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Interactive comment on "Soils from cold and snowy temperate deciduous forests release more nitrogen and phosphorus after soil freeze-thaw cycles than soils from warmer, snow-poor conditions" *by* Juergen Kreyling et al.

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Anonymous Referee #1

General comment This is a well written manuscript on a timely topic, which studies the effect of freeze thaw cycles on N and P release between snow-poorer warmer and snow-richer colder forest soils. The manuscript is very well and concisely written, clearly structured, and provides clear aims, hypotheses, and approaches. Especially the statistical analysis of the data is very strong, with a transparent use and analysis of the data. The major shortcoming of the study is lack of crucial soil data without whose, the outcome, discussion and conclusion remain speculative.

Reply: Thank you very much for this positive feedback and the thoughtful and constructive critique! Upon reading your detailed comments, we agree that additional information on soil parameters improves the interpretation of the presented results. Please compare to the replies to your specific points below for details what we have now added to the manuscript.

1. It remains unclear from which soil depth the samples had been taken and what was the criterion of the sampling. Soil temperatures in the mineral soil are known to be well buffered against air temperatures and hence it even remains unclear to which the soils studied have indeed historically experienced FTCs. Although it is described that soil temperatures had been recorded, the data are not shown except minimal winter temperatures.

Reply: To our own surprise, we indeed missed to report the sampling depth and have added this now (line 139: 0-10 cm soil depth) – thanks for spotting this! We have furthermore clarified the soil sampling design there. We further agree that the history of FTC per site is clearly relevant for the interpretation of the site effects. Our statement of recorded soil temperatures was related to the climate chamber trials while all data provided in Table 1 of the initial submission was based on gridded climate data, which generally does not include reliable soil temperature data. However, we have now added on-site soil temperature measurements for four consecutive years across three winters (2016-2019). We acknowledge that this is a short period of observations which does not shed light on historic patterns and changes, but we assume that this recent snapshot has additional value for the interpretation of our results. As already assumed in the first submission, this additional data now suggests that FTC at the studied soil depth are rare at the western end of the gradient with the warmest winter air temperatures. More importantly, FTC are also rare at the coldest sites, very probably due to the high probability of a continuous snow cover insulating the soil against air temperature fluctuations at these sites (compare to column ‘Precipitation as snow’ in Table 1). The relevant on-site FTC records have been added to the reworked Table 1 and are picked up again in the discussion (lines 377ff.).

2. In addition, data about inherent soil properties are lacking. For instance, soil organic matter contents are not given. Soil organic matter is a key soil parameter driving soil microbial communities and the release of nutrients and thus of nutrients released upon lysis of microbial cells. As SOM greatly varies with climate (and which soil depth) it seems likely that it is a key co-variable which could drive the observed responses and as such it should be reported, incorporated into the statistical model and/or discussed.

Reply: SOM, pH, and C/N have been added to Table 1 and are now discussed in light of the results (lines. 380ff): “While the soil C/N-ratio appeared irresponsive to the climatic gradient in our study, soil organic matter content increased towards the coldest sites (Table 1). High organic matter content generally increases the susceptibility of soils for nutrient loss with climate change (Liu et al., 2017). Here, we cannot answer how strongly this pattern in organic matter is driven by historic winter soil temperature and occurrence of FTC, but the expectation of increased mineralization with winter soil warming (Gao et al., 2018) would fit to the observed decrease of soil organic matter content with warmer winter climate (Liu et al., 2017). Moreover, the larger pool of organically bound nutrients at the coldest sites may contribute to their observed responsiveness to FTC warming (Gao et al., 2018).”

3. Moreover, the amount of nutrients released should be normed to the amount of nutrients present in the sample. It should be made clear which drivers are/could be responsible for the observed nutrient concentrations: for example, inherent nutrient content, C/nutrient ratios that drive the net release of nutrients, or historical FTCs. The concepts and data on other drivers should be considered in the data analysis and/or ruled out in the discussion.

Reply: This is an important point and we would agree with norming to the amount of

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nutrients present if we would only address the impact of the experimental soil temperature manipulation on soil nutrient release rates. However, in our study and in our analysis, we aimed at analyzing the impact of experimental soil temperature manipulation scenarios under explicit consideration of the soil origin. Thus, the soil origin is included in our 3-factorial modelling approach by way of including long-term winter climate variables of the soil origin. Consequently, this accounts for the different initial nutrient contents, as they indeed are characteristic for each soil origin. Standardizing to pure nutrient release rates would, therefore, be contra-productive for our analysis. Still, we agree that this aspect should be picked up in the discussion and have added there (lines 380ff, also cited in the reply above).

4. Details from the laboratory experiment are lacking or not clear. For example, was the soil moisture kept constant during the incubation, what was the reasoning for the different incubation times or what were the equidistant temperature changes?

Reply: Soil moisture was measured directly before the experiment and the samples were kept sealed during the experiment. Based on this, we see no reason to expect a change in soil moisture during the experiment. We have added this information at line 146f. See replies to the detailed comments below for all other aspects mentioned here.

Other comments 5. Provide information on effect sizes (increase of nutrient mobilization due to FTC treatments as compared to control). So far, the effect size is only shortly discussed for the most extreme site with the most extreme treatments. And, as far as I understood, for the models the absolute concentrations are used which, however, might be biased by inherent differences. What is the rationale behind the use of absolute concentrations?

Reply: See our reply to point 3 for the rationale of using absolute concentrations. Concerning effect sizes, we indeed report the increase in nutrient concentrations only for the most extreme values covered as the three-factorial interactions displayed in Figures 1-3 imply that between control conditions (no FTC) and the extremes, any effect size is possible depending on the factorial combinations of the environmental parameters. We see no option to quantify this multitude of possibilities in one or a few numbers but certainly would be interested in any idea how this could be done!

6. The reading would be facilitated to have the graphs (Fig. 1-3) at the same spot. This would allow to compare the patterns for the three different nutrients. And in general, use the same perspectives for the graphs and in the same order for all three figures.

Reply: The displayed angles of the four-dimensional models (3d plus color code) have been optimized in order to ideally show the response surfaces and their patterns. Unfortunately, we did not find a single perspective and angle which would allow for optimal visibility of the surfaces across all three response parameters. Still, we agree that the displayed views should at least be ordered as comparable as possible and have re-ordered the views to achieve this. In addition, please refer to the animated gifs in the Appendix for the same views across all parameters. While this comparison between the response parameters is of interest, we deem it more important to show each results graphic close to the text where it is presented and therefore do not combine Figures 1-3 into one Figure 1 A-C.

7. Visualization of measured analytical data is missing. The graphs from the models are great, but showing the measured data would helpful additional information for many scientists working in this field (or at least provide the information in the supplementary material).

Reply: We are happy to share our full dataset as supplementary to this paper (see Appendix B_rawdata.csv). We further tried several different options but did not find a convincing way how to display the raw data of our four-dimensional datasets. Unfortunately, all displays of scatterplots in those four dimensions are hardly digestible. Displaying the data in less dimensions, however, is hardly meaningful because of the complex three-factorial interactions (as identified in the hierarchical regression analysis).

Details/Specific comments: 8. Line 43: “more than”?

Reply: compared to their own past. We have added “with climate change” in order to specify this comparison.

9. Line 45: Nutrient limited: Could you be a bit more precise: Which nutrients, is there co-limitation and is it true for all cold-temperate deciduous forests?

Reply: We specifically refer to these points in subchapter 1.3 but have specified the limitation to N and P now already in this very first paragraph (lines 45ff).

10. Line 60: “colder temperatures” than?

Reply: We have added “than 0°C” for clarity.

11. Line 61: What about cell lysis because of drying-rewetting cycles?

Reply: We acknowledge that drying-rewetting cycles can also cause microbial lysis. In fact, it appears hardly possible to distinguish between drying-rewetting and freezing-thawing as they cause similar effects at the cellular level (with freezing-thawing causing the disappearance of liquid water as stated in the text). Still, we prefer to avoid going into details if the underlying process is driven by ‘true’ temperature effects such as physical expansion of the freezing water or by the absence of liquid water here. We rather would like to focus on the ecological consequences of altered soil temperatures here in the introduction.

12. Line 62: freeze thaw cycle: abbreviation already introduced

Reply: Thanks for spotting – we are now using the abbreviation here.

13. Line 65: physiological re-adaptation to thawing conditions may lead to microbial carbon and nutrient release – not sure what this means

Reply: Reformulated to “and the physiological re-activation of microbes when soils are thawing can lead to carbon and nutrient release”

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14. Line 89: would be interesting to directly give the amount of N deposition in Central Europe as comparison

Reply: added as suggested; 6 to 45 kg N ha−1 year−1 for European beech forests (Rennenberg and Dannenmann, 2015).

15. Line 94: P nutrition is recently decreasing: Recently it was researched, but I think the problem is not recent. It is more likely the recent change in C:N:P stoichiometry that can push P to be a limiting factor

Reply: We agree and have reformulated this statement accordingly: "Linked to the increased growth of forest trees with N deposition, phosphorus (P) nutrition of beech appears to become another limiting factor for beech growth on nutrient poor soils." (L 103ff)

16. There are also attempts to analyses biome patterns of FTC effects (e.g. meta-analysis document higher susceptibility of temperate ecosystem than arctic and high latitude; Gao et al., 2017 Global Change Biology)

Reply: Thanks a lot for pointing out this interesting reference which we now have cited several times in the introduction and discussion.

17. Line 98: FTC has been introduced before

Reply: Thanks for spotting – we are now using the abbreviation here.

18. Provide information on average snow heights or some measure of FTC frequency available. Mean winter temperature are not necessarily a good indicator for FTC.

Reply: We now present soil FTC data measured on site in Table 1. Together with the winter air temperatures and the Precipitation as snow (also Table 1), this data backs up our claim of few FTC at both ends of the climatic gradient due to warm air temperature in the west and due to consistent snow cover in the east.

19. Line 127: soil sub-samples? Could you be more precise? From which horizon(s)

where the soils taken? What was the criterion for soil sampling? This is a very crucial information for nutrient dynamics!

Reply: We agree and are surprised ourselves that this info was not given in the first submission: Per subsample, one soil core from 0–10 cm into the A horizon was taken. The whole soil sampling is now described in more detail in the methods section (lines 132ff).

20. Line 135: Information especially about organic matter content but as well of further soil parameters like microbial biomass would be crucial to draw conclusions about the impact of FTC cycles on nutrient mobilization!

Reply: The requested information has been added to Table 1. In short, soil substrate was silty sand in all cases with pHCaCl2 ranging 3.1–3.5 and organic matter content ranging 3.7–8.5% in the A horizon.

21. Line 140: Table 2: Phosphate-ion is three times negatively charged, Column soil moisture (SM): change to English punctuation

Reply: We are sorry for these inconsistencies and have corrected them here and checked the whole paper for further incidents.

22. Line 146: to clarify: equidistantly between -1.2 and -12_C : deltaT= 10.8_C / 7 = 1.542_C T intervals?

Reply: Indeed, this was the plan. However, we have now added the characterization of the single levels based on the temperatures measured during the experimental treatments in the climate chambers directly at the samples, as they were less equidistant than expected. Please note that all analyses were run with the directly measured values as stated further down in the methods section (Lines 175ff).

23. Line 160: 'Temperature directly at the soil samples'. Please provide details. Same depth, same location? How many FTCs have occurred? Why are the logged data not shown (at least in supplemental information)?

Reply: We believe that this was a misunderstanding: Here we reported the temperature measurements during the treatments in the climate chambers, not measurements at the sites of origin. The climate chamber measurements were used for all analyses. We have now added more details about the temperature measurements in the climate chamber ("Temperature was monitored for each of the 49 frequency x magnitude treatment combinations . . . with 7 sensors per FTC magnitude, directly at the incubated soil samples" Lines 175ff) and furthermore we provided a figure based on this data as Appendix A in the supplementary in order to unequivocally display the FTC treatments. We agree that soil temperature from the sites of origin would also have great value for the interpretation of the data and have now added FTC measurements over four consecutive years at the sites of origin to Table 1 (compare to point 1).

24. Line 163: Just to clarify: samples with 1 FTC were extracted after one day, samples with 5 FTC after 5 days, controls after 7 days? What is the rational behind the immediate sample extraction after the treatment has finished in comparison to the extraction for all samples after 8 days – at the end of all treatments and with the same incubation time?

Reply: Yes. We decided for this standardized sampling in terms of time after the final FTC for each treatment as otherwise the period between the last FTC and the analysis could interfere with the treatment effects due to e.g. recovery or lagged responses in microbial activity and/or community composition after frost exposition.

25. Line 178: molybdenum blue

Reply: Space added, thanks for spotting this!

26. Line 180: Determination limit: Do you mean detection limit?

Reply: We specifically quantify the determination limit here, which is the same as the 'limit of quantification' and provides the value from which the concentration was determined with sufficient precision. The detection limit or 'limit of detection' would describe the value above which the analyte is considered to be detected, i.e. significantly higher than a blank value and is calculated from the standard deviation of blank values with a safety factor (= 3 for 10 blank values). In our case, the detection limit = the determination limit / 3.

27. Line 179-180: The determination limit is 0.05 umol L-1 vs. The determination limit was slightly higher with 0.1 umol L-1? Sentences are unclear

Reply: Indeed, something went wrong here in the initial submission. We have now corrected this, it now reads: “The determination limit was 0.1 μmol l-1. The combined standard uncertainty was 4.2 % for samples and the 5 μM standards.”

28. Line 235: Quality of graphs – resolution, axis labels overlap, axis numbers difficult to read

Reply: We have now increased the resolution and tried to avoid overlaps between labels and axes. However, the latter is not always possible as we rather optimized the figures to display the response surfaces and in some incidents this leads to axes labels hidden partly behind the graphs. Please also refer to the animated graphics in the appendix.

29. Line 235: Graphs: NO3 data: is this the additional release of NO3 compared to control or just the total NO3 release? Please clarify – and I would suggest to use the numbers normed to the control data

Reply: Please compare to point 3 for our rationale to rely on the absolute concentrations measured rather than standardized data.

30. Line 270: Table 4: Would be nice to have the models numbered as done in table 3

Reply: Indeed, that was the plan – added now.

31. Line 286: copy-paste error? Should probably be phosphate instead of ammonium

Reply: True and corrected, thanks a lot for spotting it!

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32. Line 291: coldest site or sites?

Reply: The plural was used intentionally here, but we have now reformulated the sentence in order to improve clarity.

33. Line 316: How do we know its short-term? Like a flush? If there was only one measurement after the treatment?

Reply: True. What was meant was that we measured it directly after the FTC but the formulation was ambiguous and we have now deleted “short-term”.

34. Line 322: activating N and P? wording

Reply: Reformulated to “. . .processes driving the increase in N and P concentrations . . .”

35. Line 322: (1) minimum temperatures of -7 to -11_C were only reached for half of the treatments, but increase of nutrient concentration seems to increase linearly with increase FTC magnitude. . . which is also shown with the models with only magnitude as single factor where the linear model was the best. Would it not be expected to see a stronger increase of nutrient release when reaching the -7_C if this explanation (1) is right?

Reply: This is true, thanks for this helpful thought! We have now added this aspect here (now at lines 343ff).

36. Line 358: ‘Contrary, our coldest sites rarely experienced serious FTC in the past’. This seems likely but are there any data supporting this statement? As soil had been sampled in well-buffered subsoils, the FTC frequency and magnitude is open

Reply: Please refer to point 1 and the added data in Table 1.

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