Response to reviewer #2. The reviewer's comments are presented in italics, and our responses are shown as plain text. We have responded to all of the reviewer's comments.

The manuscript describes the results of a small field experiment and a model exercise that aimed at analysing the effect of variations in C:N of root exudates on microbial biomass and respiration rates. The topic itself, i.e. that exudate C:N may be an important driver for rhizosphere processes, is timely and interesting for a wider audience, but the manuscript less so. Here are my reservations and the reasons, why I am largely disappointed about the way the experiments were organised and the manuscript written:

We appreciate that the reviewer is generally interested in the topic of the manuscript. We have addressed all of the reviewer's comments and reservations, which has helped to improve the manuscript.

(1) The two hypotheses that are put forward are not really inspiring. An inspiring hypothesis, to my opinion, should allow (once its supported or rejected) gaining some insight into the mechanisms behind the relationship it describes. The two hypotheses here are both "if: : :then: : :" type of hypothesis, that are rather weak. The first one states:"if exudation alone is sufficient to stimulate microbial activity in rhizosphere soils, then the addition of exudate mimics will lead to higher microbial biomass, increased exoenzyme activities, and higher rates of C-mineralization relative to bulk soils". That's really very basic. It basically describes the well-known rhizosphere effect (that plants exude low-molecular-weight compounds to the rhizosphere supporting a certain microbial biomass and exoenzyme activities). Is there anything new that has not been tested extensively before? Despite the fact that the microbial biomass is a poor predictor of microbial activity and often the microbial biomass is lower (but more active) in times of highest plant C input into the soil. The second hypothesis then says that if microbes are N limited, exuding nitrogen-containing compounds will lead to a "higher rhizosphere response", presumably a higher microbial biomass and more investment in exoenzymes. Again, that's a very basic hypothesis. If an organism is N limited, then the addition of N per definition must lead to a higher biomass.

The original hypotheses focused the manuscript on the direct effect of exudation chemicals on the activity of soil microbes. While we think this is a novel contribution to the literature, neither reviewer found this framework particularly inspiring. We say this is novel because we have isolated the effect of exudate chemicals from other processes that naturally occur in rhizosphere soils, and we have added exudates at realistic rates to intact soils in the field with twice-daily additions. No other study has experimentally simulated root exudation in such a realistic manner. However, as per the reviewer comments, we have revised the manuscript to focus on the stoichiometric constraints of microbial response to root exudates with a single hypothesis which states **"The ability of soil microbes to utilize root exudates for the synthesis of additional biomass and exo-enzymes is constrained by N availability- roots may elicit a larger rhizosphere response by exuding N as well as C"**. This focuses the manuscript on the issue that both reviewers found compelling and novel- that exudate C:N may be an important driver for rhizosphere processes. We briefly mention that this study isolates the direct effects of exudates separate from other potentially

confounding rhizosphere processes, but this is no longer prominently presented as per the reviewers' comments.

(2) The modelling approach used here is rather basic and straightforward and largely based on an older concept, already published in 2003 by Schimel & Weintraub. The framework is so easy to understand and so well explored already, that even without any math it is clear that a pulse of DOM with a C:N of 25 (at a soils C:N of 20) would lead to a greater increase in microbial biomass and a greater exoenzyme production, than one with a C:N of 100 (Fig.2). That more biomass is build when continued pulses of DOM with a C:N of 10 are simulated than with a DOM with C only (Fig.4c) is likewise not very surprising. I did not find any new aspects in the modelling approach that is presented here.

The reviewer's criticism that our model is basic, straightforward, and already thoroughly explored is not consistent with the current state of the literature in this field. The reviewer is correct that our model uses Schimel and Weintraub (2003) as a starting point. Following this publication, other groups have developed models that include aspects of microbial physiology and exo-enzyme activity, including microbial acclimation to elevated temperatures (Allison et al. 2010), incorporating functional groups of organisms specialized on different substrates of litter decomposition (Moorhead and Sinsabaugh 2006), simulating pulsed rewetting events (Lawrence et al. 2009), and incorporating some aspects of inorganic N fertilization on decomposition in an earth system model (Gerber et al. 2010). This literature on mathematical models of microbial physiology is relatively new and currently developing; a recent synthesis paper exhaustively surveyed the literature and found only four models that explicitly couple microbes to the processes that they control and predict changes in C dynamics (Treseder et al. 2012). Thus, while our model is not revolutionary, it is certainly in line with the currently developing literature.

Additionally, the specific examples of modeling results described by the reviewer as "well explored already" and "not very surprising" are not reflective of the overall value and novelty of our manuscript. The most compelling feature of our manuscript is the agreement between a theoretical model and a field experiment on the role of exudate stoichiometry (C:N) in the microbial response to exudates. In addition, the model result that N exudation can achieve a positive return on investment (Fig. 3) is a novel contribution to the literature and supports the main conclusion of the manuscript. We included the model results of Fig. 2 partly to give the reader an understanding of the overall behaviour of the model to exudate pulses so that they can then understand why the model predicts a positive return on investment associated with N exudation (Fig. 3). In other words, we expect that not all readers will understand the Schimel and Weintraub (2003) model as well as this reviewer, and these readers would benefit with a basic demonstration of the model (Fig. 2), even if this has been well explored already.

To address the reviewer's concern regarding the novelty of the modeling, we have highlighted what is new about our work relative to the rest of the literature as follows:

- We have summarized the previous literature regarding this model in the methods section where the model is introduced and described. This will help the reader understand what has already been done.
- We have explained what is new about our modeling work later in the methods section, where we describe the simulations we performed. We highlight the novel aspects of our study, (1) the positive return on investment for N exudation, and (2) the interplay between model predictions and experimental results.

(3) The field experiments are interesting in their idea, but basic in their execution. The idea of pumping solutions through micro-lysimeters into soil to mimic root exudates, although not entirely new, is great. However, why the authors have chosen to do this in the field and then (after 50 days) taking the soil into the laboratory and measure the respiration rate in closed flasks is difficult to understand. If the authors were really setting up the experiment after the model provided support for their hypotheses (as they state on page 6909), why did they not set up the experiment in the laboratory, where they could have measured microbial respiration continuously?

We implemented this study in the field in order to work with undisturbed soils exposed to natural variation in environmental drivers. As such, our study is more closely connected to the reality of what is happening in actual forest soils than a laboratory incubation. We feel that this is a major "plus" for our study and not a limitation. Had we performed this experiment in the lab, we would have taken soil cores from the field, transported them back to the lab, sieved the soil to remove the roots (or used intact cores with the artefact of dead, decomposing roots), and incubated the soil at constant temperature and moisture (or a simulated diurnal temperature regime). We would have had less confidence that our results reflected processes actually occurring in the field. This is not to denigrate the value of laboratory incubations in general- lab work has enormous benefits regarding manipulability and control- but there is a real trade off in the degree of applicability to the real world.

The reviewer is certainly correct that we could have measured microbial respiration with more temporal resolution with a lab study, but this was not the particular focus of our study, and this we did not feel that this advantage outweighed the advantages of a field study.

There are several other aspects of the experiment that I cannot really understand. First, the reason given at page 6908 for using ammonium instead of amino acids (that are usually found in root exudates) as a N source is not comprehensible. Certainly, solutions can be prepared that contain the same amount of C at different amounts of N by using amino acids instead of ammonium.

It is not possible to independently vary the N content of the exudates using amino acids as the N source without affecting either the C content of the exudates or the chemistry of the exudates. For example, if we had used glycine as the N source, the +CN treatment would have more C and chemical energy content relative to the +C treatment, and differences between these treatments could then be attributed to the effect of C or energy and not N content. Alternatively, we could have reduced the amount of carbohydrates and organic acids included in the +CN treatment to compensate for the C added by using glycine, but then we would have changed the chemical composition of the exudate solution relative to the +C treatment. We elected to use an inorganic N source so that we could use exactly the same concentration of carbohydrates and organic acids in the +C and +CN treatments, so that differences between these treatments could be attributed solely to the N content of the solution. We have more thoroughly explained this in the methods section of the revised manuscript, and acknowledge this as a potential limitation of our study. However, there is no perfect solution to this problem.

Second, the microbial carbon use efficiency, i.e. new biomass production over substrate uptake (biomass production plus respiration), is dependent, amongst other factors, on the degree of chemical reduction of the substrates. If the degree of reduction of a certain substrate is less than the mean degree of reduction of the microbial biomass (around 4.1), then this substrate does not contain enough energy to produce a unit of biomass, thereby lowering the carbon use efficiency and the biomass production. By choosing a solution with a very low degree of reduction (dominated by oxalic acid with the lowest possible degree of reduction of 1), the production of new biomass is not favoured. That's maybe the reason even with ammonium additions the increase in biomass production was relatively small and may have been mostly due to internal carbon reserves.

The degree of reduction of a chemical substrate (γ) is defined as the number of equivalents of electrons per gram atom of carbon. In other words, a molecule's γ quantifies the number of electrons transferred to oxygen upon oxidation of the molecule to CO_2 and H_2O (Sandler and Orbey 1991, von Stockar et al. 2006). As γ declines, a larger proportion of the C atoms in the substrate molecule must be mineralized to CO_2 to transfer electrons for mitochondrial ATP production, reducing the fraction of C atoms assimilated into biomass, thus causing carbon use efficiency (CUE) to decline. Thus, γ can be used to make theoretical predictions of microbial biomass growth per unit of substrate added (yield), as the reviewer suggested (von Stockar et al. 2006). The reviewer's statement that microbial biomass growth is only possible when the substrate degree of reduction exceeds the degree of reduction in a microbial cell (4.1) is not entirely accurate; theoretical yield declines but remains positive as γ declines below a value of 4.6 (von Stockar et al. 2006). Above a γ value of 4.6, theoretical yield remains constant at 0.6 (i.e., 60% assimilation of substrate C into biomass; von Stockar et al. 2006).

We quantified γ and theoretical yield for the individual chemicals we included in the exudation experiment as well as a solution-wide weighted average (Table below).

Compound	Degree of reduction (γ)	Biomass yield*
Citric acid	3	0.39
Oxalic acid	1	0.13
Fumaric acid	3	0.39
Malonic acid	2.67	0.35
Glucose	4	0.52
Weighted Average	2.81	0.37
*Calculated from γ as in von Stockar et al. (2006)		

The reviewer is correct that oxalic acid has a very low γ and thus a theoretical biomass yield of only 13%, suggesting that it would be unreasonable to expect an observable microbial biomass growth response to oxalic acid alone. However, oxalic acid constituted only 18.75% of the C in the solution of chemicals we used as exudate mimics, and the other chemicals

were substantially more reduced (higher γ). The solution average γ of 2.81 and theoretical yield of 37% suggest that it is possible for these chemicals to stimulate microbial biomass growth.

Furthermore, we chose these chemicals because they reflect our best estimate of the actual chemical composition of root exudates (Bowen 1969, Rovira 1969, Smith 1976, Bertin et al. 2003, Jones et al. 2009). Thus, this combination of organic acids and carbohydrates is a reasonable approximation of actual root exudates.

Third, two controls are used, a water control and a disturbance control. Why? The water control includes the disturbance already. I have not found any part of the manuscript where these two controls were discussed or needed. In fact, only the water control is relevant to the experiment. I do not, in this respect, understand why the enzyme results in Figure 5 are first normalized to the disturbance and then the water treatment is shown as if it were a treatment, not a control. At least, it must be made clear in the legend, that this was not +C or +(C&N) but +(Water&C) and +(Water&C&N).

Previous work has identified a strong effect of moisture on soil respiration in the particular forest stand studied here (Borken et al. 2003, Borken et al. 2006). Thus we were concerned that delivering the exudate solutions would stimulate microbial activity because of a water effect. Thus, it was necessary to be able to separate a water effect from possible effects of the exudate chemicals dissolved in water. This was the reason for the water control. The disturbance control was necessary to quantify a potential water effect. That is, we could only identify a water effect by comparing the water control to a comparative group lacking water addition. We chose to use a disturbance control for this comparative group so that a water effect could not be confused with disturbance associated with installing the microlysimeters. That is, the water control and disturbance control differ in only one factor (water delivery), while the water control and bulk, intact soil would differ in two factors (water delivery and installation of the microlysimeter). We have more fully explained the need for a disturbance and a water control in the methods section of the revised paper.

We compared the three treatments (water control, +C, +C and N) to the disturbance control in Fig. 5 so that we could summarize all of the measured enzyme activities in a meta-analytic framework. Meta-analysis requires that each observation in an experimental group be paired with a control observation- this is necessary to combine data from different experiments. We adopted a data analysis framework using the response ratio, which is the natural log of the treatment group divided by the control group [response ratio = ln(treatment/control)]; this framework is common in the literature and has previously been applied to soil enzymes (Rustad et al. 2001, Saiya-Cork et al. 2002, Ainsworth and Long 2005). Graphing the data as we have in Fig. 5 also clearly demonstrates that there is no direct effect of the water addition on soil enzyme activities.

Fourth and finally, a proper N control is missing. That would have allowed the authors to distinguish the effect of N alone (maybe N alone was sufficient to increase microbial biomass and exoenzyme production) from the effect of C and N together (maybe there was a co-limitation?) and therefore to allow dissecting the underlying reasons for the observed pattern. It would have allowed to really address what the claimed in the abstract, namely to support a cause-and-effect relationship between root exudations and enhanced microbial biomass. As it stands it could also be that what has been found is solely an effect of fertilization.

We have addressed this limitation in our response to the first reviewer. For clarity, we repeat our response here.

The reviewer is correct- we did not include a +N treatment in our experimental design. We included four treatments: a disturbance control, a +water control, a +C treatment, and a +C and N treatment. We did not include a +N treatment partly in response to monetary constraints on the number of peristaltic pumps we could acquire to deliver the exudate solutions, but the primary reason for our decision was that a +N exudation treatment is not biologically relevant. That is, roots do not exude N in isolation; N is only exuded in combination with C. Most studies of root exudation have only been concerned with C compounds and have not considered N exudation (e.g., Jones 1998, Ryan et al. 2001, Bertin et al. 2003, Farrar et al. 2003, Phillips et al. 2011). Additionally, model simulations showed no response of microbial biomass, respiration, or enzyme activity to additions that contained only N (data not shown), as microbes were always C limited prior to exudate delivery in the model scenarios (positive rates of N mineralization are indicative of C limitation, Fig. 2m-p). Thus, given limited resources, we elected not to include a +N treatment.

The reviewer references literature concerning microbial responses to long-term N addition studies simulating atmospheric N deposition. There is a large body of literature demonstrating that surface additions of inorganic N often reduce microbial biomass, soil respiration, exo-enzyme activities, and decomposition rates in temperate forests, leading to the accumulation of soil organic matter (SOM), particularly in the organic horizon (Saiya-Cork et al. 2002, Wallenstein et al. 2006, Treseder 2008, Janssens et al. 2010, Thomas et al. 2012). A recent review largely attributed these effects to a reduction in belowground C allocation by forest trees, which reduced microbial biomass through a loss of priming (Janssens et al. 2010). The reviewer is correct that our observations of reduced lignolytic exo-enzyme activity in response to +C and N are consistent with this literature, suggesting that our observations may be driven by N alone. However, the balance of our observations are not consistent with this interpretation; we observed increases in many indices of microbial activity in response to the +C and N treatment, including microbial biomass, respiration, and labile exo-enzyme activities, while the literature on surface N additions largely show reductions in these variables (references above; but see Saiya-Cork et al. 2002). Thus, we suggest that our interpretation of the microbial response to +C and N is supported more strongly by the observed data. Additionally, we added very little N relative to this literature on N fertilization. Studies of the microbial response to N fertilization have added an average total load of ~1000 kg N ha^{-1} (Treseder 2008); we estimate that we added < 0.1% of this amount. **We have added a paragraph to the discussion to address these points, as suggested by the reviewer.**

(4) Another main point is that any description of the soils used (e.g., soil type, nutrient status, C and N content, and similar) is missing. That's certainly needed for such a paper.

All of this information has been previously published, which is why we simply cited the literature in the original manuscript. We certainly understand why a reader would want this description in the paper itself, so we have created an additional table with basic soil data for this site (horizon depths, with associated bulk density and C and N contents).

Overall, I do think that the manuscript has its merits and that this is an important topic and an interesting idea. But I also think that the manuscript needs substantial re-writing along the lines shown above.

We appreciate the reviewer's general interest. Addressing the comments and criticisms above has substantially improved the manuscript.

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