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Effects of crabs on greenhouse gas emissions, soil nutrients and stoichiometry in a subtropical estuarine wetland

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Abstract

Crabs may elicit effects on wetland carbon (C), nitrogen (N) and phosphorus (P) concentrations and associated ecological stoichiometry. In this study, we assessed effects of crabs on carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions, soil C, N, and P concentrations, and stoichiometry in upper and mid tidal flats of an estuarine wetland in China. The results showed that averaged CO_2, CH_4, and N_2O fluxes were greater in the upper and mid-tidal flats in the presence of crabs, being 46.4, 66.7, and 69.7% and 53.6, 143, and 73.1% greater than control, respectively. Mixed model analyses showed overall positive relationships between wetland soil CO_2 CH_4 and N_2O emissions (F=4.65, P=0.033; F =42.42, P=0.042 and F=10.2, P=0.0018, respectively) in the presence of crabs, taking into account season, flooding intensity, and plot effects. This may be related to the direct effects of respiration and the indirect effects of feeding, excretion, and disturbance of soil on microorganisms and/or plant roots. There were no effects of crabs on total C or N concentrations, whereas decreased soil total P concentrations, especially in the upper tidal flats (P=0.04). Crab presence was positively associated with soil C:P and N:P ratios (P<0.0001 and P<0.0001, respectively), taking into account season, flooding intensity, and plot effects. In the upper and mid-tidal flats, soil CO_2 emissions were negatively correlated with total soil C; CH_4 emissions were positively correlated with ratios of C:N and C:P; and N_2O emissions were positively correlated with N content. In general, global warming potential (GWP) of the upper tidal flats in the presence of crabs increased by 138% compared with the absence of crabs, and GWP of the mid-tidal flats in the presence of crabs increased by 99.3% compared to the absence of crabs. Global warming and associated flooding rise in several coastal wetland areas is favoring benthic fauna number enhancement and this in turn increase GWP of overall gas emissions further contributing to future warming rise.

Keywords Greenhouse gas emission · Carbon · Nitrogen · Phosphorus · Elemental stoichiometry · Wetlands

Introduction

The global average temperature increased by 0.58 °C during the period between 1880 and 2012 (IPCC 2014), and although many factors contributed to this rise in temperature, increasing concentrations of the key greenhouse gases (carbon dioxide: CO_2; methane: CH_4; and, nitrous oxide: N_2O) in the atmosphere contributed about 80% to global warming (IPCC 2014). Globally, wetlands account for 5–8% of the land surface area (RoyChowdhury et al. 2018), yet despite this relatively small area, they represent important sources and sinks of greenhouse gases (Altor and Mitsch 2008; Chen et al. 2010; Bridgham et al. 2013), due to high levels of primary productivity and soil carbon (C) and nutrient storage (Mander and Shirmohammadi 2008). Wetlands are increasingly important in the retention and filtration of excess reactive niN from surrounding soils, and may produce N_2O by nitrification and denitrification processes (Burgin and Groffman 2012; Cui et al. 2013). Moreover, CH_4 emissions are non-linearly increased with temperature (Peltola et al. 2015) that may elicit a positive feedback on climate change in wetland.

Recent studies of soil C, N, and P have explored the relative balance in concentrations of different elements and turnover processes (Elser et al. 2000) as part of the concept of ecological stoichiometry, which is based on the relationships between ecosystem structure and function, stoichiometry and use of C:N:P in different ecosystem compartments (Elser et al. 2000; Sterner and Elser 2002; Sardans et al. 2012). The relationship between ecosystem energy and multi-elemental balance described using ratios (Elser et al. 2000) has become a focus on the study of elemental ecological stoichiometry (Elser et al. 2000; Tessier and Raynal 2003; Allen and Gillooly 2009). While spatial variability in ecological stoichiometry and implications for plant growth and ecosystem function have been studied (Han et al. 2005), the effects of benthic organisms on soil nutrient stoichiometry and greenhouse gas emissions are unknown, particularly in estuarine wetlands. The influence of factors at the land-sea interface, periodic tidal inundation, and the effects of benthic fauna on soil and plants are known to affect soil C, N, and P cycling in estuarine wetlands (Liu et al. 2014). Therefore, quantification of nutrient ecological stoichiometry in these ecosystems may increase understanding of drivers and mechanisms of their ecological function and service provision.

As secondary producers in estuarine wetlands, benthic fauna (Stahl et al. 2014) is essential for a healthy ecosystem (Vermeiren and Sheaves 2015), where their bioturbation (Suren et al. 2011), excretion and other physiological activities, and biological irrigation effects impact biogeochemical cycling processes in wetlands (Hu et al. 2016), especially for soil C and N (Liu et al. 2005). Studies have shown that benthic species diversity is greater in the intertidal zone than in the upper mid-tidal zone (Wang et al. 2011; Liu et al. 2015), mainly due to flooding frequency and associated changes in salinity. For example, flooding of the mid-tidal zone brings rapid influxes of nutrients beneficial to benthic organisms and higher levels of salinity that support the growth of algae and other organisms, leading to increased abundance of crabs (Chen et al. 2008).

While the effects of benthic fauna on C and N cycling in wetlands has been studied (Mereta et al. 2013), effects on C, N, and P stoichiometry remain unclear. Crabs (Arthropoda: Brachyura) are commonly occurring zoobenthos in wetlands and they may directly affect soil properties, such as acidity, aeration, and texture, through burrowing activity that subsequently affects C, N, and P cycling (Simioni et al. 2014). For example,

CO_2 emissions from burrows in which the crab *Upogebia yokoyai* was present were greater than in the absence of the crab (Sasaki et al. 2014). CO_2 fluxes at the soil-atmosphere interface were greater in burrows containing crabs (Kristensen et al. 2008), and crab activity in upper tidal flats may promote the production and release of N_2O as a result of digging behavior, excretion, and sediment resuspension (Liu et al. 2008). Other types of benthos have been shown to elicit CH_4 by nutrient-mediated changes to the microbial cycle and coupling between the benthic upper food chain and this can lead to increased CH_4 emissions (Figueiredobarros et al. 2009). Predicted rises in sea levels due to greenhouse gas-meditated global warming are the intensity predicted to lead to increases in the intensity and frequency of flooding that may result in greater crab activity in wetlands, along with associated changes in soil properties, and production and emissions of greenhouse gases (Chen et al. 2008; Wang et al. 2011; Liu et al. 2015). Therefore, it is important to increase the understanding of the role of crabs in the emission of greenhouse gases so as to pormote mitigation. The impacts of crab activities on soil stoichiometry and C, N and P concentrations and further on greenhouse gas emissions in tidal marshes remain, however, to be studied.

We hypothesized: 1) crabs presence increases organic matter and aeration in soil, with associated increases in CO_2 and N_2O emissions and decreases in CH_4 emissions, 2) crab presence changes physicochemical properties with effects on CO_2 ,CH_4 and N_2O emissions; and, 3) crab presence increases soil C and nutrient concentrations and leads to lower soil C:N and C:P ratios and stimulates emissions of greenhouse gases. To test these hypotheses and to solve these gaps: 1) we determine the effects of the presence of crabs on soil CO_2, CH_4, and N_2O emissions in the upper and middle tidal flats, 2) we measured the impact of crab presence on soil nutrient levels and their stoichiometry, and 3) we evaluated the relationship between greenhouse gas emissions and soil nutrient concentrations and associated stoichiometry in the presence of crabs.

Materials and methods

Study sites

The study sites were located in a tidal *Spartina alterniflora* wetland in the Minjiang River estuary, China (Fig. 1), where there is a subtropical monsoon climate, with a mean annual temperature of 19.9 °C and a mean annual precipitation of 1380 mm. Precipitation is concentrated between March and September, with bimodal peaks in June in the rainy season and August in the typhoon period (Liu et al. 2006). The dominant plant species in the study area included *Kandelia candel*, *Casuarina equisetifolia*, *Phragmites australis*, *Cyperus malaccensis*, *Scirpus striquete*r, and *Spartina alterniflora*.

Three replicates of 1 × 1 m sampling plots were established in four experimental treatments that comprised combinations of presence (P) and absence of crabs (D, control) at upper (G) and mid-tidal (Z, control) flats (GP, GD, ZP, and ZD). Mid-tidal flat is the high-flooding habitat, which is flooded by intermediate tides ca. 240 d y^{-1} and is submerged beneath 10-120 cm of water for 0.5-4 h during each tidal inundation (average of 540 h y^{-1} of inundation). Upper-tidal flat is the intermediate-flooding habitat nearer to coastal line, which is flooded by intermediate tides ca. 220 d y^{-1} and is submerged beneath 10-100 cm of water for 0.5-3 h during each tidal inundation (average of 385 h y^{-1} of inundation). All measurements have been performed +/- 1h from low tide. Presence of crabs at the sampling plots was confirmed by the presence of flat holes and chimney burrows (>4 cm, and our in situ investigation showing that these holes were crab holes): in the upper tidal flats, crab burrows were larger in diameter, but fewer in number than in the mid-tidal flats (Table S1). To avoid the effects of isolated crabs, control plots (D) were covered with rust-proof barbed wire, polypropylene random (PPR) tubes, and 60-

mesh nylon mesh that was installed 70 cm above ground level and 30 cm below ground level.

Greenhouse gas sampling

Greenhouse gas emissions from the plots were collected using static chambers that consisted of an upper chamber and lower base made of opaque PVC (polyvinyl chloride), with diameter and heights of 0.2 × 0.4 m, and 0.2 × 0.1 m, respectively. There was a extraction gas and temperature measurement hole in the top of the upper chamber (Fig. S1). The base of the static chamber used to collect gas samples in the crab-free plots, whereas there was a ≥4 cm- hole in the center of the base of the static chamber used in the crab plots to facilitate normal crab activity.

We collected gas samples at low tide in 2015 on January 10 (winter), April 18 (spring), June 27 (summer), and September 13 (autumn) at 09:00, 12:00, and 15:00 hrs. At each time step, three samples were collected consecutively for 15 min; 40 ml of gas were sampled from the upper chamber and injected into a 50mL aluminum foil gas sample bag. The temperature in the chamber was recorded during gas collection by the thermometer.

We used gas chromatography to determine CO_2 and CH_4 (Shimadzu GC-2010, Kyoto, Japan) and N_2O (Shimadzu GC-2014, Kyoto, Japan) concentrations in the gas samples using a Porapak Q stainless steel column (2 m in length, 4 mm OD, 80/100 mesh). A methane conversion furnace, flame ionization detector (FID) and electron capture detector (ECD) were used for the determination of the CO_2, CH_4 and N_2O concentrations, respectively. The operating temperatures of the column, injector and detector for the determination of CO_2, CH_4 and N_2O concentrations were adjusted to 45, 100 and 280 °C; to 70, 200 and 200 °C and to 70, 200 and 320 °C, respectively. These temperatures were the optimum values for the different parts of the instrument. The gas chromatograph was calibrated before and after each set of measurements using 503, 1030, and 2980 μL of CO_2 L^{-1} in He; 1.01, 7.99, and 50.5 μL of CH_4 L^{-1} in He, and 0.2, 0.6, and 1.0 μL of N_2O L^{-1} in He (CRM/RM information center of China, Beijing) as primary standards (Wang et al. 2015a, b).

CO_2 CH_4 and N_2O fluxes and the global warming potential (GWP) estimation

The CO_2 CH_4 and N_2O fluxes were estimated by the following equation:

$$F = \frac{M}{V} \cdot \frac{dc}{dt} \cdot H \cdot \left(\frac{273}{273+T}\right)$$

where F is the CO_2, CH_4 or N_2O flux (mg/μg $CO_2/CH_4/N_2O$ m^{-2} h^{-1}), M is the molecular weight of the gas (44, 16 and 44 g mol^{-1} for CO_2, CH_4 and N_2O, respectively), V is the molar volume of gas in a standard state (22.4 mol^{-1}), dc/dt is the variation ratio of CO_2, CH_4 and N_2O concentrations (μmol mol h^{-1}), H is the height of the chamber above the water surface (m), and T is the air temperature inside the chamber (°C).

To estimate GWP, CO_2 is typically taken as the reference gas, and a change in the emission of CH_4 or N_2O is converted into "CO_2-equivalents" (Hou et al. 2012):

$$f = F \cdot t$$

Where f (kg/hm^2) represents the amount of different greenhouse gas emissions during the sampling period. t represents the time of the period. According to Ahmad et al (2009) the equation used to calculate GWP is:

$$GWP = f_{CO_2} + 34 f_{CH_4} + 298 f_{N_2O}$$

The GWP for CH_4 is 34 (based on a 100-year time horizon and a GWP for CO_2 of 1), and the GWP for N_2O is 298 (Myhre et al. 2013).

Soil sampling

More than 95% of large benthic fauna is distributed in the upper 10 cm of soil. The diameter of a crab hole is greater at the soil surface and extend to around 10 cm.

Therefore, we collected five soil samples from the upper 10 cm of soil of each plot; these samples were mixed to reduce heterogeneity and interference from crab holes (Wang et al. 2008), placed in closure pockets and transported to the laboratory. Impurities, such as stones, wood, and plastic, were removed from the soil, and after air-drying, the samples were passed through a 2-mm sieve prior to measurement of physicochemical soil properties (total C, N and P concentrations, dissolved organic C, soil exchangeable NH_4^+–N, NO_3^-–N concentrations, soil bulk density, water content, pH, salinity, and temperature). Half of the samples were subsequently passed through a 0.149-mm sieve prior to measurement of soil nutrient.

Total C and N (TC and TN, respectively) concentrations were measured using a Vario MAX CN Elemental Analyzer (Elementar Scientific Instruments, Hanau, Germany), and total P (TP) concentration was determined using Mo-Sb colorimetry (Lu 1999; Ruban et al. 1999). Soil DOC was determined by extracting the soils with deionized water (1:5 ratio) and measuring the total C concentration using a TOC-V CPH total C analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Soil exchangeable NH_4^+–N, NO_3^-–N concentrations were measured using a continuous flow injection analyzer (Skalar Analytical SAN ++ Instruments, Breda, Netherlands).

Bulk density was measured from three 5 × 3-cm cores, while soil water content and soil pH were determined using the drying method and a portable IQ 150 pH meter in a 1:5 ratio of soil:water (IQ Scientific Instruments, Carlsbad, USA), respectively. Salinity and soil temperature in the field were measured using a 2265FS salinity/temperature meter (Spectrum Technologies Inc., Paxinos, USA).

Statistical analyses

We used general mixed models (GLM) in the "*nlme*" (Pinheiro et al. 2016) R package with the "*lme*" function, with crab presence, tidal flat (upper or mid to represent flooding intensity) and season as independent fixed categorical variables, plot as independent random factor and, CO_2, CH_4, and N_2O soil gas emissions and soil physicochemical properties as continuous dependent variables. When a variable did not follow normal distribution, it was log transformed to reach normal distribution before statistical analyses. We chose the best model for each dependent variable based on the Akaike information criterion, and we used the MuMIn package (Barton 2012) in R to estimate variance explained by the mixed models. Tukey's post hoc tests in the "*multcomp*" (Hothorn et al. 2013) R package, with the "*glht*" function, were used to test for differences between the eight combinations of crab presence × season.

Associations between environmental factors and CO_2, CH_4, and N_2O emissions, and between C, N, and P concentrations were tested using Pearson correlation analysis in SPSS 20.0 (StatSoft, Inc. Tulsa, USA).

Effects of crab presence and flooding intensity on soil greenhouse gas emissions and soil physicochemical properties were tested using general discriminant analysis (GDA) in Statistica 8.0 (StatSoft, Inc. Tulsa, USA), with a supervised statistical algorithm that derived an optimal separation between groups, which was established a priori by maximizing between-group variance while minimizing within-group variance, to control for effects of season (Raamsdonk et al. 2001).

We used major axis (MA) and standardized major axis (SMA) (SMATR package; http://www.bio.mq.edu.au/ ecology/SMATR) regression to compare the slopes of the regressions of the relationships among the C, N and P with and without crab.

Results

General effects of crab presence on greenhouse gas emissions

CO_2 emissions were globally higher with than without crabs (P<0.05, Table S2). Average CO_2 emissions from soils in the presence and absence of crabs in the upper tidal flats were 125 ± 14.8 and 67.1 ± 7.67 mg $m^{-2}\cdot h^{-1}$, respectively (CVs: 23.7 and 22.9%, respectively), and in the mid-tidal flat, average emissions were 128 ± 62.4 and 58.8 ± 20.2 mg $m^{-2}\cdot h^{-1}$, respectively (CVs: 97.2 and 68.7%, respectively) (Fig. 2). There was no effect of crabs on CO_2 emissions, regardless of degree of tidal inundation (P>0.05). CO_2 emissions were higher in winter and summer than in spring and autumn (Table S2, Fig.4)

Cumulative CH_4 emissions were significantly higher *with than without crabs* (Table S2). Mean CH_4 emissions in the upper tidal flats with than without crabs were 0.35 ± 0.22 and 0.10 ± 0.05 mg $m^{-2}\cdot h^{-1}$, respectively (CVs: 128 and 98%, respectively), and in the mid-tidal flats, mean CH_4 emissions were 0.29 ±0.26 and -0.11 ±0.18 mg $m^{-2}\cdot h^{-1}$, respectively (CVs: 180 and 320%, respectively) (Fig. 3). There was no effect of crabs on soil CH_4 emissions, regardless of degree of tidal inundation.

N_2O emissions were globally higher with than without crabs (P<0.05, Table S2). N_2O emissions in the upper tidal flats were greater in the presence of crabs, especially during the autumn (Fig. 4), and overall, average N_2O emissions in the tidal flats were significantly greater in the presence of crabs (Table S2, P<0.05). N_2O emissions were higher in autumn than in the other three seasons and were higher under high tidal intensity (Table S2, Fig.4)

Under the action of crabs, the CO_2 and N_2O global warming potential (GWP) of the upper tidal and mid-tidal flats were significantly higher than those without crabs (P<0.05). GWP of the upper tidal flats in the presence of crabs increased by 138% compared with the absence of crabs, and GWP of the mid-tidal flats in the presence of crabs increased by 99.3% compared to the absence of crabs (Tables S2 and S3).

Crab presence and soil properties

In general, crab presence was associated with greater levels of soil water content, pH. Maximum and minimum soil temperatures were recorded in June and January, respectively, maximum and minimum soil salinities were recorded in January and September, respectively, and maximum and minimum soil pH values were recorded in September and June, respectively. Soil bulk density was greater in the upper tidal flat than middle tidal flat. There was no effect of crab presence on seasonal variations in soil temperature, salinity, pH, water content, or salinity, but soil water content was greater with than without crabs (Fig. S2).

There was an overall effect of crab presence on soil TP, but not on soil TC or TN through the year (Table S2, S4). Concentrations of soil TC and TN were greater in the upper tidal flats in the winter and spring than in the upper tidal flats (P<0.05). Crab presence was associated with greater levels of soil C:P and N:P ratios (Table S2).

Season had a significant influence on the total soil nutrients and their stoichiometric ratios. TN varied greatly for different tidal flats and was related to the frequency of soil flooding (P<0.05). TC and C:N were significantly influenced by different seasons, different tidal flats and presence or absence of crabs. The change of C: P was significant under the combined effect of seasons and crabs and TP in different seasons (P <0.001). The ratio of soil C:N was stable among the seasons in the upper tidal flats in the presence of crabs, while that of N:P varied; ratios of soil C:N and N:P in the upper tidal flat in the presence of crabs was stable (Fig. 5). Nutrient ratios were greater with than without crabs, and the ratio of soil C:N ratio varied with season (P<0.01, Table S2).

The y1 and y2 functions represent the linear fitting relationship of total nutrients with and without crab activity, respectively. The R^2 value in the y1 function was significantly higher than the y2 function, indicating that the crab activity helps to improve the correlation between the total soil nutrients (Fig. 6). We observed that none of the slopes of the regression lines of the TN-TC, TP-TC and TP-TN differed significantly between with and without crabs (*P*>0.05, SMA test of common slopes). Concentrations of soil TC, TN, and TP were related (Fig. 6, *P*<0.01), but the relationship between TC and TP concentrations was weaker than between TC and TN concentrations. Overall, differences in soil concentrations of TC and TN were more distinct than differences in TP during the year.

Relationship between greenhouse gas emissions and soil properties

Overall, CO_2 emissions were positively correlated with soil temperature (*R*=0.35, *P*<0.05) and negatively associated with soil bulk density (*R*=-0.36, *P*<0.05), CH_4 and N_2O emissions were positively correlated with soil temperature (*R*=0.60, *R*=0.57, *P*<0.01, respectively), and N_2O emissions were positively correlated with soil pH (*R*=0.44, *P*<0.01) and water content (*R*=0.39, *P*<0.05). However, the relationships changed in function of the presence of crabs depending on the level of tidal intensity. N_2O emissions in the upper tidal flat in the presence of crabs was positively correlated with pH (*R*=0.82, *P*<0.01) and water content (*R*=0.59, *P*<0.05), negatively correlated with salinity (*R*=-0.59, *P*<0.01) (Table 1). In the upper tidal flat without crabs, there was a negative correlation between CH_4 emissions and salinity (*R*=-0.86, *P*<0.01), and N_2O emissions were positively correlated with soil temperature (*R*=0.86, *P*<0.01) and water content (*R*=0.70, *P*<0.05). Fluxes of CO_2, CH_4, and N_2O were positively correlated with soil temperature (R=0.84, *R*=0.89, *R*=0.88, *P*<0.01, respectively) and in the presence of crabs (*P*<0.005), while N_2O emission flux was negatively correlated with soil bulk density (*R*=-0.88, P<0.01).

We found that N_2O fluxes in the upper tidal flats were positively correlated with soil concentration of TN, TP (*R*=0.75, *R*=0.79, *P*<0.01, respectively), and TC (*R*=0.63, *P*<0.05) in the presence of crabs and with soil concentration of TC, TN, and TP (*R*=0.77, *R*=0.83, *R*=0.78, *P*<0.01, respectively) in the absence of crabs (Table 2). CO_2 emissions in the mid-tidal flats were negatively correlated with soil TC (*R*= -0.58, *P*<0.05) in the presence of crabs and with soil TC concentration and ratio of N:P (*R*= -0.62, *R*= -0.68, *P*<0.05, respectively) in the absence of crabs. CH_4 emissions were positively correlated with C:N (*R*=0.71, *P*<0.01) and C:P ratios (*R*=0.60, *P*<0.05), and N_2O flux was positively correlated with ratio of C:N (*R*=0.77, *P*<0.01). Overall CO_2 emissions were negatively correlated with soil concentration of TC (*R*= -0.37, *P*<0.05), while CH_4 flux was positively correlated with C:N and C:P ratios (*R*=0.36, *R*=0.29, *P*<0.05, respectively) and N_2O flux was positively correlated with soil TN concentration (*R*=0.35, *P*<0.05). The relationships between soil physicochemical factors on soil TC, TN and TP and soil stoichiometry are provided in supplementary material (Table S4).

Overall effects of crabs and flooding intensity on greenhouse gas emissions and soil physicochemical properties

The GDA showed that N_2O emissions, soil TC, C:N ratio, water content, bulk density and temperature clustered in the four combinations of crab presence/absence and flooding intensity (Tables S5, S6). This GDA detected that the presence of crabs in upper tidal had higher overall effects in the studied variables than in the middle tidal intensity (Figure S4). Plots in the upper-tidal flats were associated with N_2O emissions, bulk density and soil C:P and N:P ratios more with than without crab presence. These great differences due to crab presence were instead not observed in the middle tidal intensity plots (Figure S4). Plots in the middle tidal flats were though correlated with higher soil water content and TP more with than without crab presence (Fig.S4).

Discussion

Effects of crab presence on greenhouse gas emissions

CO_2 emissions in wetland soils largely derives from respiration of soil animals and plant roots, and the decomposition of organic C by soil microorganisms (Blagodatsky and Smith 2012). This study showed that CO_2 emissions were greater with than without crabs, possibly due to the direct effects of respiration and the indirect effects feeding, excretion, and disturbance of soil on soil microorganisms and/or plant roots. The burrowing activity of crabs may increase the diffusion of gas and increase soil concentration of O_2 and this increases soil oxidation capacity. Similar increases in organic C mineralization due to high litter decomposition rates in crab burrows observed in other wetlands (Daleo and Iribarne 2009; Weissberger et al. 2009) depended on the stimulation aerobic bacteria respiration (Liang et al. 2015).

Estuarine wetlands are an important source of CH_4 (Du et al. 2016) and emissions of CH_4 mainly depend on the relative balance its production and oxidation processes. In this study, greater CH_4 emissions were correlated with presence of crabs throughout the year. Consumption of plant and animal litter by crabs is converted to detritus and promotes the accumulation of organic C (Moseman-Valtierra et al. 2011) and this stimulates the growth and reproduction of methanogens (Kammann et al. 2009) leading to increased CH_4 production and emission. Burrowing activities of crabs increase the sediment-water-gas contact interface, and the diffusion of inorganic N in sediments from the overlying water bodies and accelerates the rate of ammonia formation (Mereta et al. 2013) and its accumulation in soil. Studies have found that an increase in NH_4^+ inhibits methanotrophic activity and this promotes CH_4 emissions (Hu et al. 2015).

Our results showed that N_2O fluxes were greater in the presence of crabs. Production of N_2O was the result of a combination of soil nitrification and denitrification (Harley et al. 2015), which are microbial processes, so factors that affect soil microbial activity also impact soil nitrification and denitrification (Angar et al. 2016). Crab burrowing activity results in the heterogenous creation of anaerobic and aerobic micro-sites in soils that are used by a diverse range of microbes; soils surrounding burrows were compacted and anaerobic, while burrows facilitate advection and diffusion of O_2. Microbial activity in these contrasting soil environments generates N_2O through nitrification and denitrification (Liu et al. 2008). Crabs may affect microbial diversity through feeding on bacteria and fungi, mechanical action on organic matter, propagation of microbial propagules, and changes in nutrient availability that leads to increases in soil N_2O emissions (Cragg and Bardgett 2001). The O_2 exchange interface between soil and water or the atmosphere is increased in crab burrows. This may lead to increased soil nitrification at the burrow wall, while soil behind this wall may remain anaerobic (Kristensen et al. 2008) and support denitrification leading to greater production and release of N_2O. Studies have shown that crabs in upper tidal flats may elicit environmental effects. For example, high-densities of crabs at small spatial scales accelerate N_2O release in flooded tidal flats (Liu et al. 2008), and crabs fragmentation of surface accelerates litter decomposition with the release of N to the soil environment (Chen et al. 2010). In addition, direct input of excreta by crabs increases soil N concentration (Van et al. 2015) that triggers N_2O emissions due to the increased abundance and activity of nitrifying and denitrifying soil bacteria (Welsh et al. 2015).

Global warming potential (GWP) of the upper tidal flats increased by 138% with than without crabs, and GWP of the mid-tidal flats increased by 99.3% % with than without crabs (Tables S2, S3). This a very noticeable result because highlight the importance that the direct impacts of global warming on ecosystem processes can in turn exert a powerful feed-back effect on future climate change. In this case a clear positive feed-back has been detected. Rises in sea levels due to global warming are predicted and under this scenario further increases in the intensity and frequency of flooding are expected that may result in greater crab activity in wetlands, along with associated changes in soil properties (Chen et al. 2008; Wang et al. 2011; Liu et al. 2015). We have demonstrated that higher crab presence favors the production and emissions of greenhouse gases closing a positive feed-back effect.

Effects of crab presence on soil C, N, and P concentrations

Soil C, N, and P in wetlands may be affected by multiple factors, such as microbial activity, animal disturbance, decomposition of animals and plants, tidal movement, and human activities (Liu et al. 2014). In this study, the disturbance by crabs was correlated with changes in the concentrations of soil C, N, and P that varied with season. The greatest concentrations of soil C, N, and P were recorded in autumn, correlated with plant senescence and higher inputs of litter that results in larger inputs of food to be consumed and decomposed by the crabs. Overall soil TP concentrations were significantly lower in the presence of crabs than in the control. These results were not consistent with the findings of Mortimer et al. (1999) who did not observe changes in soil TP in the presence of crabs. Animal excreta (feces + urine) drive soil N:P ratios (Sitters et al. 2017). Nevertheless, the greater range in soil TC and TN than TP concentration values throughout the year is consistent with lower mobility and transformation rates of soil P than C and N.

Ecological stoichiometry of soil C, N, and P under the presence of crabs

Concentrations of soil C, N, and P vary spatio-temporally with soil factors, such as animal activity, vegetation, climate, and human interference (Cleveland and Liptzin 2007; Liu et al. 2014). We found that C:N, C:P, and N:P ratios were lowest in summer and these lower ratios were correlated with higher temperatures in summer when microbial activity is also higher, favoring nutrient use and organic mineralization. The greater release of soil C during the summer reduces its soil concentration and thus favors the drop of its ratios with nutrients, indicating that soil C:nutrient ratios are also controlled by soil C retention and input (Xiao et al. 2014). The decrease in the N:P ratio during the summer is likely due to the slower mineralization rates of P than N, while N responded more rapidly to environmental changes than P (Cleveland and Liptzin 2007). These results confirm the observed nutrient limitation that occurs during *S. alterniflora* growth in the estuarine wetlands of the Minjiang River (Wang et al. 2015c; Mactavish and Cohen 2017) that lead to greater plant N and P-uptake, especially during summer.

The ratios of C:N, and N:P were greater with than without crabs, and these ratios were greater in the crab-free upper tidal flats than in the crab-free mid-tidal flats. Thus, the presence of crabs was correlated with greater N:P ratios and thus specially with the lower TP concentration.

Nutrient ratios and C concentration have important implications for C storage. In this study, we found the ecological stoichiometric ratios (C:N, C:P, and N:P) were positively correlated with C stocks, supporting findings of Wang et al. (2016). The N:P ratio in soils of our study without and with crabs (2.03 and 2.14) were lower than the national wetland N:P ratio (13.6) (Zhang et al. 2016); in general, a lower N:P ratio indicates greater primary productivity capacity, but N-limitation may become more critical for plant growth in this wetland area under increasing temperatures due to climate change (Wang et al. 2015c).

Effect of presence of crabs on relationships between soil greenhouse gas emissions and soil nutrient and ecological stoichiometry

The availability of soil organic C is an important factor for microbial-mediated decomposition processes and CO_2 flux (Vieux et al. 2013). Crabs convert litter into debris through feeding and digestion and this debris is subsequently incorporated into the soil in the form of excrement; this process increases input to the soil of organic substances and abundance and activity of soil microbes that altogether lead to accelerated C-cycling (Žifčáková et al. 2016), accumulation of organic C in the soil, and increased CO_2 production and emissions (Mehnaz et al. 2018). When oxidation of organic matter is incomplete, as expected under flooding conditions, the production of a series of pyrolytic compounds increases and this increase the resistance to chemical and biological degradation (Shahbaz et al. 2017). However, crab activity increases aeration and destroys large aggregates, allowing greater decomposition of soil organic matter until complete oxidation occurs, resulting in greater CO_2 formation (Lehmann and Kleber 2015; Murphy et al. 2017).

Consistently, in mid flooding plots without crabs we found a positive correlation between CH_4 emission flux and ratios of C:N and C:P , indicating that increasing amounts of organic C substrates with lower nutritional quality favored growth and development of soil methanogens, and an increase in CH_4 production and emissions in absence of crabs. This was not observed in the presence of crabs suggesting the role of crabs in increasing soil aeration and destroying large aggregates.

Other drivers of greenhouse gas emissions from wetland soils

In this study, the CO_2 flux of soil was positively correlated with soil temperature, likely as a result of associated increases in soil microbial abundance and activity (Andrews et al. 2000). Increases in soil temperature to 35 °C drive increases in the abundance and activity of soil microorganisms and CO_2 emission flux (Pugh et al. 2018). Soils contain autotrophic and heterotrophic respiring microorganisms (Murdiyarso et al. 2010), and temperatures of c. 15–31 °C enhance wetland soil microbial activity and accelerate the rate of degradation of soil organic C, leading to greater releases of CO_2 (Yang et al. 2017).

We found that emissions of CH_4 from the soil were positively correlated with soil temperature, supporting a previous study in a wetland (Yang et al. 2017). The associated increase in soil microbial activity with temperature increases soil O_2 consumption and this stimulates the growth of methanogens (Zheng et al. 2018), thus observing a positive correlation between soil N_2O flux and soil temperature consistent with Tian et al (2015).

As soil temperatures rise, soil microbial nitrification and denitrification and N_2O emission rate similarly increase (Castaldi 2000). The optimum soil temperature range for nitrification is c. 15–35 °C, while that for denitrification is c. 5–75 °C (Sun et al. 2010), so it is likely that these processes were active in this study, with greater levels of microbial activity and associated N_2O emissions from soil. The soil water content, which was 44.2–59.1%, was positively correlated with N_2O flux, probably because of nitrification processes are favored in this range of soil water content (Bramley and White 1989; Szukics et al. 2010). These results support a previous study that showed N_2O production and emissions increased with increasing soil water content (Di et al. 2014). N_2O flux was positively correlated with soil pH, consistent with what observed in previous reports (Nkongolo et al. 2008; Duan et al. 2018), associated with the microbial growth (Castellano-Hinojosa et al. 2018). Moreover, the wetland anaerobic environment was rich in ammonium substrate, and then N_2O flux here is mainly produced by nitrification, and its emissions increase with the increase of soil pH.

Conclusion

This study, at the best of our knowledge is the first that have studied the shift of gas emissions and nutrient stoichiometry due to the presence of crabs in a subtropical estuarine wetland ecosystem. The presence of crabs mainly accelerates the C and N cycling probably due to increasing aeration, allowing greater decomposition of soil organic matter with greater CO_2 formation. However, crab presence, specially in up-tidal sites, decreased soil P concentration, thus rising soil C:P and N:P ratio.

Crab activity (feeding and digestion) also favored the incorporation of litter into soil in the form of excrement; this process increased the input of organic substances to soil and the abundance and activity of soil microbes, both leading to accelerated C-cycling and increased CO_2 production and emissions. Moreover, crab burrowing activity resulted in the heterogeneous creation of anaerobic and aerobic micro-sites, where soils surrounding burrows were compacted and anaerobic, while burrows facilitate advection and diffusion of O_2. This soil environment generates the conditions favorable for CH_4 production and for N_2O emission. Cumulative CH_4 emissions were significantly higher with than without crabs under both tidal intensities, whereas cumulative CO_2 and N_2O emissions were also higher with than without crabs but the impact was significantly higher under up- than under middle-tidal intensity.

The rise of sea level due to global warming may increase the intensity and frequency of flooding, thus promoting the activity of crabs in wetlands, along with associated changes in soil properties, thus inducing a positive feed-back effect on global warming.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Tables

Table 1 Correlation between greenhouse gas emission fluxes and environmental factors (Pearson correlation coefficient)

Treatment	Index	pH	Salinity	Bulk density	Temperature	Water content
GP	CO_2	-0.445	0.14	-0.347	-0.274	0.117
	CH_4	-0.258	0.407	0.315	-0.234	-0.082
	N_2O	0.816**	-0.742**	-0.162	0.02	0.591*
GD	CO_2	-0.107	0.007	-0.331	0.224	-0.228
	CH_4	0.459	-0.857**	-0.169	0.51	0.178
	N_2O	-0.454	-0.114	-0.308	0.856**	0.701*
ZP	CO_2	-0.452	-0.16	-0.430	0.654*	-0.308
	CH_4	0.235	-0.254	-0.624*	0.636*	0.267
	N_2O	-0.482	-0.052	-0.246	0.616*	-0.238
ZD	CO_2	-0.254	-0.224	-0.532	0.839**	0.012
	CH_4	0.037	-0.21	-0.451	0.887**	0.534
	N_2O	-0.247	-0.138	-0.875**	0.883**	0.374
Total	CO_2	-0.257	-0.091	-0.358*	0.352*	-0.113
	CH_4	-0.039	-0.113	-0.186	0.598**	0.134
	N_2O	0.439**	-0.143	0.005	0.568**	0.386*

GP: upper tidal flat in presence of crabs; GD: upper tidal flats in absence of crabs; ZP: mid-tidal flats in presence of crabs; and, ZD: mid-tidal flats in absence of crabs. $^*P<0.05$, $^{**}P<0.01$.

Table 2 Correlation between emissions of greenhouse gases and soil content of nutrients in the presence and absence of crabs (Pearson correlation coefficient)

Treatment	Index	TC	TN	TP	C:N	C:P	N:P
GP	CO_2	-0.282	-0.172	-0.106	-0.154	0.170	0.344
	CH_4	0.055	-0.064	-0.086	0.352	0.301	0.139
	N_2O	0.633*	0.746**	0.787**	-0.093	-0.097	-0.103
GD	CO_2	-0.300	-0.151	-0.060	0.039	-0.116	-0.206
	CH_4	-0.283	0.025	0.102	-0.440	-0.448	-0.315
	N_2O	0.773**	0.833**	0.778**	0.150	-0.227	-0.429
ZP	CO_2	-0.576*	-0.115	0.301	-0.175	0.095	0.232
	CH_4	0.473	0.476	0.488	0.055	0.111	0.114
	N_2O	-0.468	-0.071	0.340	-0.034	0.173	0.244
ZD	CO_2	-0.616*	-0.441	0.338	0.468	0.066	-0.675*
	CH_4	-0.273	-0.180	0.068	0.710**	0.601*	-0.118
	N_2O	-0.568	-0.066	0.267	0.769**	0.482	-0.187
Total	CO_2	-0.374**	-0.153	0.099	0.059	0.093	-0.022
	CH_4	0.026	0.058	0.157	0.355*	0.289*	-0.009
	N_2O	0.260	0.351*	0.283	0.082	0.097	0.042

TC: soil total C; TN: soil total N; soil TP: total P; C:N: soil C:N ratio; C:P: soil C:P ratio; N:P: soil N:P ratio; GP: upper tidal flat in presence of crabs; GD: upper tidal flats in absence of crabs; ZP: mid-tidal flats in presence of crabs; and, ZP: mid-tidal flats in absence of crabs. **P*<0.05, ***P*<0.01.

Figure Captions

Fig. 1 Location of the study area and sampling sites in southeastern China

Fig. 2 Effect of presence of crabs on CO_2 emission fluxes from soils of a *Spartina alterniflora* wetland. Different uppercase letters indicate within season treatment differences and different lowercase letters indicate between season treatment differences ($P<0.05$)

Fig. 3 Effect of presence of crabs on CH_4 emission fluxes from soils of a *Spartina alterniflora* wetland. Different uppercase letters indicate within season treatment differences and different lowercase letters indicate between season treatment differences ($P<0.05$)

Fig. 4 Effect of crabs on N_2O emission fluxes from soils of a *Spartina alterniflora* wetland. Different uppercase letters indicate within season treatment differences and different lowercase letters indicate between season treatment differences ($P<0.05$)

Fig. 5 Effect of crabs on soil TC, TN, and TP concentrations and their stoichiometric ratios. Different uppercase letters indicate within season treatment differences and different lowercase letters indicate between season treatment differences ($P<0.05$)

Fig. 6 Linear regression of the effect of crabs on relationships between soil C, N, and P. The y1 and y2 functions represent the linear fitting relationship of total nutrients with and without crab activity, respectively.

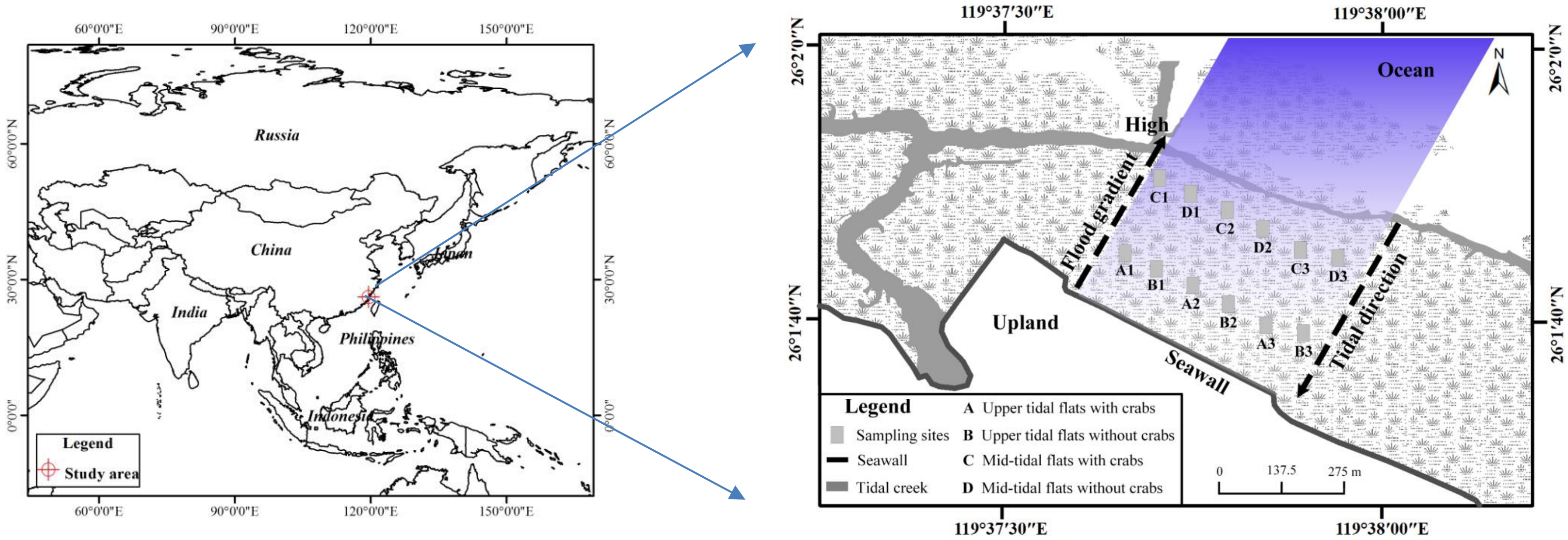
60°0'0"E
90°0'0"E
120°0'0"E
150°0'0"E
60°0'0"N
30°0'0"N
0°0'0"
Russia
China
Japan
India
Philippines
Indonesia
Legend
Study area
119°37′30″E
119°38′00″E
26°2′0″N
26°1′40″N
Ocean
N
High
Flood gradient
Tidal direction
Upland
Seawall
C1
D1
C2
D2
C3
D3
A1
B1
A2
B2
A3
B3
Legend
Sampling sites
Seawall
Tidal creek
A Upper tidal flats with crabs
B Upper tidal flats without crabs
C Mid-tidal flats with crabs
D Mid-tidal flats without crabs
0
137.5
275 m

Fig. 1

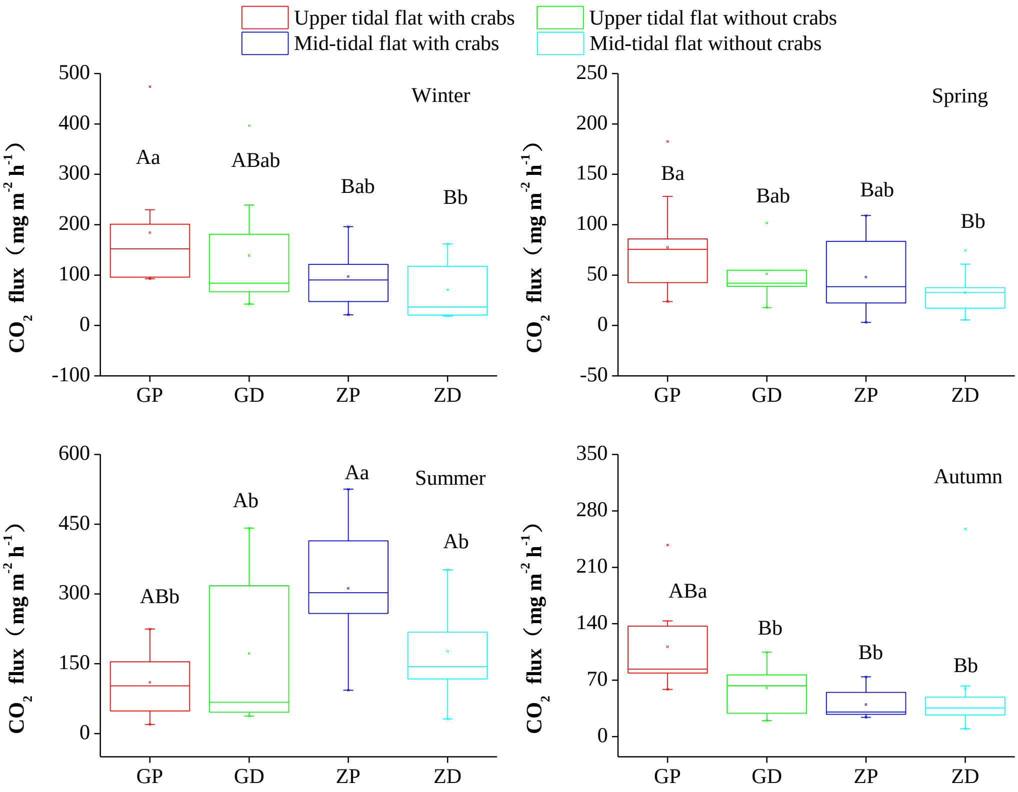
Upper tidal flat with crabs
Upper tidal flat without crabs
Mid-tidal flat with crabs
Mid-tidal flat without crabs
Winter
Spring
Summer
Autumn
CO$_2$ flux（mg m^{-2} h^{-1}）
500
400
300
200
100
0
-100
250
200
150
100
50
0
-50
600
450
300
150
0
350
280
210
140
70
0
Aa
ABab
Bab
Bb
Ba
Bab
Bab
Bb
ABb
Ab
Aa
Ab
ABa
Bb
Bb
Bb
GP
GD
ZP
ZD

Fig. 2

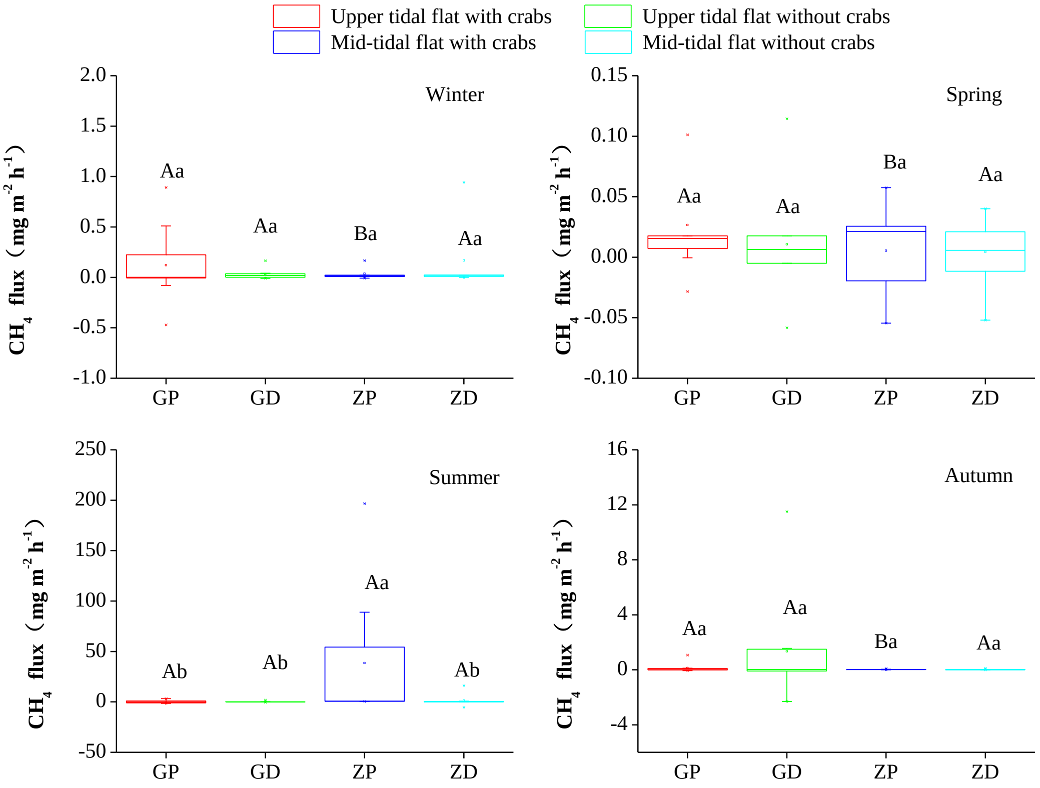
Upper tidal flat with crabs
Upper tidal flat without crabs
Mid-tidal flat with crabs
Mid-tidal flat without crabs
Winter
CH4 flux（mg m-2 h-1）
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Aa
Aa
Ba
Aa
GP
GD
ZP
ZD
Spring
0.15
0.10
0.05
0.00
-0.05
-0.10
Aa
Aa
Ba
Aa
GP
GD
ZP
ZD
Summer
250
200
150
100
50
0
-50
Ab
Ab
Aa
Ab
GP
GD
ZP
ZD
Autumn
16
12
8
4
0
-4
Aa
Aa
Ba
Aa
GP
GD
ZP
ZD

Fig. 3

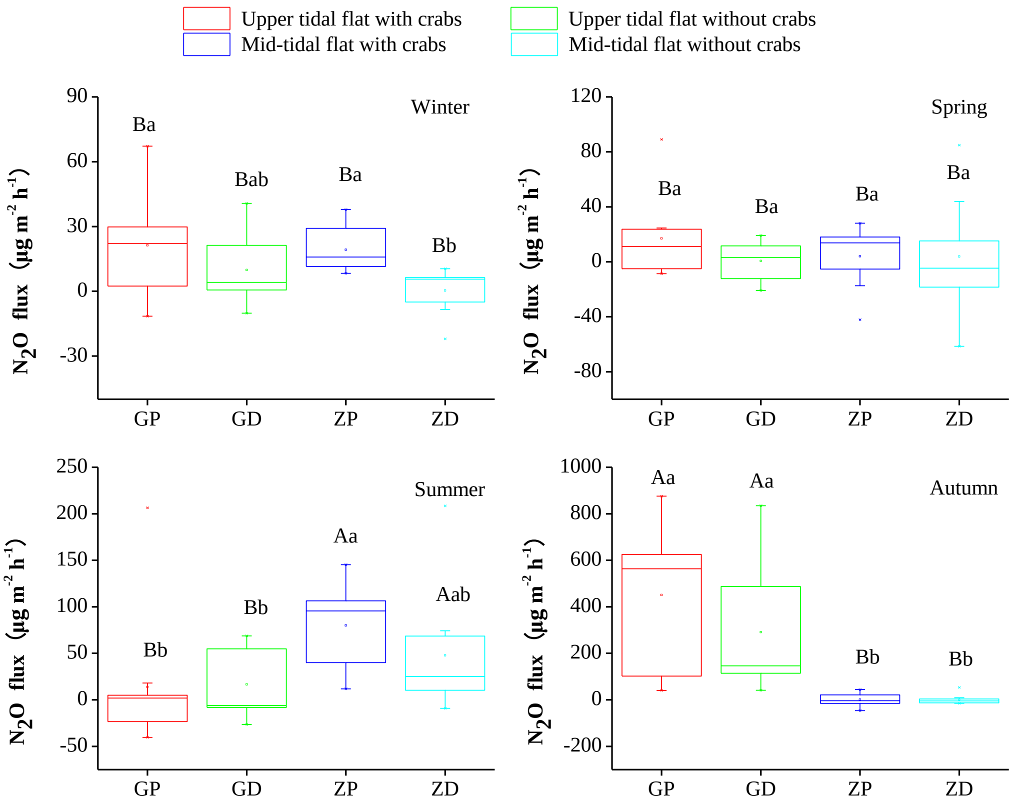
Upper tidal flat with crabs
Upper tidal flat without crabs
Mid-tidal flat with crabs
Mid-tidal flat without crabs
Winter
Spring
Summer
Autumn
N2O flux (μg m-2 h-1)
90
60
30
0
-30
120
80
40
0
-40
-80
250
200
150
100
50
0
-50
1000
800
600
400
200
0
-200
Ba
Bab
Ba
Bb
Ba
Ba
Ba
Ba
Bb
Bb
Aa
Aab
Aa
Aa
Bb
Bb
GP
GD
ZP
ZD

Fig. 4

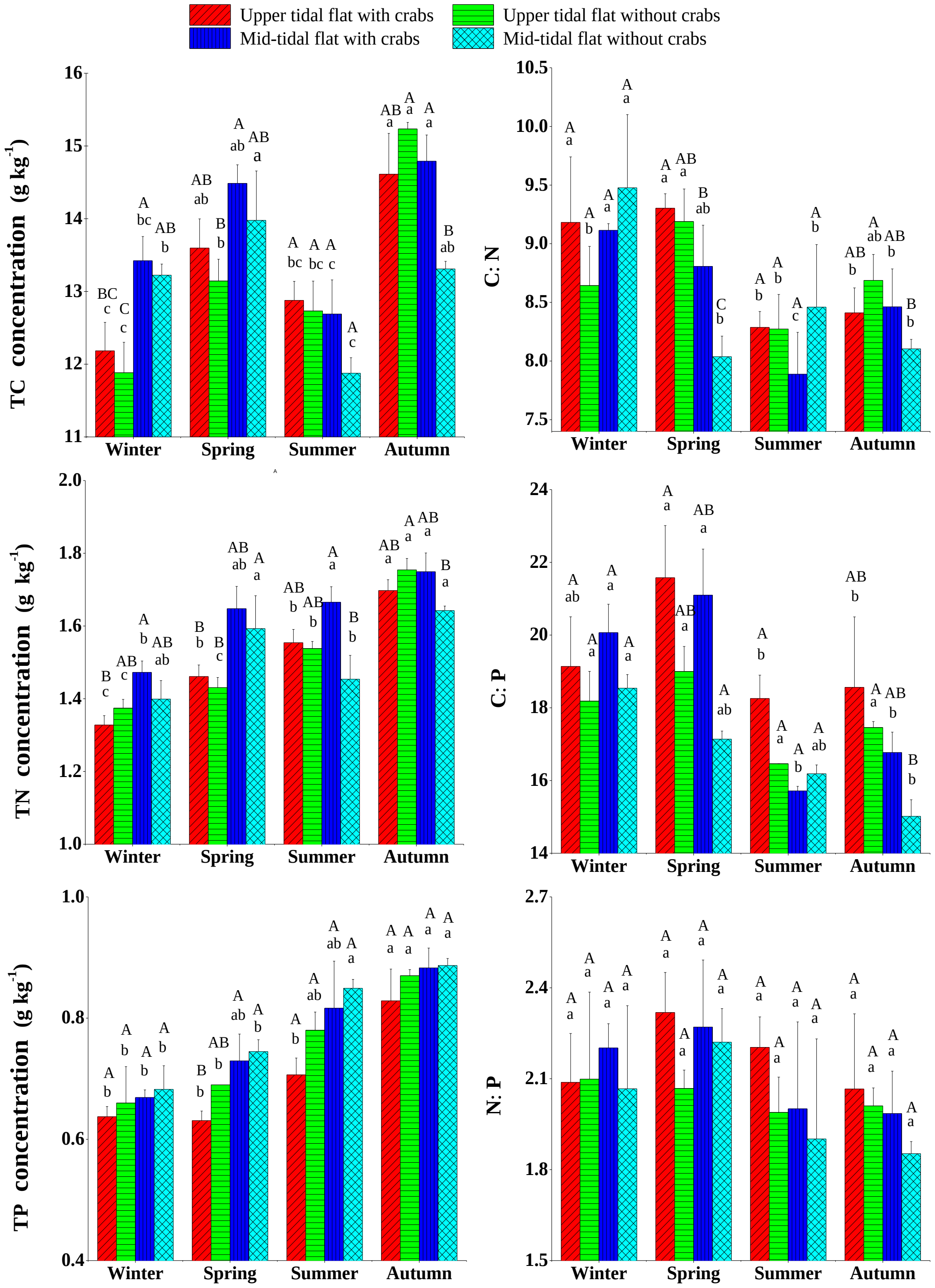
Upper tidal flat with crabs
Upper tidal flat without crabs
Mid-tidal flat with crabs
Mid-tidal flat without crabs
TC concentration (g kg^{-1})
11
12
13
14
15
16
C: N
7.5
8.0
8.5
9.0
9.5
10.0
10.5
TN concentration (g kg^{-1})
1.0
1.2
1.4
1.6
1.8
2.0
C: P
14
16
18
20
22
24
TP concentration (g kg^{-1})
0.4
0.6
0.8
1.0
N: P
1.5
1.8
2.1
2.4
2.7
Winter
Spring
Summer
Autumn

Fig. 5

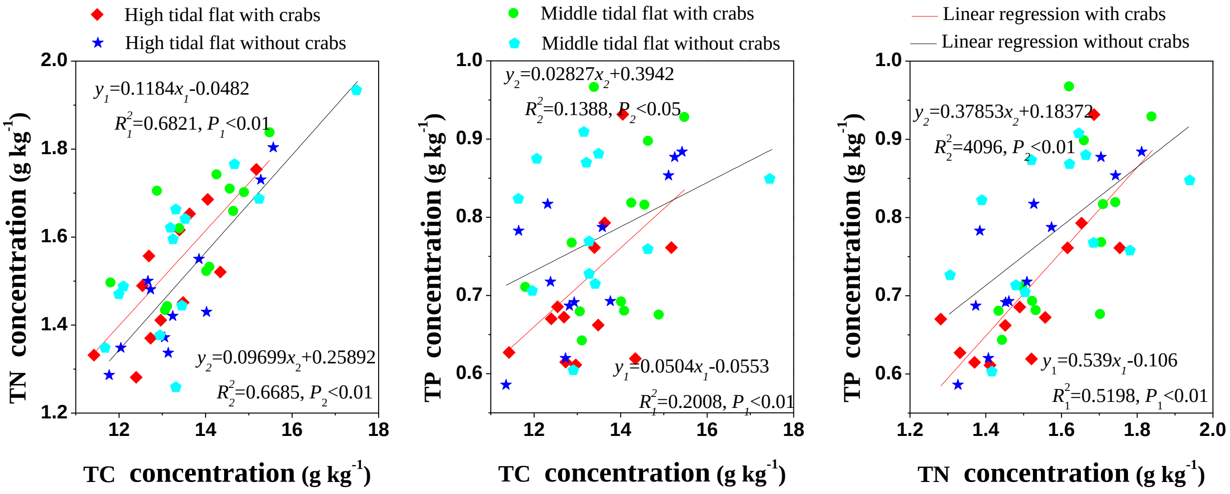
High tidal flat with crabs
High tidal flat without crabs
Middle tidal flat with crabs
Middle tidal flat without crabs
Linear regression with crabs
Linear regression without crabs
y1=0.1184x1-0.0482
R1²=0.6821, P1<0.01
y2=0.09699x2+0.25892
R2²=0.6685, P2<0.01
TN concentration (g kg-1)
TC concentration (g kg-1)
2.0
1.8
1.6
1.4
1.2
12
14
16
18
y2=0.02827x2+0.3942
R2²=0.1388, P2<0.05
y1=0.0504x1-0.0553
R1²=0.2008, P1<0.01
TP concentration (g kg-1)
TC concentration (g kg-1)
1.0
0.9
0.8
0.7
0.6
12
14
16
18
y2=0.37853x2+0.18372
R2²=4096, P2<0.01
y1=0.539x1-0.106
R1²=0.5198, P1<0.01
TP concentration (g kg-1)
TN concentration (g kg-1)
1.0
0.9
0.8
0.7
0.6
1.2
1.4
1.6
1.8
2.0

Fig. 6