Interactive comment on “Root biomass responses to elevated CO₂ limit soil C sequestration in managed grasslands” by W. M. A. Sillen and W. I. J. Dieleman

Anonymous Referee #1
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Report BGD “Root biomass responses to elevated CO2 limit soil C sequestration in managed Grasslands.” The manuscript provides a meta-analise of 69 grassland manipulation experiments (published between 1998 and 2008) on elevated CO2 and N fertilisation. Meta analyses comprised a gradient of fertiliser application (between zero and 560 kg N ha⁻¹ yr⁻¹) and fertiliser types (i.e. urea-N, NPK, KSO₄, MgSO₄, P), management disturbance (i.e. non, cut, burning, grazing) and irrigation. The manuscripts aimed to summarize overall outcome of those 69 grassland experiments in terms of aboveground biomass, root and microbial biomass and soil C content. The effect of CO2 an N by taking also into account the management practice (i.e. irrigation, dose of fertiliser applied, defoliation). The manuscript is interesting, synthetic and summarises nicely main effects (biomass, SoilC) of FACE experiments published in the last 10 yrs.

Some extra experiments were added to the analysis, bringing the total number of experiments to 77 on 31 experimental sites (publications up to 2010).

- At some points (MM, Results), however, the manuscript is a bit too synthetic and I would have preferred to have further details on data acquisition, variables tested (see also comments). Along the same lines, Figs/Table legends would need some clarification to help reader though the significant results (see also comments). The discussion, needs to my opinion, to be synthesised to avoid repeating of results and interpretation. Nevertheless, the manuscript is worth to be published. I recommend (minor) revisions before publication.

General comments
My first comments involve several points which would need more detail or to be changed in the whole manuscript.
- To what is shown here, the manuscript does not deal with C sequestration, as soil C content is not C sequestration. However, as this variable was not explained in the MM section I did not know what authors tested. Most likely, studies (i.e. 69) mentioned changes in soil C during their experiments. In this case I accept that authors used C storage throughout manuscript. If this is not the case, this needs to be changed.!

The manuscript discusses changes in soil C content/concentration (in % change), i.e. compares soil C content in ambient and treatment conditions. For all given response variables (AB, RB, MB and SOC), we discuss the natural logarithm of the ratio treatment/ambient (unlogged to obtain % change). As such, we talk about potential changes in soil C content as a consequence of elevated CO2 and/or N fertilization, which meets the criteria reviewer 1 outlined.

We have also added a limited descriptive paragraph about the response variables tested:

“Figures and tables within articles were used as a source for data. Aboveground and root biomass were expressed on a dry weight per area basis. Microbial biomass was expressed on a dry weight per unit of soil weight basis, and SOC was expressed on a dry weight per area, or dry weight per unit of soil weight basis. For SOC data
expressed on an area basis, we assumed that soil density was not affected by elevated CO2 treatments. This resulted in …"

- Along the same lines, I wonder why soil texture was not tested. I suppose, depending on soil (Cl/ Sa/Si) an experiment was carried out, effects of N and CO2 will be more or less pronounced. May Authors comment on this.

There was not enough soil textural data reported for the studies we used. Quite often, soil type or a description of soil (e.g. sandy) is given, but quantitative data is often lacking. Stratifying in sandy, loamy, silty, and clayey soils might be possible, but would often reduce the sample size for each group to <5. Although the authors agree that soil texture could be important, we argue that including it in the analyses was not possible here.

- The same for rain/irrigation, authors tested irrigation but not annual precipitation neither temperature. May Authors comment on this. Also, biomass (and C storage) depends on water availability and temperature. So, I would have expected to see climatic variables (rain, soil moisture) in the tests.

An important point to consider is that the authors did not design the individual experiments, and are therefore limited by the available data. While we agree that water/climatic variables might play a role here, rainfall is not necessarily the most informative parameter in regard to water availability for plant growth (e.g. soil water potential is more useful, but is rarely reported in CO2 studies). In addition, the frequency and magnitude of rain events will determine whether a site will experience periodic droughts over the year, or experienced drought at all.

However, to meet the note of the reviewer, we have tested the relationship of plant and soil responses to MAP and MAT (see table below). We found that the AB response to CO2 (without N fertilization) became smaller in sites with more rainfall, possibly indicating smaller positive effects of CO2-induced water savings. In sites that also received N fertilization, we found an increased RB response with MAT, possibly as a response to reduced water availability in warmer sites.

Both findings suggest water availability is of major importance in relation to CO2 effects, and we will incorporate this information in further versions of the manuscript.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tested Factor</th>
<th>P</th>
<th>slope</th>
<th>n</th>
<th>Treatment</th>
<th>Factor</th>
<th>P</th>
<th>slope</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>CF MAT</td>
<td>0.846</td>
<td>+</td>
<td>25</td>
<td>MB CF MAP</td>
<td>0.453</td>
<td>+</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.114</td>
<td>-</td>
<td>30</td>
<td></td>
<td>MAP</td>
<td>0.935</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>CO2 Duration</td>
<td>0.328</td>
<td>-</td>
<td>44</td>
<td></td>
<td>CO2 Duration</td>
<td>0.695</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>CO2 Intensity</td>
<td>0.898</td>
<td>+</td>
<td>44</td>
<td></td>
<td>CO2 Intensity</td>
<td>0.403</td>
<td>+</td>
<td>18</td>
</tr>
<tr>
<td>C</td>
<td>MAT</td>
<td>0.193</td>
<td>-</td>
<td>20</td>
<td>C</td>
<td>MAT</td>
<td>0.721</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.027</td>
<td>-</td>
<td>16</td>
<td></td>
<td>MAP</td>
<td>0.562</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CO2 Duration</td>
<td>0.153</td>
<td>-</td>
<td>25</td>
<td></td>
<td>CO2 Duration</td>
<td>0.823</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>CO2 Intensity</td>
<td>0.852</td>
<td>-</td>
<td>25</td>
<td></td>
<td>CO2 Intensity</td>
<td>0.128</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>RB</td>
<td>CF MAT</td>
<td>0.044</td>
<td>+</td>
<td>19</td>
<td>SOC CF MAP</td>
<td>0.805</td>
<td>-</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.811</td>
<td>+</td>
<td>23</td>
<td></td>
<td>MAP</td>
<td>0.849</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>CO2 Duration</td>
<td>0.825</td>
<td>-</td>
<td>34</td>
<td></td>
<td>CO2 Duration</td>
<td>0.027</td>
<td>+</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>CO2 Intensity</td>
<td>&lt;0.001</td>
<td>+</td>
<td>34</td>
<td></td>
<td>CO2 Intensity</td>
<td>0.058</td>
<td>+</td>
<td>21</td>
</tr>
</tbody>
</table>
We have tested the relation of CO2 effects to the intensity and duration of the treatments (see table above), and found an increasingly positive effect on RB with treatment intensity (both with or without N fertilization). In addition, we found an increasingly more positive RS response to root compartments with treatment intensity (more C allocation to RB) (only with N fertilization). Both findings confirm functional responses of root biomass in relation to resource needs (water and/or nutrients).

We also found an increasingly more negative RS response (more C allocation to AB) (with or without N fertilization) with treatment duration, and an increasingly positive SOC response with treatment duration (only with N fertilization). This might suggest that positive effects of water savings increasingly become more important over time.

We will also incorporate these results in further versions of the manuscript.

Title. I suggest, changing title and running title as the root biomass (and C storage) is a bit misleading to the overall subject of the paper. Ex: Responses to elevated CO2 and fertilization in managed grasslands – a meta analyses on limits of C sequestration potential

New title: Responses of grassland C pools to elevated CO2 and N fertilization: a meta-analysis on limits of soil C storage.

Running title: managed grassland under elevated CO2

New running title: Managed grasslands under elevated CO2

Outset hypotheses are very long and do not need (to my opinion) an introduction of forests. Hypothesis. Reading through hypothesis reader gets impression to read through results and discussion. Moreover, hypothesis highlight variables which were not tested in this study (i.e. LAI, nutrient depletion). I suggest to shorten and reword (see below).
We have rewritten the hypotheses along the line of the suggestions (see remarks below).

-MM, authors used weighted means for experiments with data from different years. I suppose that interannual variability has an effect on biomass ect (see comment on other variables above).

I agree that inter-annual variability might be important to find out about links to climatic variables, but this is not the focus of this paper. Here, we try to discover whether C storage is affected on an annual or longer-term basis. Variability of climatic variables inherently is part of natural ecosystems. Therefore, as indicated before, broad patterns related to climatic stratification makes sense here to discern between effects in different climatic regions, but we think smaller resolution climatic data are not directly useful for the questions asked in this manuscript.

-I suggest detailing 2.1 Data acquisition and criteria used for tested variables (Csoil is not described here). I also recommend to add a list of variables this would help reader going though results and reduce length of legends (Example Tab3 is not clear at all what is CF, Cf, pureC ect). However, I agree that most of site-information should be kept in the supplementary material.

See comments above: we included a limited description of included response variables.

To clarify different treatment combinations discussed, we will include the following table and consistently use these codes in the manuscript.

<table>
<thead>
<tr>
<th>Treatment Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Elevated CO2 treatment without any nutrient addition</td>
</tr>
<tr>
<td>CF</td>
<td>Elevated CO2 treatment with nutrient addition (amount of N addition not specified)</td>
</tr>
<tr>
<td>F</td>
<td>N fertilization treatment (amount of N addition not specified)</td>
</tr>
<tr>
<td>CF-L</td>
<td>Elevated CO2 treatment with N fertilization &lt;50 kgN m-2 yr-1</td>
</tr>
<tr>
<td>CF-H</td>
<td>Elevated CO2 treatment with N fertilization &gt;50 kgN m-2 yr-1</td>
</tr>
<tr>
<td>CF-N</td>
<td>Elevated CO2 treatment with pure N fertilization</td>
</tr>
<tr>
<td>CF-NPK</td>
<td>Elevated CO2 treatment with NPK fertilization</td>
</tr>
<tr>
<td>C-M</td>
<td>Elevated CO2 treatment with biomass removal component</td>
</tr>
<tr>
<td>C-m</td>
<td>Elevated CO2 treatment without biomass removal component</td>
</tr>
<tr>
<td>C-I</td>
<td>Elevated CO2 treatment with irrigation treatment</td>
</tr>
<tr>
<td>C-i</td>
<td>Elevated CO2 treatment without irrigation treatment</td>
</tr>
<tr>
<td>F-N</td>
<td>N fertilization treatment with N fertilization &lt;50 kgN m-2 yr-1</td>
</tr>
<tr>
<td>F-NPK</td>
<td>N fertilization treatment with N fertilization &gt;50 kgN m-2 yr-1</td>
</tr>
<tr>
<td>F-L</td>
<td>N fertilization treatment with pure N fertilization</td>
</tr>
<tr>
<td>F-H</td>
<td>N fertilization treatment with NPK fertilization</td>
</tr>
</tbody>
</table>

-Results, just a little comment. If I have not mistaken the Fig legends, significant differences between different treatments are highlighted by stars. Confidence intervals (CI, results of bootstrapping)) are the horizontal line going through each point. If this line does not go trough the vertical zero(It took my quite a while to understand this (2hours). Probably,
authors need to clarify how their Fig were made by putting part of the legend into MM section. Example Tab2 and Fig6 is especially difficult to understand from legend and symbols

The table above should solve the issue with symbols. In addition, in each figure legend it is clearly stated that the treatment effect is statistically significant when the CI does not encompass zero.

-Suggest to find another wording for “pure N fertilizers “

The authors think that in order to maintain readability pure N fertilizer is recommended. Otherwise, something like “fertilizer solely containing nitrogen” will have to be used, making sentences more cumbersome than needed. Alternatively, N fertilization only, would then confuse between single factor or combined fertilization+elevated CO2 treatments.

Authors should stick to one variable/treatment name throughout the manuscript. Got the impression that (suggest to add a table of abbreviations, see above) - elevated CO2 kept changing between C, CO2, - so did CO2 and irrigation with CI/Ci: : : : - CO2 and Fertiliser with CF, CO2 and Fert , Cf : : : - Is Fig 6 really necessary, as not easy to read. The fig repeats (in a more synthetic way) what was shown in Figs before. I suggest showing only Fig6 RB. (see comments)

See previous comments: We have included a table with the consistent treatment codes.

Further, we have decided to use the information in table 3 to represent data in figure 6. Therefore figure 6 is removed from the manuscript and the discussion related to it will be performed in the text only.

-Discussion . discussed results are repeated in various discussion chapter (more or less detailed) and paragraphs. I recommend grouping those paragraphs to avoid repeating.

Example:
P364 L25ff Chapter 4.1 was already mention and does not need to be repeated but merged together (i.e limit of non N nutrients) (see also comments to this first section)  P365 chapter 4.2 seems to re-discussion in tw different was the effect of N on MB and soil C. I suggest to merge the two paragraphs together P366L22-27 re- discusses the same results as mentioned above but brings this time priming effect into the play, while reapeting the C storage in long living tissues (already discussed P365L25ff) , P367LFF repeats inhibiting effect of N already mentioned P366L1ff

*Accordingly discussion section may be grouped as followed *Short overview of main results *Role of nutrients on C allocation under elevated CO2 - effect of management practise - effect on C storage (Soil C??)

Ok, changed this as suggested. Paragraphs are merged and redundancies reduced as much as possible.

*Specific comments
Abstract L 9 : : : monitors carbon storage potential in grasslands, and considered the influence

Ok, changed this as suggested
Abstract L16 An important role was attributed to responsive of root biomass to elevated CO2, since this implied higher potential for increases in soil C content when root biomass increased.

Ok, changed this as suggested.

Abstract L21 .. but the potential soil C storage limited due to concomitant increase in microbial biomass (?)

No, due to the relation to root biomass responses.

P359L24ff. However, their study : : : in C sink strength between both system types. DELET sentences on trees as this study does not compare grass <> tree responds

We limited the comparison with forests in the introduction as far as possible.

P360 L3ff I suggest to shorten and reword. In the present study we use data from 69 published grassland studies, to investigate whether CO2 elevation and/or nitrogen fertilization is likely to change carbon storage potential in managed grasslands. More precisely, we analyse effects of elevated CO2 concentrations and N fertilization (i.e. combined and individually) on above and below ground biomass production, microbial biomass and soil C. We test following hypotheses: 1) elevated CO2 will stimulate plant production and will increase allocation of C to root compartments, (2) addition of N solely, will stimulate plant productivity but will leave microbial biomass unaffected due C limitation in below ground (3) the combined CO2 and N treatment will strongly stimulate above and below ground biomass production, which in turn stimulates soil C storage (4) management practices (i.e. defoliation, irrigation) shifts C allocation towards aboveground plant compartments and reduces C soil.

Ok, changed this as suggested (see also previous comments).

P362L23 Pure N is strange wording I suggest to use N fertilisation solely or as a single factor (see also Tab2)

The ‘pure’ stresses the fact that the fertilizer used only consists of N (contrasting with NPK). ‘N fertilization solely’ or ‘as a single factor’ thus completely missed the purpose here.

L25 root biomass increased more when low high doses of N (i.e. C-H) were applied

This is now clarified with consistent treatment codes.

L363L25 .. and a tendency to increase soil C content : : : is this true???

Changed this statement to ‘tendency for small increases or no changes in soil C content.’

L364 L2-25 I suggest to restructure to highlight main results. Ex: However, considering CO2 as a single factor we found a decrease in root biomass as a consequence of elevated CO2 concentrations, which is in sharp contrast to most other studies. Interestingly, when excluding experiments that were irrigated or where biomass was removed, root biomass was no longer significantly decreased by elevated CO2 (data not shown). (L10) This offered support to our hypothesis that plants deprived of their shoots by harvest,
burning or grazing, allocate proportionally more energy to aboveground biomass for repair and regrowth, which could impair root growth by lowering the amount of C available for belowground biomass. As with irrigation (L13) root biomass tends to decrease, compared to non-irrigated systems as a results of increased WUE which does not necessarily need an extensive root network. In contrast with unfertilized systems, fertilised systems displayed an increase in root biomass in response to elevated CO2 (de Graaff et al., 2006), and showed a clear dependence on N additions (van Groenigen et al., 2006).

Ok, changed this as suggested

Table 2 legend has point 1-3 but not the table

Ok, changed this as suggested

Table 3 very difficult to understand, soil C responses, Pure C, Cf, What is C+Cf+CF??

Now clarified with consistent treatment codes

Fig 2 legend CO2 OR N fertilization responses ??? does x-axis means CO2 divided by

This means CO2 response for C to C-H or N response for N-N to N-H, this is now clarified in the figure legend

Fig 6 what is (Ln(E/A))

Not applicable anymore as Figure 6 is removed. Ln(E/A) means natural logarithm of elevated/ambient (see also methods)