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## ***Interactive comment on “Increases in the abundance of microbial genes encoding halotolerance and photosynthesis along a sediment salinity gradient” by T. C. Jeffries et al.***

### **Anonymous Referee #2**

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Review bg-2011-277

Title: "Increases in the abundance of microbial genes encoding halotolerance and photosynthesis along a sediment salinity gradient"

by T.C. Jeffries et al.

This manuscript submitted by Jeffries et al. presents selected data of microbial metagenomic sequence libraries generated from a natural salinity gradient of Australian Coorong lagoon sediments. Taken that salinity is a major determinant of microbial composition and activity hypothesis of the authors was that shifts in salinity will also alter the bulk biogeochemical potential of microbial assemblages. In order to

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test this hypothesis four sediment samples (one sample per sediment type) were taken throughout the salinity gradient with salinities of 37, 109, 134, and 136. DNA was extracted, pyrosequenced, and gained reads bioinformatically as well as statistically analysed. Moreover, cell numbers and N,P concentrations were determined.

I read the excellent review by A. Oren on this manuscript already and would like to avoid redundancies. Therefore, the focus of my review is a different one, in the hope that we will gain a deeper insight into the quality and scope of this manuscript.

In the case of Coorong lagoon, but also in general, it is interesting to see how the functional potentials of microorganisms coded on the metagenomes may vary throughout a salinity gradient. Taken this, the approach of Jeffries et al. with the aim to identify key functions for distinct salinities or habitats, respectively, is interesting and important. Unfortunately, the approach used is inappropriate because of two fundamental reasons:

1. Based on this dataset it remains unproven if salinity really determines microbial composition and functions. Other physicochemical parameters have to be taken into account, too. The potential sedimentary redoxcline which has very probably been sampled is, for instance, often a much more important determinant than salinity.
2. Statistical analyses were used to determine significant differences between sediments of increasing salinity. In fact, statistics is a powerful tool for the interpretation of environmental datasets; however, especially in ecology independent replicates are essential for significant statistical analyses. If I got it right one single sample, maybe even only a single core was taken for each of the four sediment types. From this, one metagenomic library was generated per sediment. Thus, we have no idea on the mean or variances of gene abundances (how can confidence intervals be presented based on one sampling?). This is simply insufficient for statistical analyses and the whole statistical part has to be removed from the manuscript. It is for sure that metagenomics in general generates large numbers of reads often leading to the assumption that this

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could be of relevance, but statistics is a question of random sampling, not of the amount of reads in one sample.

Taken together, Jeffries et al. should rigorously rewrite this manuscript and present a more descriptive comparison of potential physiological capacities of Coorong lagoon salinity gradients.

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Interactive comment on Biogeosciences Discuss., 8, 7551, 2011.

**BGD**

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