Response to referee #2 (D. S. Macholdt et al., Artifacts from manganese reduction in rock samples prepared by focused ion beam (FIB) slicing for X-ray microspectroscopic analysis)

We appreciate the very thorough and helpful comments by referee #2, which have been considered carefully and helped to improve the quality of our manuscript. The referees' comments and our responses are outlined in detail below:

[1.1] Referee comment: Page 8 line 32: "To verify whether a layer of modified material is actually distributed homogeneously on the surface of the sample" Why would you assume an even distribution? Please justify.

Author Response: In the course of our extensive rework of the manuscript, the above-mentioned statement was omitted. We must admit that we are not capable of proving how deep the damaged layer reaches or if the damaged layer thickness is similar across the sample. However, the spatial distribution is obviously even enough to show clearly the mentioned thickness dependency (refer to Fig. 2. and 3). We cannot exclude that areas with different chemical composition inside the varnish coating behave differently on ion beam exposure. Furthermore, the resolution of the here shown STXM stacks is not high enough to clearly resolve the submicron to nanometer-sized layering with good statistics, so any differences in beam damage sensitivity because of compositional fluctuations in the varnish got averaged and therefore remain invisible in our measurements.

[1.2] Referee comment: Page 11 line 10: "As there is at this time no alternative to FIB as sample preparation technique to produce intact ultra-thin slices of rock samples," The reader might wonder which are the benefits provided by FIB compared to, e.g., Argon ion slicing that has been also used in production of thin foils especially for TEM. According to this statement, you don't consider Argon ion slicing as an alternative to FIB. However, if this is the case especially for the samples used in this study, the reader would appreciate some reasoning.

Author Response: We appreciate this helpful comment by the referee. Indeed, Argon ion slicing could be a suitable alternative to prepare ultra-thin varnish slices. Accordingly, the section#

> "As there is at this time no alternative to FIB as sample preparation technique to produce intact ultra-thin slices of rock samples, one needs to be aware of these problems and choose preparation parameters that help to keep damage to a minimum. To reduce or minimize the damaged area, the preparation procedure could be conducted using lower voltages during preparation with the FIB and SEM or, if available, a cryo-FIB (Bassim et al. 2012). However, it is left to further studies to investigate whether oxidation states can indeed be kept unchanged using more gentle preparation approaches."

has been changed to

> "As FIB is a widely used technique to produce ultra-thin slices of rock samples, one needs to be aware of these problems and choose preparation parameters that help to keep damage to a minimum. To reduce or minimize the damaged volume, the preparation procedure could be conducted using not only low currents, but lower voltages during FIB preparation. In contrast, lowering the accelerating voltage in SEM might have an opposing, more damaging effect (Joy and Joy, 1996). If available, a cryo-FIB approach (Bassim et al. 2012) could be applied. Sezen et al. (2011) showed, however, that cryogenic conditions could not prevent or even slow down the degradation of conjugated polymers during FIB milling. Alternatively, Argon ion slicing (Stojic and Brenker, 2010) may be a more gentle and, therefore, suitable approach to reduce beam damage (e.g., Mn reduction) in the

preparation of ultrathin varnish slices. Even less damaging might be iodine ion milling as mentioned in Barber (1993). Fischione et al. (2017) established a method in which the damaged surface layers can be removed after FIB milling by a small spot Argon ion milling process. However, it is left to further studies to investigate whether oxidation states can indeed be kept unchanged using such more gentle preparation approaches."

[1.3] Referee comment: Page 3 line 33: "Here we report about our findings observed during the investigation of the Mn oxidation states in 14 rock varnish samples, collected in different environments and countries." and Page 4 line 2: "For the sake of brevity, and since all samples showed the same phenomena, these findings will be exemplified using measurements on one of the samples." It is interesting that no differences between the varnishes were found especially as you have previously reported (Macholdt et al. 2017a) that layers of Mn-rich material and structures like cavities vary significantly between coatings of rock samples collected from different environments and regions. Perhaps you could refer to your earlier study to emphasize the importance of the finding of this manuscript - that the sample preparation of this sort produces similar kind of artifacts no matter what the structure of the varnish is.

Author Response: Thanks for this thought. As a response to comment [1.1] by referee 1, we included further plots from other varnish types into the manuscript text and discuss to what extent similar beam damage patterns have been observed for most samples, with few exceptions. Please refer to response to [1.1] for details.

[1.4] Referee comment: Page 10 line 8: "we found that artifacts are produced during the preparation of the samples by FIB and monitoring by SEM, which create a high degree of uncertainty for oxidation state analyses." The reader would appreciate a quantitative estimate. Would it be possible to give a rough estimate on how much sample preparation of this kind adds to the total uncertainty – on the basis of the case presented in the manuscript?

Author Response: Unfortunately, our attempts to directly compare microtomes vs. FIB slides failed to provide a direct measure of beam damage (from FIB and/or SEM) relative to the native oxidation state. In lack of suitable reference substances and without a detailed understanding of the damaging mechanisms and their proportionate amounts of contribution, any quantitative statement would be highly speculative. Typical for ambient samples is their heterogeneity and diverse composition, which adds another dimension of uncertainty we do not oversee.

References:

Barber, D. J.: Radiation damage in ion-milled specimens: characteristics, effects and methods of damage limitation, Ultramicroscopy, 52(1), 101–125, doi:10.1016/0304-3991(93)90025-S, 1993.

Bassim, N. D., De Gregorio, B. T., Kilcoyne, A. L. D., Scott, K., Chou, T., Wirick, S., Cody, G. and Stroud, R. M.: Minimizing damage during FIB sample preparation of soft materials, J. Microsc., 245(3), 288–301, doi:10.1111/j.1365-2818.2011.03570.x, 2012.

Fischione, P. E., Williams, R. E. A., Genç, A., Fraser, H. L., Dunin-Borkowski, R. E., Luysberg, M., Bonifacio, C. S. and Kovács, A.: A Small Spot, Inert Gas, Ion Milling Process as a Complementary Technique to Focused Ion Beam Specimen Preparation, Microsc. Microanal., 23(4), 782–793, doi:10.1017/S1431927617000514, 2017.

Joy, D. C. and Joy, C. S.: Low voltage scanning electron microscopy, Micron, 27(3–4), 247–263, doi:10.1016/0968-4328(96)00023-6, 1996.

Macholdt, D. S., Jochum, K. P., Pöhlker, C., Arangio, A., Förster, J.-D., Stoll, B., Weis, U., Weber, B., Müller, M., Kappl, M., Shiraiwa, M., Kilcoyne, A. L. D., Weigand, M., Scholz, D., Haug, G. H., Al-Amri, A. and Andreae, M. O.: Characterization and differentiation of rock varnish types from different environments by microanalytical techniques, Chem. Geol., 459, 91–118, doi:10.1016/j.chemgeo.2017.04.009, 2017a.

Sezen, M., Plank, H., Fisslthaler, E., Chernev, B., Zankel, A., Tchernychova, E., Blümel, A., List, E. J. W., Groggera, W. and Pölta, P.: An investigation on focused electron/ion beam induced degradation mechanisms of conjugated polymers, Phys. Chem. Chem. Phys., (13), 20235–20240, doi:10.1039/C1CP22406A, 2011.

Stojic, A. N. and Brenker, F. E.: Argon ion slicing (ArIS): a new tool to prepare super large TEM thin films from Earth and planetary materials, Eur. J. Mineral., 22(1), 17–21, doi:10.1127/0935-1221/2009/0022-2004, 2010.