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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.

Anonymous Referee #1

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General Comments:

I think the authors did an excellent job expanding on earlier work by the same group that investigated the effects of mixed populations of ureolytic and non-ureolytic bacteria on biomineralization and growth. I would be interested to see future work from this group using mixed populations of known (wild type) ureolytic bacteria in natural soils to see to what degree there is synergy between the bacterium in a consortium. Overall, the paper represents a very good contribution to understanding the effects on MICP by mixed populations of bacteria.

Specific Comments:

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

Pg. 17251, Lines 15-16: I agree that the distribution of ureolytic bacteria is probably world wide 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. 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.

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?

Pg. 17255, Line 1: General comment: Typically three replicates should be used.

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.

Pg. 17262, Line 25: The studies using low concentrations of carbon sources is very relevant to real life biostimulation 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.

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