**Biogeosciences
Discussions**

Interactive
Comment

Interactive comment on "High diversity of nitrogen-fixing bacteria in upper reaches of Heihe River, Northwestern China" *by* X. S. Tai et al.

X. S. Tai et al.

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Dear Anonymous Referee #1: Thank you very much for your helpful suggestions. We will deal with the technical comments seriously in revised paper. For the general comments and questions, our replies are as follows: 1. Give some explanation on why an RDA was chosen and not a CCA? Reply: A redundancy analysis (RDA) was performed using CANOCO (version 4.5, Microcomputer Power, Ithaca, NY, USA) to analyze the bacterial taxa combined with the environmental parameters (Braak and Smilauer, 2002). An initial detrended correspondence analysis (DCA) revealed that the data exhibited a linear, rather than a unimodal, response to the environmental parameters. The RDA showed linear models of species response to environmental gradients. How was the RDA plot done? Reply: CanoDraw for Windows can draw ordination di-

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Interactive Discussion

Discussion Paper

agrams based on linear model. The RDA plot was done with biplots method and joint plots between species and environmental variables by CanoDraw. How confident is the analysis -did you do some ANOVA? Reply: Testing significance of the relation with environmental variables can be done during RDA analysis (use Monte Carlo Permutation Tests) with Canoco (version 4.5) software. In addition, we did some ANOVA with SPSS (version 16.0), the data could be supplied in revised paper if needed. Is there a reason that the sampling sites are missing? Reply: We chose biplots and joint plots between species and environmental variables with CanoDraw. Clustering of sites were done with Hierarchical Cluster analysis method by SPSS (version 16.0) software (Fig.3), so that triplots and joint plots among sampling sites, species and environmental variables was missed. 2. Was it to be expected that they would mainly target Proteobacteria? Reply: There have been several researches demonstrated that specificity of nifH-F/nifH-R primers are nitrogen-fixing Bacteria and Archea, targeting a wide range of nitrogen-fixing bacteria and including Proteobacteria, α, β-, γ-subdivision; Firmicutes, Actinomycetales; Firmicutes, Bacillus/Clostridium group and Cyanobacteria. Previous studies have proved that the nifH-F/nifH-R primers would mainly target Proteobacteria (Rösch et al., 2002;Kizilova et al., 2012) , and in this study, the results confirmed it. We will provide the gel picture and melting curve data from the q-PCR analysis in revised paper for the review process. 3. In equation 2 (calculation of H), what does p stand for? Reply: The "pi" in equation 2 means how much the clone numbers of i-th OTU account for in the clone library of a sampling site. The nifH OTU calculation was based on RFLP patterns. Clones with same RFLP pattern were cluster into one OTU. 4. Do you have any data on nitrogen fixation activity supporting the qPCR results? Reply: It is a pity that we have not designed any rate measurements in this study and we will dispose it seriously. 5. Some general statement or concluding remark referring to the objectives of the study mentioned in the Introduction should be added at the end of the abstract. Reply: We will deal with the problem in revised paper. 6. What was the reason for the statistical analysis? Reply: This kind of constrained ordination including RDA (Redundancy analysis) target for finding out species variations response to

environmental gradients, and looking for potential or indirect environmental gradients which can explain changes of species data. 7. Does this mean that there was only a single qPCR run? Reply: The q-PCR run was done in triplicate for both standard and unknown samples, and the standard curve was just an average. Why are there different values for efficiencies? Reply: "R and efficiency were 0.996 and 105.1% respectively." (p:5021, l: 14-15) showed that the PCR efficiency is 105.1% and R^2 of the standard curve is 0.996. 8. The last paragraph of the discussion section (p: 5026, l: 3-13) is redundant to the conclusion section. Also, the conclusion rather reads like a summary, not like conclusions. Either just omit this part or rewrite in a concluding manner. Reply: It's a helpful suggestion and in revised paper, we will make a choice seriously. 9. If possible, include some discussion on what is known about nitrogen fixation in association with Potentilla and Carex as the two main plant taxa. Reply: It is a pity that we have not come across some reports about nitrogen fixation in association with Potentilla and Carex, but we will try our best to found out some reference. If possible, in revised paper, we will add some discussion on what is known about nitrogen fixation in association with Potentilla and Carex. 10. What do the numbers stand for? Reply: In figure 1 and 2, the numbers reflected significance. 11. Is the tree based on nucleotides or amino acids? Reply: The tree is based on nucleotides. What was the amount of sequences used for tree calculation? Reply: A total of 64 nifH clones of the unique RFLP patterns were sequenced and used for tree calculation. How was the alignment done? Reply: The representatives which most closely related to the nifH sequences in GenBank database were obtained by BLAST, coupled with the nucleotides of sequenced clones, the alignment was done with ClustalX (version 1.8). Phylogenetic dendrogram based on a comparison of the nifH sequences of clone libraries from shrub and meadow soils and some of their closest phylogenetic relatives. The tree was created using the neighbor-joining method. The numbers on the tree indicate the percentage of bootstrap sampling derived from 1000 replications. In case any questions arise, please contact me! Best wishes for you! Sincerely yours, X. S. Tai

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