

***Interactive comment on “ Effects of stoichiometry
and temperature perturbations on beech litter
decomposition, enzyme activities and protein
expression” by K. M. Keiblinger et al.***

Anonymous Referee #1

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The paper represents an attempt to combine enzyme activity measurements, respirometry and metaproteomics in the research on the effects of severe climatic episodes on decomposition. Theoretically, the methods applied here may represent an interesting alternative to other approaches but in my opinion, the paper in its present form has many limitations. Most importantly, the nature, size and setup of microcosms can not be found so one can not see if they were open systems or closed ones with gas exchange only (as I assume). This is of crucial importance since in the latter case, C compounds can exit the system as CO₂ but the N and P can not so there is no reason to speak about stoichiometry: the total N and P in the system remains the same,

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only C content changes along with respiration. Consequently, the C/N and C/P ratios mentioned in the text would behave in exactly the same way and many significant correlations are due to these constraints. I am also not sure about the natural relevance of the treatments used. Although temperature can rise to >30°C temporarily, in forests, it is usual that these periods are not continuous but limited to <2 h in a day with drops to 10–20°C during nights even during hot summers. I wonder whether the total mass loss of the system was also recorded, if so, it should be noted. The authors should clearly identify the use of proteomics instead of DNA/RNA for microbial community analysis. As apparent from the remains of plant protein in the samples, some proteins can sustain the treatments. If the plant proteins are stable, how much of the microbial protein analysed stems from the period before treatment? This should be made clear or at least discussed. I have also reservations about the evaluation of the proteomic data. Since there were no replicates from treatments, the significance of observed changes can not be tested and no conclusions can be made. This might be possibly overcome by combining samples of different litter undergoing the same treatment and recording the direction of their development, e.g. the increase of bacteria, fungi, etc. In Table 3, the authors should note that there are not enough degrees of freedom to test by linear correlation. This is since the litter samples before/after treatment are not independent (the high C/N litter will still have relatively high C/N even if frozen or heated). As minor comments, I recommend to avoid the term "stoichiometry" and use the C and N content instead. In litter, there is usually no limitation of C, so the "stoichiometric" use of C and N or C and P can not be achieved. I also suggest to use "freezing" as a term for one of the treatment, not "freeze".

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