Response to Referee #1

The authors again thank the referee for submitting helpful and constructive comments, which lead to further improvements and clarifications within the manuscript. Below, we provide our point-by-point responses. For clarity and easy visualization, the Referee's comments (***RC***) are shown from here on in black. The authors' responses (***AR***) are in blue color below each of the referee's statement. In addition to the responses to referees' comments, we further modified the manuscript to increase its clarity and readability. The summary of major and minor changes is included at the end of this document. We introduce the revised materials in green color along/below each one of your response (otherwise directed to the Track Changes version manuscript). All references are available in the end of this AR document.

RC: Concerns I had raised in my earlier review were taken seriously. The manuscript is is better shape now than initially. Still, there are numerous issues left to solve before it might be in a form where it can recommended for publication. I have to say that my earlier review did not address all issues in detail because the manuscript seemed to require more than one iteration anyway, initially a coarse one and then one to solve remaining intermediate and minor issues.

My main concern with the revised manuscript is its still insufficient focus. It should focus on the immediate objective of the study and the progress made towards achieving it. New insights generated by the study is what the reader is interested in. Currently, these insights are diluted with lengthy explanations of general issues that are well known to most of those who chose to read papers on ice-nucleating particles. For readers, who are not yet familiar with INPs, there are numerous excellent review papers available for a general introduction. I see no need for explaining in the Introduction sections of a specific study in detail what INPs are or why immersion freezing is important.

AR: The authors agree with the reviewer that there are many other great review articles regarding atmospheric INPs. Nevertheless, our general introduction of INPs (Sect. 1.1), immersion freezing (Sect. 1.2), and precipitation INPs (Sect. 1.3) contain invaluable information for the reader to follow the story without heavily reading and referring to other papers in parallel. The authors would like to retain these information. In any case, the referee makes a good point, and the authors took the referee's word for it to clarify and extend our study focus in Sect. 1.4.

RC: Also section 2.5 can be shortened substantially. I would not call it "IN Parameterization" because what the section describes is the estimation of INP concentration in cloud volume from INP concentration measured in precipitation samples.

AR: The authors concur. We now rephrased the subsection title to "*2.5 Precipitation $n_{\mathrm{INP}}(T)$ Estimation,*" and shortened the contents accordingly. We keep the most discussion of CWC as this part was extended based on the previous review comments, but decided to move the following content to Sect. 3.5:

"…while assuming a constant CWC may be reasonable to study precipitation INPs (i.e., Sect. 2.5), it is necessary in the future to further investigate in cloud specific CWCs incorporating with loss of water through partial evaporation of raindrops during free fall based on vertical vapor deficit profiles to conclusively assess if this assumption is fair or not. Precipitation evaporation rate might introduce bias in n_{INP} for precipitation systems with high cloud base, and the correction can be applied accordingly (Petters and Wright, 2015). Direct comparison between INP measurements in cloud water samples and those in precipitation samples might also be key to answer this question (e.g., Pereira et al., 2020)."

RC: If I was to revise the manuscript, I would start with Section 1.4, Study Objectives. This section summarises what has been done (not necessary in such a section), but does not convincingly state why it has been done. The only statement somewhat pointing in such a direction needs to be specified ("...help understanding of ambient INPs in the West Texas region..."). Perhaps it means something similar to: "...to understand whether the high density of animals on dusty ground in large feed yards (feedlot), which are typical for Western Texas, has a discernible impact on regional atmospheric INP concentration and composition near the gound and in clouds." If this was indeed the objective, it should be introduced by a short description of feedlot operations, the extent of these operations in terms on number of animals and their spatial extent in Western Texas to give the reader a flavour of these features in the landscape. In most other regions of the world feedlot operations of that kind are unknown. Once the objective is clear, all other changes to the manuscript follow naturally from that point onwards. As I am not sure whether my idea of the specific objective of this study is correct, it makes little sense for me to datail all changes that would follow.

AR: Indeed, cattle feedyards can act as a significant point source of local PMs, and they can represent important perturbations to other agricultural INPs - thereby potentially influencing INPs in precipitation if PM reached out to the cloud height and fell back to the ground as part of precipitation particles. Investigating in the potential contributions of cattle feedyard PMs to INPs was definitely one of major research objectives and motivations of the presented study. To incorporate with the reviewer's comment, the authors extended our motivation by revising Sect. 1.4 as follows:

"It is noteworthy that adjacent cattle feedyards (> 45,000 head capacity) are located within 33 miles of our sampling site, and the role of cattle feedyard dusts in atmospheric INPs is described in more detail in Hiranuma et al. (2020)."

→

"In this study, we characterized properties of INPs in precipitation samples collected in the Texas Panhandle region to understand whether the high density of cattle in large open-lot concentrated feeding operation facilities (cattle feedyards hereafter), where often >45,000 head capacity can be seen in a single facility in this region, has a discernible impact on regional atmospheric INP concentration and composition near the ground and in clouds. This region significantly contributes to U.S. cattle production, and the total cattle population of 11 million head accounts for 42% of cattle in the U.S. (according to cattle feedyard research experts at Texas A&M AgriLife Research). Adjacent cattle feedyards are located within 33 miles of our sampling site, and the impact of cattle feedyard dusts in ambient particulate matter (PM), frequently exceeding 1200 $\mu g\ m^{-3}$ (24-hour averaged-basis), and aerosol particle composition as well as an overall regional air quality is described in Hiranuma et al. (2011) and Von Essen and Auvermann (2005). Moreover, the emission flux of PM smaller than < 10 µm diameter (PM_{10}) is typically high in the range of 4.5 $\mu g\ m^{-2}\ s^{-1}$ up to 23.5 $\mu g\ m^{-2}\ s^{-1}$ depending on stocking density, creating PM-laden ambient conditions in this particular region (Bush et al., 2014)."

The authors also added the following sentence in the second paragraph of Sect. 1.4 to clarify that we compared the metagenomics result of precipitation to that of cattle feedyard PM samples in this study:
"Some of water-suspended cattle feedyard PM samples were also analyzed with metagenomics to find bacterial microbiome that may appear in precipitations."

In Sect. 2.6, the authors clarified the focus of our metagenomics analysis - "The overall goal of our metagenomics analysis was to identify known ice-nucleation-active bacterial species in cattle feedyard dust, collected in commercial cattle feedyards located within 33 miles from the precipitation sampling site and suspended in the high-performance liquid chromatography grade water (Hiranuma et al., 2020), …"

The authors replaced "feedlot" with "cattle feedyard" to specify the type of animal feeding activities discussed in this manuscript in more accurate and descriptive manner (based on advice provided by a local cattle feedyard research expert). Additionally, we also renamed the subsection title to "*1.4. Study Motivation and Objectives*"

RC: Line 17: Please specify that "nINP" stands for INP concentration in air.
AR: Specified and corrected – it now reads INP concentrations per unit volume of air (n_{INP}). The authors also reflected this change to its first appearance in the main text (Sect. 1.3); increased n_{INP} → ambient INP concentration (n_{INP})

RC: Lines 78 to 82: I do not understand the argument. There is a high number concentration of INPs (up to 20'000 per litre) and at the same time there are liquid droplets. Why should this be an argument for immersion freezing to be important? If it had happened in these clouds, there would have been up to 20'000 ice crystals formed per litre and the clouds would quickly have completely glaciated, so liquid droplets would not have been observed.
AR: The discussion on raised topics is removed in the revised manuscript. Instead, we have added a more relevant reference (Westbrook and Illingworth, 2011) in Sect. 1.2.
"Similarly, an importance and predominance of supercooled liquid droplets as for a prerequisite of atmospheric ice formation is reported in Westbrook and Illingworth (2011). The authors verified it based on radar and lidar observations of clouds over the U.K. at temperatures relevant to immersion freezing."

RC: Line 92: What is an "INP episode"?
AR: We have removed the "INP episode" word, and clarify the sentence by extending the sentence as follows:
This latent heat is further influenced by INP episode, thus affecting the dynamics of the precipitation system.
→
When immersion freezing occurs, the latent heat of freezing energy can be released. Thus, INPs themselves can impact the dynamics of the precipitation system.

RC: Lines 98 to 100: Do you mean "...have shown that the addition of INPs at the base of warm clouds would result in stronger updrafts and lead to increased amounts of precipitation..."?
AR: Yes. The referee is right. Thank you. We corrected the sentence as suggested.
"…have shown that the addition of INPs at the base of warm clouds results in stronger updrafts and lead to increased amounts of precipitation…"

RC: Line 121: "studied" or "compared"?
AR: "Compared" would be more appropriate word choice. Thank you.

RC: Line 123: What is "bio-speciation"?
AR: We meant taxonomic identification. We have replaced all other "speciation" words to either analysis or characterization for clarity.
e.g., P1L27: metagenomics analysis of ambient dust samples → metagenomics characterization of the bacterial microbiome in suspended ambient dust samples

RC: Line 263/264: What are the feedlot samples, airborne dust collected next to a feedlot or soil samples taken from the surface of a feedlot?
AR: These are airborne particulate matter collected at the downwind location of typical large commercial cattle feedyards in West Texas (1.5 m above ground level). We have revised the sentence as:
"…we have examined a heterogeneous set of samples including four airborne PM samples locally collected at the downwind location of typical commercial cattle feedyards in West Texas on March 28, 2019 and…"

RC: Line 267: "tornado warning", not "tornado warming"
AR: Corrected. Thank you.

RC: Lines 218 to 220: I do not understand why in the case of overlapping INP spectra those with the "lower nINP values" were taken for merging when they had the "lowest confidence intervals". Did you mean "lower uncertainty"?
AR: Yes. Thanks for catching this. We meant lower uncertainty. Corrected as suggested/stated.

RC: Line 229: Please say what "CINP" stands for.
AR: It is defined in the second sentence in Sect. 2.5 as "the nucleus concentration in precipitation suspension (L^{-1} water) at a given *T*."

RC: Line 239: What is a "factor of few more"?
AR: It means 0.2 to 0.8 g m^{-3}. We rephrased the senescence as:
"…typical values of CWC for different cloud types could narrowly range within a factor of two from 0.4 g m^{-3}…"

RC: Line 346: A standard deviation that is larger than the mean implies that there is a substantial fraction of data with negative values. This is not possible when the unit of the data is mass per volume. Most likely, the data does not have a normal, but a log-normal distribution. If so, it should be treated and reported accordingly (cf. Limpert et al, 2001, https://doi.org/10.1641/0006-3568(2001)051[0341:LNDATS]2.0.CO;2, or Limpert et al., 2008, DOI 10.1007/s10453-008-9092-4)
AR: Those negative powers of hundredth and tenth are only effective for the standard error values. So all means are larger than error values. We apologize for causing this confusion. We have corrected our text and numerical expressions as:
$3.9 \pm 0.0_9$ μg m^{-3} ($PM_{1.0}$), $4.0 \pm 0.0_5$ μg m^{-3} ($PM_{2.5}$), and $10.0 \pm 0.2_2$ μg m^{-3} (PM_{10}).

RC: Lines 350 to 362: A range of potential sources of particulate matter in the sampled air are discussed, but why not soil dust from feedlot? I thought this study intends to understand the possible impact of this source on regional INP concentrations in air and precipitation (or clouds)?
AR: The referee is right about PM from cattle feedyard to be another significant PM source in this region. Admittedly, we overlooked to mention it. We have updated the text as:
"Besides the local PMs originating from cattle feedyards as described in Sect. 1.4, other prominent local sources include harvesting crop fields and agricultural burning In the Great Plains region nearby West Texas..."

RC: Line 367: What do you mean with "surface material rupture"?
AR: We meant "surface material ejected by water impaction of rainfall (e.g., Huffman et al., 2013; Wang et al., 2016)." For clarity, we added another reference (Wang et al., 2016) for supporting this sentence.

RC: Section 3.4: Here, I am missing a discussion of the results of the present study in a wider context. There have been others, who analysed the microbiome of precipitation samples (e.g. Woo and Yamamoto, 2020, https://doi.org/10.1186/s40793-020-00369-4). How do their results compare to your results. Is there much similarity? Are there major differences, perhaps pointing at the influence of dust from feedlot operations? I am not expecting a list of names and percentages of microorganisms found in numberous other places ot the world, but a discussion focused on what I presume is the aim of your study: do feedlot operations affect what is found in terms of microorganisms and INPs in air and precipitation in Western Texas.

AR: In Abstract, we added "...Some key bacterial phyla present in cattle feedyard samples appeared in precipitation samples. However, no known ice nucleation active species were detected in our samples."
IN activity of *Massilia* reported in Woo and Yamamoto is not conclusive yet, but the authors added the following sentence along with another new citation (Jimenez-Sanchez et al., 2018).
In Sect. 3.4, we added "Genera *Massilia* and *Sphingomonas* have been reported as weak IN active species (Jimenez-Sanchez et al., 2018), but these results are inconclusive and the discussion is ongoing at this stage (Woo and Yamamoto, 2020)."

RC: Line 460: I do not understand the statement in brackets: Did the back-trajectories show a possible marine influence or not?
AR: Yes, they did. For our back-trajectory analysis we used the HYSPILT-READY model with Global Data Assimilation System (1 degree) meteorological data as input (Stein et al., 2015; Rolph et al., 2017). The back-trajectory analysis for our precipitation sampling periods (i.e., PCPT 1-4 in Fig. 6) was carried out at different heights over our precipitation sampling location; i.e., 500, 1000, and 3000 m above ground level (assuming these as the typical cloud heights). For example, as represented in the PCPT 4 example shown below, our back-trajectory analysis indicates a possible maritime influence from the Atlantic Ocean through the Caribbean Sea and Gulf of Mexico.

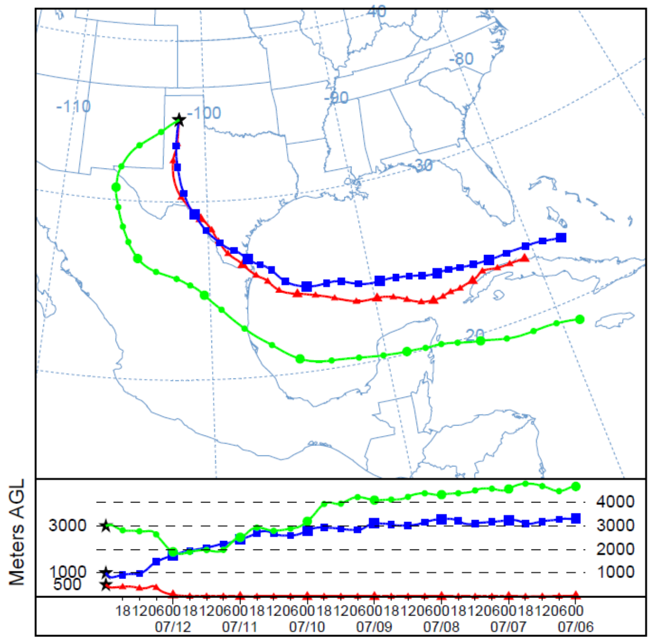

Figure. NOAA HYSPLIT 7 day backward trajectory calculations are given for our precipitation samples of PCPT 4 which corresponds to our Sample# 7 for 500, 1000, and 3000 m above ground level (AGL).

Furthermore, for the cattle feedyard samples 1-4 (Fig. 6), the back-trajectory analysis was carried out at 1.5 m above ground level (i.e., our sampling height at cattle feedyards). The authors note that the back-trajectories for the cattle feedyard samples are intentionally not included anywhere in this response and our manuscript to protect the royalty of our commercial cattle feedyard partners and not to disclose any geographical information of the cattle feedyards. Regardless, the results from all of our back-trajectory analyses indicated that the air masses were originated from the Atlantic Ocean (through Gulf of Mexico and Caribbean Sea) and/or the Pacific Ocean. Overall, these results support a possible marine influence in our cattle feedyard and precipitation samples.

We have extended the back-trajectory discussion in Sect. 3.4 as:
"Additionally, in one hailstorm sample, we also identified *Gilvimarinus*, which is another marine genus of *γ-Proteobacteria* (**Table S9**). These results indicate some connection with air mass originating from ocean. To verify this point, we performed back-trajectory analysis using the HYSPILT-READY model with Global Data Assimilation System (1 degree) meteorological data as input (Stein et al., 2015; Rolph et al., 2017). The analysis for our precipitation sampling periods (i.e., PCPT 1-4 in **Fig. 6**) was carried out at different heights over our precipitation sampling location; i.e., 500, 1000, and 3000 m above ground level (assuming these as the typical cloud heights). Furthermore, for the cattle feedyard samples 1-4 (**Fig. 6**), the back-trajectory analysis was carried out at the sampling height, which is 1. 5m above ground level. Overall, all these back-trajectories indicate a possible maritime influence through the Caribbean Sea, Gulf of Mexico and/or the Pacific Ocean (not shown). Thus, these results support a possible marine influence in our precipitation and cattle feedyard samples."

RC: Line 459: What was the limit of detection again?
AR: Theoretically, there is no detection limit in any metagenomics studies. Even if the DNA from a single cell is extracted and amplified, these species would be detected. In our supplemental table S9, the 0% of several bacteria phyla is exactly this case: a very low percentage of these phyla in the microbiome, which nevertheless were detected. For this study, we only report >0.1% OTU/ASV.

RC: Section 4. Conclusion: The first paragraph (lines 510 to 533) is a summary of results and only the second, much shorter paragraph contains conclusions. I would suggest to shorten the first and to strengthen the second paragraph.
AR: The authors agree. We changed the section tile to Summary and Conclusion, and excluded the following sentences from the first paragraph to shorten texts:
"Our disdrometer measurements showed a clear variation in the precipitation properties among the four different categories of precipitation samples. Severe precipitation, such as hail/thunderstorms, had the highest rainfall intensity (mm hr^{-1}) and the number of precipitation particles were highest in the snow samples. We also found an increased number of large hydrometeors (> 10 mm in diameter) in both the snow and hail/thunderstorm samples. In contrast, there were no precipitation particles > 6.5 mm in diameter observed in the weak rain samples. Our PM concentration measurements implied some possibilities of wet deposition (but neglected). The IN spectra from each precipitation category in this study were compared with the IN spectra from previous precipitation-based INP studies (Petters and Wright, 2015; Vali, 1986)."

Furthermore, as suggested, we extended our second paragraph as follows; "Our metagenomics results suggest the presence of marine genera *Marinoscillum* and *Gilvimarinus* in precipitation and cattle feedyard PM samples. These genera may have derived by an influence of air mass originating from maritime regions. Marine bacteria in inland sampling sites have been identified in previous studies (e.g., Cho and Jang, 2014). We also identified bacterial genera common in our precipitation as well as the local cattle feedyard dust samples, while the microbiome composition in one feedyard sample (Feedyard 3 **in Fig. 6**) was considerably different from the microbiome composition in precipitation samples. The difference of the microbiomes in dry and wet deposition samples, suggesting a non-local origin of bioaerosols in precipitation, has also been observed previously over crops (Constantinidou et al., 1990), as well as in urban precipitation samples (Cho and Jang, 2014; Woo and Yamamoto, 2020). While we cannot conclude if local cattle feedyard dust contributes to precipitation formation, we also found some indications of the inclusion of agricultural dust in our precipitation samples. Regardless, we did not find previously known bacterial INPs, such as *Pseudomonas* and *Xanthomonas* (Morris et al., 2004) in either the precipitation or cattle feedyard samples. To further seek a connection between local dust and precipitation, it is worthwhile to characterize the local cattle feedyard dust in cloud water samples, as it can be the source of INPs and may impact the local hydrological cycle. Collecting long-term pollen and other biogenic aerosol particles samples (i.e., *Fungi* and *Archaea*) and associated observational data for multiple years may add important knowledge regarding the role of local bioaerosols on precipitation INPs. Besides DNA analysis, analysis of RNA by metatranscriptomics will provide insights on the active life of the microbiome in clouds and precipitation. Ultimately, both DNA and RNA analysis of the microbe in ice crystal residuals would offer a direct link between naturally-occurring biological particles and INPs."

RC: Lines 514 to 515: Is it not self-evident that severe precipitation has the highest rainfall intensity? It is measured rainfall intensity that leads to the categorisation of an event as severe rainfall. Or, was there any other criteria for that category?
AR: As summarized in Table S3, the Hail/Thunderstorm precipitation type showed the highest average intensity as well as maximum intensity when compared to other precipitation types beyond standard error.

RC: Table 1: Are the PM really reliable to a precision of 1 ng/m3? If not, reduce the number of digits.
AR: This is a good question and suggestion. A previous publication reported only one decimal digit for PM in µg m^{-3} measured by an identical sensor to correctly represent the detectable PM by this particular sensor (Hegde et al., 2020). The authors decided to follow the same procedure to report only one decimal place and changed all numbers accordingly as shown in the table below:

Table 1. Adjacent hourly averaged PM values (with one decimal point) before and after each precipitation event. We excluded 14 data where PM data were not recorded due to technical issues etc. (ID# of 6-7, 17, 20, 22-24, 26, 28-33).

			PM_1 (µg m^{-3})		**$PM_{2.5}$ (µg m^{-3})**		**PM_{10} (µg m^{-3})**	
ID#	**Sample#**	**Precipitation type**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	PCPT_NSB_1	Hail/Thunderstorm	2	0.1	4.1	1.7	6.2	2
2	PCPT_NSB_2	Hail/Thunderstorm	<0.1	0	1.8	<0.1	2.1	<0.1
3	PCPT_NSB_5	Long-Lasted Rain	4.7	0.7	5.7	1.9	10.8	3.7
4	PCPT_NSB_6	Long-Lasted Rain	3.8	3.8	6	5.7	8.9	8.6
5	PCPT_NSB_7	Hail/Thunderstorm	0	N/A	0.6	N/A	0.7	N/A
8	PCPT_NSB_10	Long-Lasted Rain	7.5	1.5	9.9	3.4	14.8	4.7
9	PCPT_NSB_11	Weak Rain	5.8	3.8	8.2	6.2	12.8	9.4
10	PCPT_NSB_15	Hail/Thunderstorm	14.3	4	16.1	5.1	30.8	9.3
11	PCPT_NSB_16	Hail/Thunderstorm	4.9	N/A	5.4	N/A	10.5	N/A
12	PCPT_NSB_17	Long-Lasted Rain	4.6	N/A	6.4	N/A	10.6	N/A
13	PCPT_NSB_19	Weak Rain	<0.1	N/A	1.3	N/A	6.3	N/A
14	PCPT_NSB_20	Long-Lasted Rain	1.8	N/A	4.3	N/A	5.9	N/A
15	PCPT_NSB_23	Hail/Thunderstorm	3.9	2.2	5.7	5.7	9.6	7.2
16	PCPT_NSB_24	Hail/Thunderstorm	1.6	0	5	<0.1	5.8	<0.1
18	PCPT_NSB_26	Long-Lasted Rain	0.7	0	2.8	0	3.2	0
19	PCPT_NSB_27	Snow Sample	0	N/A	<0.1	N/A	0.1	N/A
21	PCPT_NSB_30	Snow Sample	0.8	0	2.6	0.3	3.2	0.3
25	PCPT_NSB_46	Weak Rain	1.5	0	4.5	1.2	5.4	1.2
27	PCPT_NSB_48	Hail/Thunderstorm	0	0	0.4	<0.1	0.4	<0.1
34	PCPT_NSB_57	Hail/Thunderstorm	29.6	13.5	29.6	13.8	58.9	26.6
35	PCPT_NSB_58	Hail/Thunderstorm	12.5	0.7	13.2	1.4	24.4	2.9
36	PCPT_NSB_59	Long-Lasted Rain	10.5	6.9	11.5	7.9	21.2	12.9
37	PCPT_NSB_60	Hail/Thunderstorm	9.7	3.4	10.7	4.4	18.8	7.3
38	PCPT_NSB_61	Long-Lasted Rain	4.4	0.2	5.9	1.2	10.1	2.1
39	PCPT_NSB_62	Hail/Thunderstorm	<0.1	N/A	1.6	N/A	1.8	N/A
40	PCPT_NSB_63	Hail/Thunderstorm	2.2	1.4	4.3	2.5	6.5	4.8
41	PCPT_NSB_65	Hail/Thunderstorm	1.7	0	4	0.3	5.3	0.3
42	PCPT_NSB_66	Hail/Thunderstorm	1.8	0.1	2.9	1.5	5.8	1.5

NOTE: N/A: either below detection sensor failure return values (i.e., detection limit of our PM sensor).

In addition, the authors made a few technical language/grammar changes without changing context since the referee rated the presentation quality fair but not good yet**.** Our changes include:

- Throughout the manuscript, the use of appropriate articles, plural nouns, and pre-position words has been corrected wherever applicable. An appropriate use of English language was re-checked by native English speakers.
- P1L4: Greg D. Mayer → Gregory D. Mayer
- P1L23: lowest at -25 °C → lowest n_{INP} values at -25 °C
- P1L28: to check… → to ascertain whether local cattle feedyards can act as
- P4L143: examine → determine
- P4L147: → ranging from several hundred to several thousand
- P8L303: → 5µM primer mix
- P27L890: Metagenomics analysis → Bacterial community analysis

The authors will use an external data depository to increase a public awareness of our data (e.g., https://issues.pangaea.de/). Therefore, we rephrased our Data Availability from "Original data created for the study will be available in a persistent repository upon publication within www.wtamu.edu. → Original data created for the study are or will be available in a persistent repository (pangaea.de) upon publication.

Naruki Hiranuma now acts as a single corresponding author for the revised manuscript as he led the revision effort.

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