Reply to Referee Comment

Referee:

The sc metric relies on taking square root of integrated SFG area) as proportional to interfacial oscillator number while treating orientational factors as constant across natural samples. Could the authors provide polarization-resolved SFG checks (e.g., ssp vs ppp) or an external standard spike to demonstrate that orientation changes with composition/wind history do not bias sc, especially at low coverage ($< 20\,\%$) where baseline subtraction issues are acknowledged?

Response:

The referee raises an important and interesting point. In fact, it is remarkable that simply integrating the (square-rooted) C-H intensity in the VSFG spectrum truly succeeds in yielding a practically useful measure for the surface coverage of natural films. In particular for readers with a strong background in VSFG spectroscopy, our assumption that structural effects or orientation effects of the detected molecules can be neglected for the sc metric, can be surprising. It is well known from the literature (and it is a particular strength of VSFG) that VSFG can be inherently sensitive to such effects when investigating organic monolayers of pure surfactants. By building on previous findings, it is in fact an important side aspect of this paper to reaffirm that structural/orientational effects play a minor role for natural SML samples and that our sc metric is indeed a robust measure for surfactant surface coverage.

In the following, by looking again to the presented findings from a different (spectroscopic, physico-chemical) perspective, we briefly address several aspects related to the referee's question and refer to the corresponding sections in the submitted paper. Stimulated by the referee's comment, we have now also recorded additional ppp-spectra for selected Helgoland samples. They were still available as frozen samples in our laboratory. Unfortunately, this was not the case for the SURF mesocosm samples, as these were used for further investigations and were completely consumed in the process.

Selection of ssp polarization combination: VSFG measurements in ssp polarizarion are most common because they are known to yield high signal intensities and relatively rich spectral information for surfactants with alkyl chains. When we began measurements on natural seawater samples [1], we initially had carried out exploratory, yet unpublished measurements in ssp, ppp, and sps polarization combination. Since the ssp spectra provided the best signal-to-noise ratio (as expected) and also seemed the most promising in terms of the contained spectral and orientational information, we decided to focus further studies on this polarization combination. Surprisingly, however, measurements on natural SML samples showed that the spectral signatures hardly depend on the measured total intensity, as reported in our previous paper [2]. That's what originally gave us the idea to derive an operational measure for the surface coverage based on integrated C-H VSFG intensity alone. We have reported such an initial attempt in a paper from 2018 [3] and also used integrated VSFG intensities in a time-series study of SML abundance in 2013 [4]. Building on our previous work, we have now further substantiated the sc metric by means of systematic measurements and by comparison with simple laboratory standards (DPPC and Triton-X).

VSFG data in ppp polarization combination: Motivated by the referee's comment, we have re-measured selected samples for high, medium, and low surfactant surface coverage, both in ssp and ppp polarization combination. We plan to include these data in the Supplementary Information in the revised version of the paper. A direct comparison of ssp and ppp data is presented in Fig. RC1). Similar to our early explorative measurements noted above, the polarization-resolved measurements show that the ssp and ppp spectra exhibit the spectral signatures expected for alkyl-bearing surfactants. In particular, the ppp spectrum mainly consists of one strong peak, which can be assigned to the antisymmetric CH_3 stretching mode at 2960 cm^{-1}. Note that the intensity trend of the ppp spectra follows the

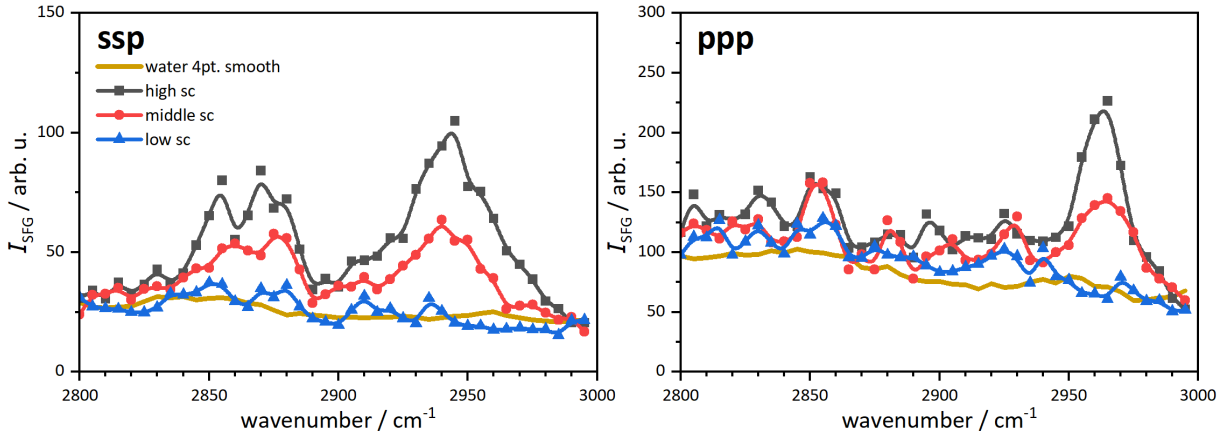

Figure RC1: Comparison of VSFG measurements in ssp and ppp polarization combination for selected Helgoland SML samples exhibiting high, medium, and low surfactant surface coverage. Each SML spectrum corresponds to a single measurement and the solid lines represent B-spline fits to the data to guide the eye.

same trend as observed for the ssp polarization combination for all investigated SML states (low, medium, and high surface coverage). The absence of additional modes and the consistent intensity trend suggest that the ppp-VSFG spectra do not help to identify potential compositional changes or – to answer the referee's question – provide additional information on a distinct structural/orientational change of the detected alkyl groups in our natural SML samples.

VSFG response to changes in SML composition: Similar to literature investigations on natural variability of SML composition (e.g., [5–8]), clear trends related to carbohydrate and amino acid abundances have been reported for the SURF mesocosm study as well [9, 10]. However, as demonstrated by the data presented in this work, the corresponding VSFG spectral shape remained largely invariant despite this substantial compositional change and the increase in surface coverage. This can be best seen in Fig. 3 and in Fig. S1 in the Supplementary Information. The latter clearly demonstrates a linear increase of the individual vibrational intensities with increasing total spectral area for the SML samples. This linearity suggests that the dominant effect on signal intensity is simply an increase in interfacial molecular density. Note that this conclusion is in contrast to the behavior of the monolayers of pure DPPC and Triton-X (as discussed in section A of the Supplementary Information)

A comparison with the yet rather limited set of VSFG spectra of natural SML samples in the literature draws a similar picture [2, 3, 11, 12]. Both for SML and and SML derived aerosol samples, neither substantial shifts in the observed vibrational frequencies nor the appearance of additional modes is observed. However, the VSFG spectra from previous work exhibit some variability in terms of the relative intensities of the different observed alkyl bands, which may explain part of the scatter in Fig. 8.

Low and high surfactant surface coverages: The linearity of the VSFG response is maintained even at low surface coverage values (Fig. S1). However, as outlined in the main paper, surface coverage values below $< 20\,\%$ are comparable to the uncertainty limits (due to low signal-to-noise and necessary water-background correction) such that potential orientational effects are difficult to detect. Note that such low surface coverages go along with a very limit impact of the SML on air-sea gas exchange. As shown in a wind-wave tank study [12], sc values $< 50\,\%$ do not alter the mean-square slope of the wave field such that the SML impact on the gas transfer coefficient diminishes. In this sense, the uncertainties at low surface coverage are less critical for the application of the sc metric in terms of air-sea gas exchange.

Our choice of the $100\,\%$ surface coverage reference point was operationally defined using a DPPC monolayer at the *onset* of its structural transition to a well-ordered lipid monolayer. This transition is going along with a strong

increase in SFG intensity and molecular order. It is therefore important to realize that the observed VSFG intensities of natural organic layers are in a range in which orientational/structural effects are less pronounced, even for simple surfactant layers. In natural systems, we have observed sc values of over 100 % only in a very few cases. Obviously, highly ordered layers, such as those easily achieved by compressing simple dry surfactant monolayers, do not present a suitable reference system for natural films.

Summary: Taken together, while minor compositional differences between sites are reflected in small variations of relative CH_2/CH_3 intensities, the polarization-resolved measurements and the observed linear scaling behavior (despite compositional and surface coverage changes in the SML during the SURF experiment) suggest that both orientation and compositional changes do not dominate the sc metric within the investigated and environmentally relevant coverage regime, which includes low surface coverages. Our pragmatic choice of the reference point ensures that for measurements of environmental SML samples, an sc value of about 100 % can be understood as a fully established organic nanolayer at the air-water interface. If this approach can be generalized must be demonstrated by further studies. Here, it would be particularly interesting to examine SML samples from diverse biogeochemical provinces – a line of research that we plan to continue in the future.

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