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Discussions
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# *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.

**C. Lauritano et al.**

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Dear Dr. Jardine, we are grateful for your new comments to our manuscript and we modified the text accordingly. Here we stated the actions taken to improve the manuscript. Besides the points you raised we also added few more lines in the introduction and in the discussion, to better explain the function of single gene categories. The new text is available and will be submitted when required. Best regards, Gabriele Procaccini.

Please see Q for questions and A for answers:

Q1. Before the manuscript can be considered as an article in Biogeosciences, please



respond the comments of anonymous reviewer 1. A1. We have answered to the comments of anonymous reviewer #1 in the reply to the Editor. We have uploaded the answers again the 9th of May as reply to reviewer #1.

Q2. abstract; list examples of ROS metabolism genes activated at the Panarea site A2. We have now included examples of ROS metabolism genes activated at the Panarea site: "The up-regulation of genes involved in the free radical detoxification response (e.g. CAPX, SODCP and GR) indicates that, in contrast with Ischia, P. oceanica at the Panarea site faces stressors that result in the production of reactive oxygen species, triggering antioxidant responses."

Q3. the introduction if fairly well written but the results section needs to be completely rewritten and expanded to include the details of the results for each gene studied. For example, the authors claim the results show an enhanced expression of genes involved in ROS metabolism, but fail to acknowledge the suppression of many ROS genes relative to the controls. A3. The results section has been rewritten as suggested and is reported below: "P. oceanica samples collected for gene expression analyses were previously genotyped using microsatellite markers, assuring that there were at least 3 distinct genotypes for each gene expression replicate. Results obtained from all distinct genotypes, using the site with normal pH as control, show that different gene category or specific gene functions have different behaviour in the two sampling sites. Opposite patterns of expression levels between the two sites were observed for many HSP (Fig. 1a). In Ischia, many HSPs were significantly down-regulated. In particular, HSP90, HSP83 and the transcription factor HSFA5 were 2-fold down-regulated at both S2 and S3 sites ($p<0.001$), while DNAJ was significantly down-regulated only at S3 site ($p<0.001$). On the contrary, HSP83 ($p<0.05$) and DehSP ($p<0.01$) were significantly up-regulated in the Panarea site (Fig. 1a). The other HSPs did not show significant changes. For the primary metabolism genes, ABC and CYP were significantly up-regulated in the Panarea site ($p<0.01$ for both), while CYP was down-regulated only in the Ischia S2 site ($p<0.01$). ABC did not show significant expression level changes

in Ischia, and ALDH both Ischia and Panarea (Fig. 1b). Regarding genes involved in the antioxidant response (Fig. 2a), CAT did not show significant changes both in Ischia and Panarea, while among the SOD isoforms analysed (SODCP, CSD1, FSD and MSD), only the Cu-Zn chloroplastic one (SODCP) was down-regulated in Ischia S3 site ($p<0.001$) and up-regulated at Panarea ($p<0.01$). For the glutathione-related enzymes (GST, GPX, GSH-S and GR), GST was significantly down-regulated only at S3 Ischia site ($p<0.001$), GPX was up-regulated at all sites ($p<0.05$ for Ischia S2, $p<0.01$ for Ischia S3 and Panarea), GSH-S did not show significant variations and GR was down-regulated at both Ischia sites ($p<0.001$ for both) and up-regulated at Panarea site ($p<0.01$). Regarding the ascorbate-related enzymes (AR, APX3 and CAPX), AR did not show significant changes, APX3 was significantly down-regulated only at Ischia S2 site ($p<0.001$), while CAPX was down-regulated at Ischia S2 site ($p<0.001$) and up-regulated at Panarea ($p<0.01$). Finally, Prx Q was up-regulated in all the sites ($p<0.05$), while GLP was down-regulated at both Ischia S3 and Panarea sites ($p<0.001$). DSP5 and LPX did not change significantly. For the metal-related genes (Fig. 2b), HMA was down-expressed both at Ischia and Panarea ($p<0.001$), NRAMP1 only at Ischia sites ($p<0.001$ for S2 and $p<0.01$ for S3)while HMATPase5 was down-regulated at S2 and up-regulated at S3 ($p<0.05$ for both). The other genes did not change significantly."

Q4. Figure 1. Please explain the x-axis and why do you choose to use a log scale. A4. The X-axis represents gene expression levels in the respective control sites in Ischia and Panarea sites. Relative expression is computed by REST (Relative expression software tool) tool (Pfaffl et al., 2002) on a log 2 scale. The logarithmic scale is regularly used to visualize gene expression data because of the symmetry of magnitude for up and down regulated genes. Accordingly, the relevance of biological effects is graphically best represented as logarithms.

Q5. Figure 2. Should not be included as a figure but rather summarized in the text. This leaves only a single figure for the paper. Thus the authors are strongly encouraged to expand the figure list to enable the presentation of the results much more clearly. A5.



Figure 2 has been removed as suggested. Figure 1 has been divided in two different figures and each one in two panels, where gene categories have been represented separately (i.e. Heat shock proteins, primary metabolism, antioxidant and heavy metal-related genes). We think that in this way the results are presented more clearly. Figure legends have been modified accordingly.

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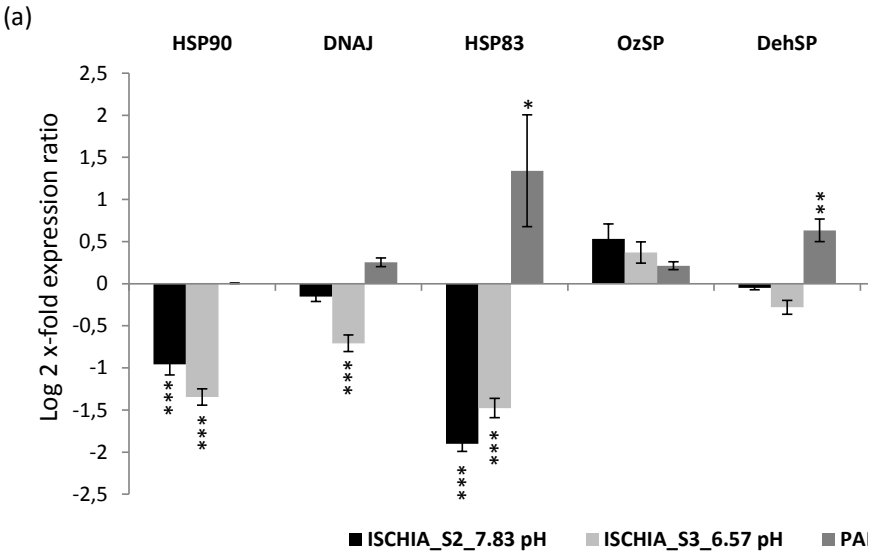
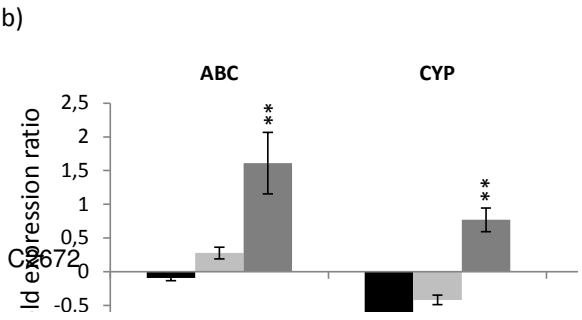
Interactive comment on Biogeosciences Discuss., 12, 4947, 2015.


(a)
HSP90
DNAJ
HSP83
OzSP
DehSP
Log 2 x-fold expression ratio
2,5
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
-2,5
ISCHIA_S2_7.83 pH
ISCHIA_S3_6.57 pH
PA


(b)
ABC
CYP
ld expression ratio
2,5
2
1,5
1
0,5
0
-0.5

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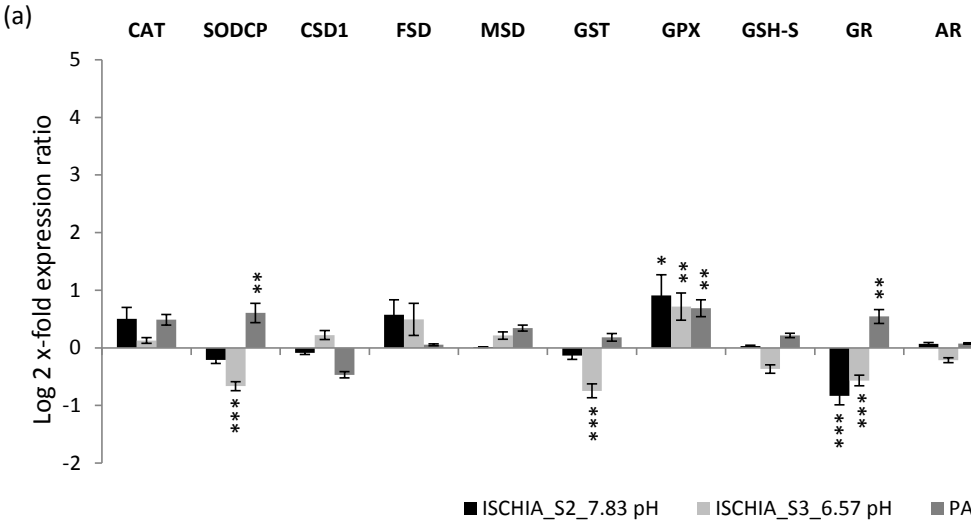
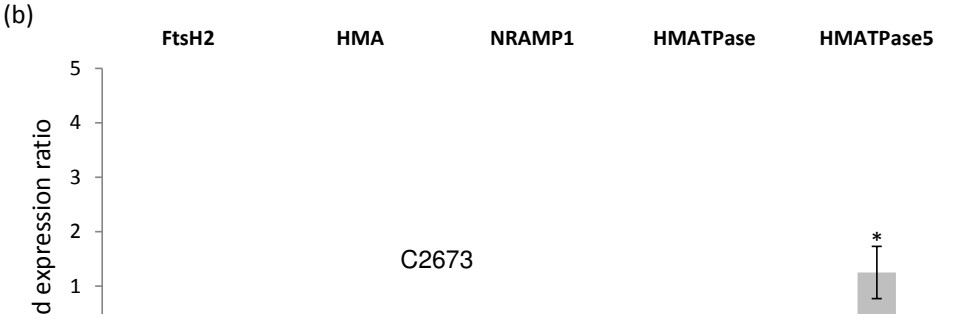

(a)
CAT
SODCP
CSD1
FSD
MSD
GST
GPX
GSH-S
GR
AR
Log 2 x-fold expression ratio
5
4
3
2
1
0
-1
-2
ISCHIA_S2_7.83 pH
ISCHIA_S3_6.57 pH
PA


(b)
FtsH2
HMA
NRAMP1
HMATPase
HMATPase5
d expression ratio
5
4
3
2
1

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