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SOIL Discussions

Interactive comment on "Low molecular weight organic anions (carboxylates) increase microbial activity and alter microbial community composition in uncontaminated and diesel contaminated soil" *by* B. C. Martin et al.

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Response to referee #1

We thank the reviewers for their useful and positive comments. We have made several changes to improve the manuscript based on their suggestions. A response to each comment is provided below.

1. "The authors appear to be treating this as a model system for rhizosphere activity and phytoremediation rather than a test of a simple process for remediation by direct addition of carboxylates to contaminated soil. While the authors do discuss the

limitations of the study on page 10, the experiment is so simplistic as to be of very limited value. At the very least, we would have liked to have seen, as one treatment, a more complex mixture of carboxylic acids, amino acids, and carbohydrates, all of which are substrates for microbial growth, and which would be more representative of a real rhizosphere exudate. If the authors were determined to only study carboxylates, which we see no justification for, then a wider range of carboxylates including a complex mixture of many carboxylates found in rhizosphere exudates would have been an improvement."

Author response: We do not disagree with the referee's comments about the limitations of using a model system. However, we believe that our approach is valid for providing indications into drivers of microbial function and communities that can occur in rhizospheres. We do not attempt to test all possible experimental options, nor would this be logistically possible, rather we are taking the first steps in unraveling the mechanism involved in rhizoremediation with well-considered experimental factors. Previous work using model systems has used a combination of rhizosphere components (e.g. carboxylates in combination with carbohydrates and amino acids) (Joner and Leyval, 2003; Miya and Firestone, 2001; Xie et al., 2012). By using this approach, it is difficult to separate which component is causing the greatest shifts in microbial community structure and function. Our study is unique in that it specifically attempts to isolate the effects of two carboxylates that are commonly exuded by plant roots (Jones, 1998; Ryan et al., 2001). This is important because, whilst many studies have investigated the role of carboxylates in nutrient acquisition and aluminium detoxification by plants (Pang et al., 2010; Pearse et al., 2006; Shane and Lambers, 2005), their impact on the soil microbial community has received considerably less attention. Those studies that have examined microbial biodegradation of carboxylates have done so from a context of contrasting soil types (Fujii et al., 2010, 2012; Hashimoto, 2007; Jones and Darrah, 1994), soil horizons (Evans, 1998; van Hees et al., 2002), or in variations in the initial pH of the organic acid applied (Ström et al., 2005). As far as we are aware, there are no studies examining in detail the biodegradation of carboxylates in the context of

polluted soils, and we therefore believe that this paper makes a valuable contribution to the literature. It was beyond the capacity of this study to include every carboxylate that is potentially exuded by plant roots. Instead, we chose to focus on two of the most commonly exuded carboxylates; citrate and malonate (Jones, 1998). We also selected these carboxylates as one is a tri-carboxylate, the other a di-carboxylate and therefore expected they may provide different results due to their differing chelating abilities. It should also be noted that while a large number of carboxylates can be exuded by plant roots, typically there are only five or six present in the rhizosphere in detectable concentrations and, often, the rhizosphere carboxylate profile is dominated by only one to three carboxylates. For instance, in a study of 11 perennial legumes, native and agricultural, Pang et al. (2010) found that >95% of rhizosphere carboxylates for each species consisted of the sum of malate, malonate and citrate. Similarly, Kidd et al.(2016) found that citrate and malonate together contributed from 80% to close to 100% of the rhizosphere carboxylates in 19 out of 25 pasture legumes and grasses.

2. "The title of the paper “Low molecular weight organic anions. . ..” suggests that the results of this paper are general enough to apply to a wide range of carboxylates. But the authors only tested two carboxylates, hardly enough to generalize from. The authors should consider rewriting the title to make it more specific."

Author response: We agree with the referee’s comments in that the title could be misleading. We have changed the title to read “Citrate and malonate increase microbial activity and alter microbial community composition in uncontaminated and diesel contaminated soil”

3. "Only one soil was studied. Therefore, the results of this study can only be applied to that one soil. The study would be vastly improved by conducting it across a range of soil types and edaphic soil properties."

Author response: The soil selected for use in this study is representative of the majority of soil types that are present across the coastal plains of Western Australia

(Schoknecht and Pathan 2013). As this state has significant onshore oil fields, this study was aimed to be applicable to real world applications where contamination from oil pipelines is a potential issue.

4. "page 3 – soil type must be specified."

Author response: The soil type was specified in the paper as a loamy sand with 5% clay collected from the top mineral horizon. Additionally, nutrient concentrations were provided, as well as EC, pH and water retention. The pedological classification of grey chromosol (Australian soil classification) has now also been included in the paper to further define the soil.

5. "The concentration of diesel and of organic acids used in the experiment should be justified. Why were these concentrations chosen, and are they in any way realistic?"

Author response: The concentration of diesel (10,000 mg kg-1) was selected to make results comparable to previous studies on diesel bioremediation and rhizoremediation (Bento et al., 2005; Boopathy, 2004; Seklemova et al., 2001; Tesar et al., 2002). The concentrations of organic acids were selected based on concentrations that are typically found in the rhizosphere (0.1 – 50 μM), although the rhizosphere around cluster roots can contain concentrations in excess of 600 μM (Dinkelaker et al., 1989; Grierson, 1992; Jones, 1998; Strobel, 2001). It must be noted that obtaining reliable estimates of rhizosphere carboxylate concentrations is extremely difficult. Root concentrations are typically in the range of 10 – 20 mM (Jones, 1998; Neumann and Römheld, 1999; Shane and Lambers, 2005). The manuscript has been updated to include the justification for using these concentrations.

6. "The role of pH in controlling microbial communities cannot be overstated. Two more recent references are: Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Applied and Environmental Microbiology 75, 5111-5120. Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso,

J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4, 1340-1351."

Author response: We agree that pH is an important variable in influencing microbial processes and the references suggested have been included in the manuscript to further exemplify this point.

7. "On page 10 you state that “further investigation is warranted. . .”. While further investigation is certainly warranted into the role of rhizosphere exudates on microbial community structure and function, including but not limited to carboxylates, it is not at all clear from the results of this paper that there is any need to continue looking at the effects of carboxylates on PHC degradation."

Author response: Although, the adding carboxylates had little direct effect on biodegradation of saturated hydrocarbons present in diesel, the microbial community did shift. As root exudates are often cited as being a major contributor to rhizoremediation and microbial functioning in the rhizosphere in general, further investigation into how these compounds may affect microbial communities in contaminated soils is warranted. Also, as the reviewer states, there is much more to learn about what might occur under different edaphic environments, hydrocarbon types and exudates.

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