic processes in the first rapid phase compared to the leaf litter ($p < 0.001$). This was not surprising since we expected that only a small portion of the ^{14}C in the leaf litter would be initially leached out into soil solution and available for respiration.

Substrates in the soil solution were respired at the same rate irrespective of grazing regime, since the results of a two-way ANOVA with grazing and substrate as factors revealed no significant effect of grazing on k_1 ($p = 0.104$) or the half-life of either substrate

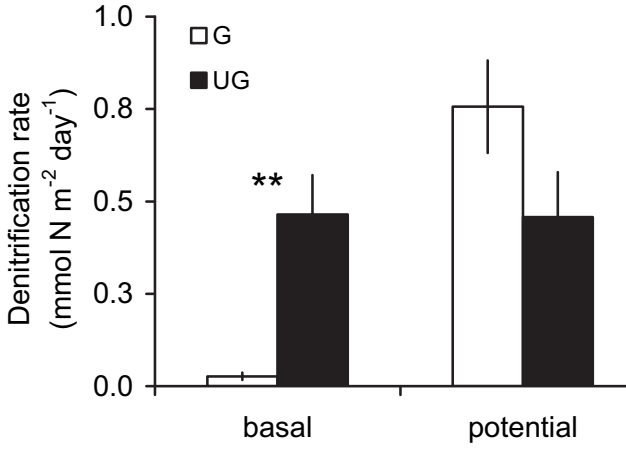

Fig. 5. Basal and potential denitrification rates (means ± SE) in grazed (G) and ungrazed (UG) salt marsh ($n = 6$). Basal denitrification rates were significantly different between grazed and ungrazed marsh as denoted by **(*t*-test; $p < 0.01$). There was no significant difference between the potential rates (*t*-test; $p = 0.118$).

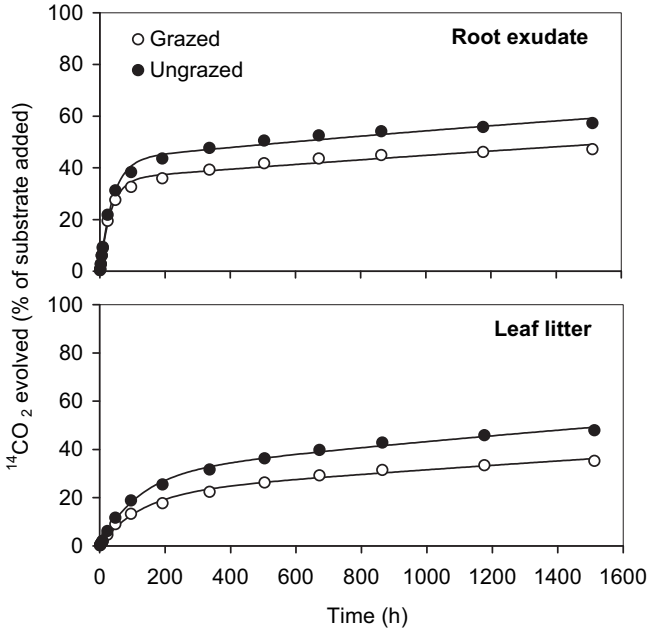

Fig. 6. Comparison of $^{14}CO_2$ evolution after the addition of ^{14}C-labelled root exudates (top) and leaf litter (bottom) to soil from grazed and ungrazed salt marsh. Values represent means ($n = 6$). Standard error bars are too small to be shown.

Table 3
Parameters (means ± SE) of the double exponential equations fitted to the decay curves for ^{14}C-labelled root exudates and leaf litter added to soil from the grazed and ungrazed salt marsh ($n = 6$). The soil solution for leaf litter refers to the low molecular weight carbon that is rapidly leached from the solid plant litter. The microbial pool includes carbon contained in microbial structure and storage pools. The effects of treatment (grazed vs ungrazed) and substrate (root exudate vs leaf litter) were evaluated using two-way ANOVAs. Different superscript letter represent significant differences between values within a column ($p < 0.05$).

Substrate	Soil solution			Microbial pool	
	a_1	k_1	half-life (h)	a_2	k_2
Root exudate					
Grazed	35.62 ± 0.62[a]	0.03 ± 0.002[a]	23.0 ± 1.3[a]	64.41 ± 0.59[a]	0.00016 ± 0.00001[a]
Ungrazed	43.16 ± 1.43[b]	0.03 ± 0.001[a]	26.8 ± 1.3[a]	56.90 ± 1.31[b]	0.00022 ± 0.00001[b]
Leaf litter					
Grazed	21.21 ± 0.88[c]	0.01 ± 0.001[b]	87.3 ± 5.8[b]	78.42 ± 0.92[c]	0.00014 ± 0.00001[a]
Ungrazed	29.24 ± 2.11[d]	0.01 ± 0.000[b]	79.5 ± 3.5[b]	70.63 ± 2.09[d]	0.00022 ± 0.00002[b]

in soil solution ($p = 0.542$). The results show relatively rapid turnover of the root exudates in soil solution with an average first phase half-life of 23.0 h for the grazed and 26.8 h for the ungrazed treatment (Table 3). The half-life for the labile component of leaf litter was higher, averaging 87.3 h and 79.5 h for grazed and ungrazed treatments respectively. Substrate type did not affect the rate of turnover of the microbial C pool (k_2) (in the leaf litter treatment, this slow phase of turnover includes microbial biomass and non-decomposed leaf material), but grazing had a significant negative effect ($p < 0.001$) on k_2 suggesting a much slower turnover of the microbial biomass in the grazed marsh.

There was a significant effect of grazing regime on the half-life describing the residence time of the total substrate in soil ($p < 0.001$) with root exudate-C being turned over at a more rapid rate than leaf litter ($p < 0.001$). Overall, grazing had a negative impact on the rate of turnover of both substrates with half-lives for both substrates being significantly lower in soil from the ungrazed marsh. The microbial yield was also significantly affected by both grazing regime and substrate type (Table 3). Grazing increased the microbial yield of both substrates ($p < 0.001$) and higher yields were observed following addition of leaf litter compared to root exudates ($p < 0.001$).

4. Discussion

4.1. Microbial biomass and respiration

The comparatively large biomass of fine root material in the grazed marsh suggests high rates of root turnover and root exudation, both of which represent major sources of C addition to soil (Bardgett et al., 1998). Microbial activity in soils is generally limited by C availability and rhizodeposition can significantly influence microbial community respiration (Raich and Tufekcioglu, 2000). We measured higher microbial biomass and rates of soil respiration under grazed conditions, which are both consistent with higher root turnover and exudation after leaf clipping or defoliation (Bardgett et al., 1998; Hamilton and Frank, 2001; Frank et al., 2002). Deposition of urine and dung by the cattle may also have provided a source of labile carbon that stimulated the microbial community. Grazing therefore appears to have increased microbial biomass and activity by increasing the quantity and improving the quality of resources to the microbial community. The increased root biomass and finely branched root structure in the grazed marsh also provided additional surface area and microbes are typically found in higher concentrations on the surface of roots (Toal et al., 2000).

Table 4
Substrate half-lives and microbial yield (means ± SE). The effects of treatment (grazed vs ungrazed) and substrate (root exudate vs leaf litter) ($n = 6$) were evaluated using two-way ANOVAs. Different superscript letters represent significant differences between values within a column ($p < 0.05$).

	Half-life (d)	Microbial yield
Root exudate		
Grazed	69.44 ± 6.57[a]	0.64 ± 0.01[a]
Ungrazed	24.38 ± 4.21[b]	0.57 ± 0.01[b]
Leaf litter		
Grazed	139.49 ± 7.97[c]	0.79 ± 0.01[c]
Ungrazed	67.22 ± 4.76[d]	0.71 ± 0.02[d]

4.2. Impact of grazing on N cycling

Potential mineralization rates, measured *ex situ* showed that grazing might enhance soil mineralization activity. This agrees well with results from grasslands, where grazing by ungulates has been shown to stimulate microbial processes and N mineralization due to increased root exudation of labile organic C and N and enhanced root turnover (Frank and Groffman, 1998b; Hamilton and Frank, 2001). However, in the field, we did not see a significant effect of grazing on N mineralization. The reason for a lack of response could be because grazing may have had both positive and negative effects on net mineralization rates (Kiehl et al., 2001). While increased availability of labile sources of C from faeces and root exudates can stimulate gross mineralization (Frank et al., 2000; Bakker et al., 2004) it can also be associated with increased immobilization of N by bacteria, which would reduce net mineralization (Tracy and Frank, 1998). The apparent lack of influence of grazing on net mineralization may also be partially attributed to differences between the plots in the ungrazed marsh. Plot UG1 displayed much higher rates of mineralization compared to the other two plots in May, June, and August (Fig. 3, top). Plot UG1 differed from the other ungrazed plots in several important characteristics such as having higher moisture content, lower bulk density, higher NH_4^+ concentrations and soil organic matter content. There were also important differences in the vegetation of the plots. Plot UG1 was established in a portion of the marsh dominated by *F. rubra* (L.) (71%) and *T. maritima* (L.) (48%) whereas the vast majority of the surface of the ungrazed marsh was dominated by 55–86% *E. repens* (L.), which has a high lignin content and slow decomposition rates (Valéry et al., 2004). The presence of large amounts of *Elymus* may have contributed to the lower rates of N mineralization measured in UG plots 3 and 4. It is however, difficult to disentangle the influence of vegetation quality and soil conditions. We speculate that net mineralization rates across the majority of the ungrazed marsh would be more similar to those measured in plots 3 and 4, which suggests that for the majority of the marsh surface, grazing stimulates net N mineralization across a large portion of the growing season.

Denitrification rates measured here (0.03–0.46 kg N ha^{-1} d^{-1}; the equivalent of 0.19–3.31 mmol N m^{-2} d^{-1}) were within the low

to mid-range of values previously reported from salt marshes (Valiela et al., 2000). Denitrification in the grazed marsh was limited by NO_3^- supply, which has been identified as a major factor controlling denitrification rates in freshwater wetlands (Seitzinger, 1994) and intertidal sediments (Cabrita and Brotas, 2000). Nitrification, which is an important delivery mechanism for NO_3^- to the soil, was not directly measured in this study, but the net production of NO_3^- measured after the *in situ* incubations was very low or even negative in the grazed treatment and high in the ungrazed. The presence of an anoxic layer in the grazed marsh may have limited nitrification and explain the low concentrations of NO_3^- measured. Similar results have been found elsewhere - a reduction in nitrification rates by sheep grazing and high soil water content in a salt marsh (Kiehl et al., 2001) and reduced NO_3^- availability has been observed after grazing (Bakker et al., 2004). In contrast to these results, studies carried out in grasslands have shown increased denitrification in response to grazing most likely due to increased amounts of labile C (Frank and Groffman, 1998a; Frank et al., 2000; Le Roux et al., 2003). It is therefore possible that the effect of grazing on denitrification and the mechanisms that regulate its rates differ between grasslands and salt marshes. The waterlogging of the compacted grazed saltmarsh soils could be a key factor in reducing denitrification rates by limiting nitrate availability in the soil. Grazing may therefore lead to a reduction in coupled nitrification–denitrification, increase export of N and diminish the role of salt marshes as protection for coastal waters that are highly sensitive to inputs of N (Valiela and Cole, 2002).

A large number of mechanisms are involved in regulating N cycling and there are inherently complex linkages between different species of N as well as between different storage compartments within the salt marsh, making it difficult to predict how grazing affects uptake and exports of N. We found a significant effect of grazing on potential mineralization rates measured in the laboratory, but this difference did not translate to the *in situ* measurements. The *in situ* measurements revealed a shift in the peak mineralization rates in grazed and ungrazed marsh, but there were no obvious changes in the abiotic conditions that could be responsible for this change.

4.3. Turnover of C in saltmarsh systems

Turnover rates of litter and low molecular weight C were slower in saltmarsh soils compared to rates previously measured in terrestrial soils. The half-lives of LMW substrates in soil solution (root exudates $t_{1/2} = 23.0 \pm 1.3$ h for grazed and 26.8 ± 1.3 h for ungrazed) were much longer than the half-lives reported for a range of LMW substrates tested for non-saline agricultural soils (amino acids $t_{1/2} = 2.3 \pm 0.5$ h; Jones et al., 2005), temperate grassland soils (amino acids $t_{1/2} = 0.43 \pm 0.06$ h; Boddy et al., 2007) and arctic tundra soils (amino acids $t_{1/2} = 0.49–2.71$ h; glucose $t_{1/2} = 0.54–2.72$ h; Boddy et al., 2008). The rate constants that describe the turnover of the microbial pool (k_2), assumed to be the result of turnover of C immobilized within the microbial biomass (Boddy et al., 2007), were an order of magnitude lower in salt marsh compared to rate constants from agricultural and grassland soils (Jones et al., 2005; Boddy et al., 2007). As a result of the slow turnover of the microbial biomass, half-lives of the substrates in the salt marsh were also far slower than typical values measured in terrestrial soils (Boddy et al., 2007, 2008; Oburger and Jones, 2009). A major difference between salt marshes and most other terrestrial environments is that saltmarsh soils tend to have limited oxygen penetration. This may explain why rates of turnover were slower in saltmarsh soil, since efficiency of organic matter decomposition is lower under anaerobic conditions. Salt marshes and terrestrial environments also differ in the level of activity and abundance of microbial-feeding organisms. The breakdown of leaf litter is mediated by mechanical breakdown by invertebrate and protozoan grazers contribute to around 30% of net N mineralization in a range of ecosystems (Griffiths, 1994). Long bacterial turnover times in salt marshes appear to be associated with low abundances of protists relative to bacteria and result in a community with reduced rates of organic matter decomposition (First and Hollibaugh, 2010). This likely contributes to slower turnover of C saltmarsh soil compared to terrestrial soils.

4.4. Impact of grazing on C turnover in salt marshes

Grazing affected the overall turnover of leaf litter and root exudates in two ways: Firstly, more C was immobilized into microbial biomass and storage. More ^{14}C was used for respiratory processes rather than for storage and growth in the ungrazed marsh, which is indicative of higher maintenance costs for microorganisms in this treatment. We expected respiratory demand and rates of soil solution turnover to be higher in the grazed treatment, which had the highest microbial biomass and basal respiration rate, but the rate of depletion of C in the rapid phase (k_1) did not differ between treatments. Whereas root respiration may have contributed significantly to the basal respiration measurements, we can assume that roots did not contribute significantly to $^{14}CO_2$ respiration (Nguyen and Henry, 2002). Overall, the results are indicative of there being no difference in C availability with grazing treatment.

The second major effect of grazing was to slow down the rates of turnover (k_2) of C that was immobilized in microbial biomass and storage. This result could be related to faster turnover of the microbial community in the ungrazed marsh versus a more stable community in the grazed marsh. This may be due to differences in the populations of microbial-feeding grazers, which may substantially increase turnover of the microbial biomass and play an important role in nutrient cycling. Soil compaction by grazers reduces soil pore space available for small invertebrates leading to increased physical protection of organic matter and microbes from attack by grazing nematodes and has been shown to reduce mineralization rates (Breland and Hansen, 1996). Invertebrate activity measured with bait lamina sticks in our study site showed that invertebrate activity was restricted to the top 4 cm in the grazed marsh whereas the ungrazed marsh had high activity levels throughout the 8.5 cm depth measured (Walsh, 2009; H. Ford, unpublished data). Our results therefore suggest lower activity of microbial-feeders in the grazed marsh. Another possibility is that there are differences in the partitioning of the substrates in medium- and long-term C storage pools (e.g. storage polymers, which are more labile than structural compartments such as cell walls) (Oburger and Jones, 2009).

The rates of turnover in the slow phase of C cycling (k_2) were not affected by substrate type. This further supports the notion that what limits the overall turnover of C in the grazed marsh is mainly immobilization within soil microbial biomass and that the rate of processing is dictated by turnover of C that is released as necromass as soil microbes die.

The results of the ^{14}C experiments suggest overall slower turnover of C in the grazed salt marsh. This was surprising considering the higher microbial biomass measured in the grazed treatment. At this stage, we do not have a clear explanation for this result and several factors may have contributed to this difference. The measurements of ^{14}C mineralization were made in the laboratory, which could bias the results. Removal of soil samples from the field have been shown to significantly alter the kinetics of mineralization either due to differences in substrate utilization and storage pathways in the microbial community or due to differences in the rates

of microbial turnover in the laboratory (Oburger and Jones, 2009). To our knowledge, this study represents the first attempt to characterize C turnover in a salt marsh using a ^{14}C-label technique in the laboratory or the field. Our results do suggest that grazing changes the dynamics of the soil microbial community, but at this stage we cannot say whether the half-lives measured are truly representative of rates of C processing under field conditions.

Presence or absence of vegetation can significantly affect microbial activity and the longevity of C in soil (Oburger and Jones, 2009). Our results suggest that biotic and abiotic changes resulting from grazing also affects C storage and utilization pathways in the microbial community. Grazing may therefore ultimately affect the longevity of C in the soil, but based on our results it does not appear that cattle grazing would reduce the large capacity for C sequestration and storage in salt marshes (Chmura et al., 2003; IUCN, 2009). Further work is required to identify how saltmarsh grazing alters microbial communities and their metabolism, and to evaluate any potential seasonal variation in mineralization dynamics.

5. Conclusions

We demonstrated here that long-term grazing by cattle led to changes in the structure and composition of the saltmarsh plant community, abiotic conditions of the soil, and soil microbial biomass and respiration. Our study did not support the notion that grazing increases C and N cycling rates through the provision of large quantities of high-quality organic matter, although it is possible that microbial immobilization compensated for increased gross mineralization rates. The major effect of grazing on N cycling appears to be in limiting the production of NO_3^- through mineralization of organic matter, thereby limiting rates of denitrification in the grazed marsh. The implications of limited denitrification may be enhanced nutrient availability to saltmarsh plants and a loss in the ability of the marsh to intercept land-derived nutrients from adjacent watersheds.

Grazing also increased microbial immobilization of C and slowed down the overall turnover of C. Grazing may therefore ultimately affect the longevity of C in the soil, but it does not appear that cattle grazing would significantly reduce the large capacity for C sequestration and storage in salt marshes.

Acknowledgments

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