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Interactive comment on “Soil microbial communities following bush removal in a Namibian savanna” by J. S. Buyer et al.

J. S. Buyer et al.

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We thank Referee #1 for reviewing the manuscript and for the kind comments regarding the paper.

Referee: Specific comments: Page 1398, line 6-10. Provide more details on how you are going to analyse the soil properties. The reader will be interested to know what volume/mass of soil you used and how you did it.

Authors: We propose an amended paragraph as follows: Soil pH was measured with a combination electrode after shaking 1 g soil in 10 ml 0.01 M CaCl₂ for 1 hour and letting solids settle for 15 min. Total C and N were measured on an Elementar VarioMax CNS analyzer (Elementar Americas, Mt. Laurel, NJ, USA), using duplicate 0.5 g samples.

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No inorganic C was detected by reaction with acid, so the soil was noncalcareous, and total C equaled organic C (Nelson and Sommers, 1996). Soil texture was analyzed on 50 g samples taken from a single sampling date using the hydrometer method (Gee and Bauder, 1986).

Referee: Page 1398, line 24-25. On DNA extraction, how was it done? Please give details or refer the reader to a previous work where exactly the same method has been used. Page 1398, line 27. Again give the name and sequences of the primers used. State exactly what part of the ribosomal DNA you amplified. Did you use the same primer pairs for all three taxa, Bacteria, Achaea and Fungi. Please clarify. What was the composition of the PCR mix, PCR conditions used. Technical comments: Page 1399, line 2. Life technologies is now part of ThermoFisher Scientific.

Authors: We propose the following amended paragraph to answer all of these points: Soil DNA was extracted and purified using 0.25 g samples and the PowerSoil-htp 96 Well Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). Multiplex terminal restriction fragment length polymorphism analysis was performed for Bacteria, Archaea, and fungi as previously described (Singh et al., 2006). Briefly, bacterial, archaeal, and fungal ribosomal DNA sequences were amplified by polymerase chain reaction using fluorescent dye-labelled primers. Each PCR reaction contained: 1X Amplitaq Gold[®] 360 master mix (ThermoFisher Scientific, Waltham, MA), 0.2 μ M eubacterial forward (63fVIC) and reverse (1087r) primers, 0.4 μ M archaeal forward (Ar3f) and reverse (Ar927rNED) primers, 0.4 μ M fungal forward (ITS1fFAM) and reverse (ITS4) primers, 10 ng of template DNA, and nuclease-free water to adjust to a total reaction volume of 50 μ L. Primer sequences are given in Singh et al. (2006). Amplification was accomplished on a Techne thermal cycler (Bibby Scientific US, Burlington, NJ, USA) according to the following protocol: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 45 s, annealing for 45 s at 55°C, and extension at 72°C for 1 min; and final extension at 72°C for 10 min. The amplicons were restricted with the enzymes MspI and HhaI and purified using a Performa[®] DTR Edge Plate (Edge

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BioSystems, Gaithersberg, MD). The dye-labelled restriction fragments were analyzed on an ABI 3730 Prism Genetic Analyzer (ThermoFisher Scientific).

Interactive comment on SOIL Discuss., 2, 1393, 2015.

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2, C699–C701, 2016

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