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Interactive comment on "Accelerated microbial-induced $CaCO_3$ precipitation in a defined co-culture of ureolytic and non-ureolytic bacteria" *by* D. Gat et al.

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We thank the anonymous referees for the important and helpful remarks made on this manuscript. The following is our reply to the remarks and suggestions by the referees. Referee #1 "One consideration that should be discussed with regard to 1) OD and 2) biological activity during prolonged experiments with endospore formers such as were used is that depletion of nutrients (nitrogen, carbon, cations) will trigger sporulation. Sporulation will affect both biological activity and absorbance. 80 hour experiments without changing media or removing potentially toxic byproducts of metabolism can affect the experiments". It is very likely that both bacterial species in this experi-

ment sporulated towards the end of incubations. However, when examining changes in ammonium concentration over time as an indication for bacterial activity, both urea-hydrolysis and amino-acids catabolism, up until the 80th hour of the experiment no changes in ammonium production rate were observed, suggesting that any sporulation that might have taken place during this period of time did not affect the activity significantly. With regard to OD measurements, it is likely that OD should decrease as a result of sporulation, whereas plate-counts should remain stable. Based on our data from plate counts and OD measurements we learn that the bacteria remained viable, in accordance with our results of ammonium measurements. "Pg. 17251, Lines 15-16: I agree that the distribution of ureolytic bacteria is probably worldwide but Lloyd & Sheaffe's study only looked at 6 soils, all from their area so the source does not adequately support the statement. I would recommend that in addition to Lloyd & Sheaffe, that you cite a couple more papers that support the broad distribution of these organisms." We agree that the citation of Lloyd & Sheaffe is only partial. The broad distribution of urea hydrolyzing bacteria in soils is also reported in many other studies such as Bremner, J. M., and R. L. Mulvaney. 1978. Urease activity in soils, p. 149-196. In R. G. Burns (ed.), Soil enzymes. Academic Press, Inc., New York, and Mobley, H. L. T. and Hausinger, R. P.: Microbial ureases: significance, regulation, and molecular characterization, Microbiol. Mol. Biol. R., 53, 85–108, 1989.Following your recommendation we will add these citations to the revised manuscript. "You may also want to add some discussion with regard to the actual subset of ureolytic bacteria/organism that can participate in this reaction for MICP. For example, most ureolytic bacteria can only hydrolyze urea when they are starved of nitrogen and the urease enzyme can be repressed by the presence of ammonium/ammonia. Only those organisms whose regulation of urease is constitutive or inducible can fully participate in a meaningful way in MICP." We agree with this important suggestion. In this study we used S. pasteurii because of their constitutive urease production and the widespread use as a model ureolytic bacterial species. The manner of regulation of the production of urease is particularly important when MICP is achieved by bio-stimulation, as it relies on indigenous microbes.

In a natural environment we might see several stages of urea hydrolysis: initially all urease-positive bacteria might take part in urea-hydrolysis, once a threshold amount of ammonium is released the repressible ureases could be inhibited; as urea hydrolysis continues urea concentration might decrease below the threshold required for urease expression in urease-inducible bacteria, finally only constitutive regulated urease could continue hydrolyzing urea regardless of ambient concentrations of ammonium or urea. However, other scenarios could occur, depending on the initial composition of the different urea-hydrolyzing fauna. We intend to address this issue in the revised version of the manuscript, thank you for the constructive remark. "Pg. 17254, Line 23: How much inoculum of each bacterium was used? What was the final bacterial concentration of each bacterium? Were they equal in concentration?" The inoculum size, for both bacteria, was 109 bacteria ml-1, to produce a final concentration of 107 bacteria ml-1 of each species. Therefore, in the mixed treatments NBps and 1/3NBps the concentration of S. pasteurii and B. subtilis were each 107 bacteria ml-1 and in the control treatment the concentration of S. pasteurii was 107 bacteria ml-1. We realize that this important information is unclear in the original text. We will clarify it in the revised version of the manuscript. "Pg. 17255, Line 1: General comment: Typically three replicates should be used." We are aware that batch experiments similar to ours are usually conducted with three replicates; however, the high similarity between our replicates (maximal RMS of residuals of 3.0% and 1.3% for Ca2+ and NH4+ measurements, respectively) attests the reliability of our results. "Pg. 17255, Line 22: S. pasteurii can be grown on media without urea as long as the media has high concentrations of ammonium as noted by Jans in his citations: Bornside, G. H., and R. E. Kallio. 1956. Urea hydrolyzing bacilli. II. Nutritional profiles. J. Bacteriol. 71:655–660.; Gibson, T. 1934. An investigation of the Bacillus pasteurii group. II. Special physiology of the organisms. J. Bacteriol. 28:313–322. Please add “or high concentrations of ammonium salts” to that sentence. This correction will be made. Our intention in the text was to emphasize that the two plate types would show different growth because the NB-Agar plates cannot sustain S. pasteurii, we realize that the phrase was inaccurate. "Pg. 17262, Line 25: The studies

using low concentrations of carbon sources is very relevant to real life bio-stimulation since an over abundance of carbon, particularly of reducing sugars (glucose, fructose for example in molasses) can deplete the soil of oxygen and kill or suppress the activity of the aerobic soil organisms. Typically soils are deficient in nutrients and natural populations of soil bacteria (as opposed to laboratory adapted strains) are not easily enriched or stimulated by relatively nutrient dense solutions. In addition, nutrient broth is expensive for scaling up compared to sodium acetate and molasses." We very much agree with this statement. We believe that up-scaling of MICP cannot take place using rich media such as nutrient broth, and that any carbon source should be added to the treated soil in a low concentration for the reasons stated by referee #1. We therefore addressed this issue in our discussion indicating that the results of treatment 1/3 NBps could be more applicable to in-situ MICP.

Interactive comment on Biogeosciences Discuss., 10, 17249, 2013.