

## ***Interactive comment on “Response of key stress-related genes of the seagrass *Posidonia oceanica* in the vicinity of submarine volcanic vents” by C. Lauritano et al.***

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We are grateful for Anonymous Referee #1's comments. As we stated in the file attached as Supplement in response to the Editor comments, we have followed all the suggestions and we think the present version of the manuscript is much improved in respect to the previous one. See below our response to specific comments. The manuscript has been modified and will be uploaded when required from the editorial system. In particular, see Q for questions and A for answers:

1. General comments Q1. While gene expression information is a novel addition to this science, as written, this manuscript weakly justifies the use of such information. A1.

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We agree that the rationale of the manuscript was not clearly stated. The motivation of our work was the fact that submarine volcanic vents are being widely used as natural laboratories to assess the effects of increased ocean acidity on organisms and that the observed responses have been attributed exclusively to CO<sub>2</sub> without considering putative stressors associated with the proximity to volcanic vents that may be confounding the CO<sub>2</sub> effects. The hypothesis tested in our work was that the seagrass *P. oceanica* in the proximity of volcanic vents is under higher stress levels than in control areas away from vents. We used the expression of stress genes related to heavy metals, oxidative stress and general stress-related genes to test the hypothesis. We concluded that in fact, *P. oceanica* is under high stress near the vents and that different vents cause different stresses. As CO<sub>2</sub> is expected to be beneficial to seagrasses and cannot explain the gene expression results, care must be taken to interpret its responses near vents as unknown stressors may confound the effects of CO<sub>2</sub>. Our results indicate that the stressors are not related to heavy metals in neither vents. The plants near the Panarea vents are under higher stress levels than at Ischia, related to free radical toxicity. This study constitutes a first step for using stress-related genes of seagrasses as indicators of environmental pressures in a changing ocean. We have modified the abstract, the Introduction and the Discussion to make sure the manuscript

Q2. Additionally, English grammatical errors and a lack of references are found throughout manuscript. A2. The manuscript has been revised for English grammatical errors. In addition, more references have been added along the text.

Specific comments Q3. Page 4948, line 4: “should positively react” is a vague statement A3. The sentence has been rephrased as “ However, in the vicinity of volcanic vents other factors in addition to CO<sub>2</sub>, which is the main gaseous component of the emissions, may directly or indirectly confound the biota responses to high CO<sub>2</sub>. Here we used for the first time the expression of antioxidant and stress-related genes of the seagrass *Posidonia oceanica* to assess the stress levels of the species. . . .”

Q4. Page 4948, line 21 – 22: “environmental and evolutionary. . . .sites”, vague state-

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ment A4. The sentence has been deleted and a new sentence has been added: "This is the first study analysing the expression of target genes in marine plants living near natural CO<sub>2</sub> vents. Our results call for contention to the general claim of seagrass as "winners" in a high-CO<sub>2</sub> world based on observations near volcanic vents. A careful consideration of factors that are at play in natural vents sites other than CO<sub>2</sub> and acidification, must be undertaken. This study constitutes also a first step for using stress-related genes of seagrasses as indicators of environmental pressures in a changing ocean." Differences between Ischia and Panarea sites are reported throughout the text.

Q5. Page 4951, line 25: define "low quantities" A5. The sentence has been rephrased as follows "The accumulation of high quantities of ROI can be very damaging to DNA, RNA and proteins and may activate programmed cell death (PDC)."

Q6. Page 4953, line 14: remove "little spot" and specifically define area A6. "Little spot" has been deleted and the area has been defined (< 5 m<sup>2</sup>).

Q7. Page 4953, line 20: add "relatively" in front of "acidified" A7. "Relatively" has been added.

Q8. Page 4957, line 4: define "adult intermediate leaves" A8. The term has been now specified as follows "For both sites, tissue from the youngest fully mature leaves of the shoots (usually the second-rank leaf)" in paragraph 2.1.

Q9. Page 4958, line 22 – 27: lacking references A9. New references have been added: Rouhier, N., Gelhaye, E., Gualberto, J.M., Jordy, M.N., De Fay, E., Hirasawa, M., Duplessis, S., Lemaire, S.D., Frey, P., Martin, F., Manieri, W., Knaff, D.B. and Jacquot, J.P.: Poplar Peroxiredoxin Q. A Thioredoxin-Linked Chloroplast Antioxidant Functional in Pathogen Defense, *Plant Physiol.*, 134, 3, 1027–1038, 2004. Lubos, E., Loscalzo, J. and Handy, D.E.: Glutathione Peroxidase-1 in Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities, *Antioxid Redox Signal*, 15, 7, 1957–1997, 2011.

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Q10. Page 4959, line 2: "although in one. . ." please specify which site. A10. The site has been now specified as follows: "although in S<sub>2</sub>, pH 7.83, it was not significant".

Q11. Page 4959, lines 22 -25: Without any quantitative measurements of the mentioned factors, this argument is not supportable. In addition, as written, "heat shocks" is considered not probable, however this needs to be reconciled with the paragraph beginning on the same page, line 28. A11. To avoid confusion, the sentence has been deleted.

Q12. Figure 1 is unreadable. A12. Figure 1 has been modified.

Q13. Figure 2 is not helpful as constructed and should be omitted. A13. Figure 2 has been removed from the text, as suggested.

Q14. Table 1 and Figure 1 need to be better connected so that the reader can easily understand the site differences within each vent vicinity as well as cross vent comparisons. A14. Table 1 and Figure 1 now report gene names in the same order, to make easier to follow and understand the results.

Best regards, Gabriele Procaccini

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