

Comments R1:

This is a promising study of carbon emissions and soil mineralization potential from non-tidal salt marshes which offers unique data with which to improve understanding of relationships between gas fluxes, plant physiology, and salinity. Marshes in the region are relatively understudied and the non-tidal system is also poorly known in terms of methane dynamics. The measurement of CO₂ exchange from woody plant tissues reveals new insights about the capacity for carbon uptake throughout the plant body in the salt marsh species studied here.

Thank you very much for the feedback on the value of the study.

One major finding is that the salt meadow and halophilous scrub had lower soil mineralization potential but higher soil CO₂ emissions than the glasswort sward. This result seems counter-intuitive and I would like to see more discussion to potentially explain how longer term carbon could be sequestered despite the shorter term CO₂ fluxes from soils.

We agree that, at first glance, this result may seem counter-intuitive, but it is important to remember that the mineralization quotient is calculated as the ratio between carbon emitted and carbon stored as soil organic carbon (SOC) (Pinzari et al., 1999). In a parallel study, we found significantly higher amounts of carbon in the soils of the halophilous scrub and the salt meadow than in the glasswort sward, in accordance with a much higher aboveground, belowground and litter biomass in the former two habitats (Carrasco-Barea et al. 2023). Therefore, despite the higher soil CO₂ emissions in the halophilous scrub and the salt meadow, they showed lower mineralization quotients due to their much greater amount of SOC. We have clarified this in the discussion in lines 404-409 (“*Despite the halophilous scrub and the salt meadow had higher soil CO₂ emissions than the glasswort sward, they showed lower mineralization quotients due to a much greater amount of SOC (Table S3), which was in accordance with a much higher aboveground, belowground and litter biomass (Carrasco-Barea et al., 2023). Hence, our results would indicate that soils of the halophilous scrub and the salt meadow would have a higher carbon sequestration potential, despite their higher soil carbon emissions*”).

Authors meanwhile found higher CO₂ uptake from woody tissues of the plants in the former two habitats. I wonder whether this might contribute to or be related to the higher carbon sequestration rates in those soils?

As commented above, soil carbon sequestration capacity is related to soil CO₂ emissions and soil organic carbon content, which is directly linked to the amount of organic matter that arrives to the soil surface, ready to be decomposed and integrated into the soil. The CO₂ fluxes from woody stems measure the net CO₂ exchange between the woody living surface of plants and the atmosphere, and thus are not directly related to what happens at the soil compartment. Nevertheless, we could speculate that higher CO₂ uptake by woody tissues might lead to increased woody tissue biomass, potentially resulting in greater incorporation of more recalcitrant organic matter into the soil. However, this remains speculative, and we have decided not to include it in the discussion.

Another highlight from the study is that relatively large methane emissions were observed despite the salinity of the marshes. Authors partially attribute these high methane fluxes to the influence of low salinity groundwater, which is logical. However, I am concerned that the value of the methane emissions may be over-estimated due to

the long duration of chamber closure (24h). Taking only 2 samples (initial and final) over this 24 period limits the precision of the methane fluxes as well. Authors should take care to interpret their emissions in relative terms (comparison between habitats) rather than drawing comparisons with literature, unless they find studies that have employed similarly long chamber deployments.

We agree that using different methodologies is a handicap when comparing results from various studies. Thus, we have included a sentence indicating which of the mentioned studies collected samples after 24h of chamber closure as we did, in lines 453-455 (*“although it is worth mentioning that only Hirota et al. (2007) took samples after 24h of chamber closure, as it was performed in the present study”*).

The data presentation does need to be improved. Please use different symbols or colors to distinguish the marsh habitats or plant species in all data figures. With the current version (all gray), one cannot discern these groups.

Following your suggestion, we have improved data presentation using different types of lines to distinguish the different habitats and species in all the figures.

Additional detailed suggestions are attached.

Review for 2024-1320

Abstract

Line 14: Clarify that *H. portulacoides* and *E. atherica* are part of the same habitat (similar to line 85)

Done.

Introduction

Consider also methylotrophic methanogens which persist in saline environments.

We have included a sentence about the predominance of methylotrophic methanogens in saline environments in lines 64-67 (*“Specifically, acetoclastic and hydrogenotrophic methanogens, with their lower energetic yields, are more susceptible to increasing salinity than methylotrophic methanogens, which explains the predominance of methylotrophic methanogens like Methanohalophilus spp. in hypersaline environments (Mcgenity and Sorokin, 2018).”*

Line 68- How extensive are non-tidal marshes in the Mediterranean? Elsewhere?

This information has been added to the text in lines 72-74 (*“Hence, considering the extensive coverage of non-tidal salt marshes in the Mediterranean Basin, which has been estimated in approximately 19 million hectares (around 2.5% of the total area of the 27 Mediterranean countries and 1 to 2% of wetlands in the world; Geijzenborffer et al., 2018)”*).

Line 85-90: Which plant species are C3 vs C4?

It is specified in lines 91-92 that all species are C3 plants.

Line 93: Specify the temperature and salinity ranges typical of this region. Are there any salinity differences in the marsh soils between habitats or seasons?

We have included the lowest and the highest mean temperatures in the text (line 98) and we have added a Figure (supplementary material, Figure S2) showing monthly mean daily temperatures and total rainfall for the previous 10 years and for the study year (2017). Besides, we have included the groundwater salinity in section 2.1 (lines 103-105). Soil salinity differences between habitats and seasons (represented as variations in soil electrical conductivity) are shown in section 3.2.1 (Figure 3).

Section 2.2

How does severing the plant stems and leaves affect the CO₂ fluxes? How long were they stored in the refrigerator?

(Were any measurements done on live, intact plants in the field?)

All measures of net CO₂ fluxes from vegetation were conducted in the field using intact and attached plant tissues. We have clarified this in the text in lines 108-109 (*“Measurements were performed in the field, using attached living green and woody plant tissues”*). After measures were performed, we collected the measured plant fractions, and we stored them in a fridge until the sampled leaf area was determined (within the next 24h). We have included this information in line 119.

Line 110-111: How was stomatal conductance measured?

We measured stomatal conductance with the same infrared gas analyser (IRGA) used for net CO₂ exchange measures (CIRAS-II, PPsystems USA). We have clarified this in the text (line 107).

Line 126-130: Specify make/model of the gas chromatograph- was it using flame ionization detector?

We have specified the model of the gas chromatograph used (Agilent 7890A, Agilent Technologies USA) and that it was connected to a thermal conductivity detector (lines 143-144).

Two methods for soil respiration rates are reported: The soda lime method was used when soils were not flooded. A gas chromatography based method was used when soils were flooded. How was “flooded” defined?

How many measurements were made with each method? (This information will help readers to understand whether flooding frequent or infrequent).

We considered a soil as flooded when it was covered by water. We have included this information in line 140. The number of measures performed with each method are detailed in Table S2. A sentence has been added to inform readers about this Table (lines 154-155).

I am not familiar with the soda lime method. Authors should better support their statement that gas chromatography underestimates CO₂ fluxes relative to the soda lime method. With only an initial and final time point, over 24 hours, the fluxes are not very precise. They may be affected by artifacts such as accumulation of pressure or altered temperatures, both of which could influence the gas fluxes measured.

We agree that the statement about gas chromatography has not enough support, and thus we have decided to remove the sentence “it has been observed that gas chromatography can underestimate CO₂ emission rates by up to 45% in comparison with the soda-lime method (Lou and Zhou, 2006)”. Instead, we have focused on explaining why we chose the

soda lime method, rather than gas chromatography, in order to have an integrative measure of soil CO₂ fluxes throughout the whole day (day and night), see lines 151-154 (“Gas chromatography analyses were not used to estimate soil respiration when the soil was not flooded because temperature and humidity variations throughout the day and night could affect the concentration of gas components in the sample (Rochette and Hutchinson, 2005), not being this a problem by using the soda-lime method, which can integrate soil CO₂ fluxes over long periods, such as 24h (Keith and Wong, 2006)”). We have also added a sentence clarifying that previous studies have demonstrated that soda lime is a reliable method for estimating soil CO₂ fluxes, see lines 128-129 (“This method gives a reliable and integrative measurement of soil CO₂ fluxes throughout the whole day (Keith and Wong, 2006)”).

Results

Figures: Colors or symbols are needed to distinguish the species represented by each line.

We have improved the data presentation by using different types of lines to distinguish habitats and species in the figures.

Figure 4a: Since these are different methods used to measure CO₂ fluxes from flooded vs unflooded soils, the study should not make claims about differences in soil respiration between flooded and exposed conditions. Likewise, authors should omit the flooded data points from Figure 4a to avoid direct comparison with the non-flooded data.

We understand your concern, but we believe this comparison is interesting despite the different methodological approaches applied. For this reason, we have decided to keep these data points in Figure 4a, but, in order to clarify that measurements were performed using two different methods, we have added a sentence in the figure caption and we have highlighted this limitation in the discussion in lines 427-428 (“However, since different methods were used to measure soil respiration in flooded and non-flooded soils, this comparison should be interpreted with caution.”).

Discussion

Could the higher photosynthetic rates be related to C4 metabolism in *E. atherica* (in addition to structural difference in stomata?) Which species are C3 vs C4?

As commented before, all the studied species are C3 plants (lines 91-92).

Line 327: Listing the species in consistent order of water use efficiency would be clearer for the reader

Thank you for your suggestion. We have ordered the species from high to low WUE values (Lines 344-345).

Line 397-399: Avoid direct comparison of flooded and unflooded CO₂ fluxes (as mentioned above) due to differences in methods. Authors might rather consider that flooding waters are a known physical barrier to gas exchange.

As commented above, we have highlighted this limitation in the discussion (lines 427-428) and explained the effect that flooding has on the diffusion of CO₂ molecules in lines 426-427 (“A reduction of soil CO₂ emission to the atmosphere during flooding conditions can be explained by the fact that CO₂ molecules diffuse 10000 times slower in water than in air (Kathilankal et al., 2008).”)

Table 1: Which methods were used in the studies on this table? Are they comparable to those in this study?

The most common method used in these studies is gas chromatography for both CH₄ and CO₂ measures, with the infrared gas analyser being also used to determine CO₂ fluxes in one study. To highlight these methodological differences, we have included this information in Table 1. Despite the time period in which the chamber was closed was generally shorter than in our study, the study by Hirota et al. (2007) also kept the chamber closed during 24h. We have added some comments about this to the discussion in lines 420-423 (*“Nevertheless, it should also be noted that the methodology used to determine soil CO₂ and CH₄ fluxes differs from that generally employed in the studies listed in Table 1, since most of them used gas chromatography for both CH₄ and CO₂ measurements. Thus, an effect caused by these methodological differences cannot be excluded”*), in lines 427-428 (*“However, since different methods were used to measure soil respiration in flooded and non-flooded soils, this comparison should be interpreted with caution.”*) and in lines 453-455 (*“although it is worth mentioning that only Hirota et al. (2007) took samples after 24h of chamber closure, as it was performed in the present study.”*).

Discussion of methane lines 401-414: Most of the methane fluxes were positive, and so authors should not mislead the reader by first discussing negative fluxes (indicating consumption). Similarly, the methane emissions did not differ statistically between habitats. Discussion in this section should therefore focus on what might have been similar between habitats and/or how the general magnitude of fluxes falls within the range reported in other marshes.

This part of the discussion has been rewritten following your suggestion (lines 429-455). Thank you for the comment.

Line 425-435: This paragraph about salinity relationships to methane emissions is useful for readers to place this study site and its findings in context. This information about the site salinity should be incorporated into the methods/ site description.

We have included this information within section 2.1 (lines 103-105).

Conclusions

Authors should discuss the possible relationship of the high CO₂ uptake of woody tissues with the high carbon sequestration potential (as reflected by low mineralization quotients) for the salt meadow and halophytic scrub. Can this help to reconcile the finding of lower soil mineralization quotients despite the high respiration and methane emissions observed?

As we mentioned above, soil carbon sequestration capacity is related to soil CO₂ emissions and soil organic carbon content, while CO₂ fluxes from woody stems measure the net CO₂ exchange between the living woody surfaces of plants and the atmosphere and are therefore not directly related to the soil compartment. Hence, although we could speculate that higher CO₂ uptake by woody tissues might lead to increased woody biomass and potentially more organic matter being incorporated into the soil, this remains purely speculative. Therefore, we have decided not to include this speculation in the discussion.

CO₂ emissions may be higher in this study than in other previous studies due to the long period of chamber closure (24h) and associated artifacts discussed above.

The soda-lime method has been proved to be a reliable way to integrate daily soil CO₂ fluxes (as commented above, see for instance Keith and Wong, 2006). Previous studies have also used 24h of chamber closure, as we have now highlighted in the discussion (lines 453-455) (Hirota et al., 2007). In response to your comments, we have also included several sentences to inform the reader about the different methodologies used in the studies cited throughout the manuscript (lines 420-423 and 427-428).