Manuscript bg-2013-433 – Response to reviewers' comments

Interactive comment on "Weathering by tree root-associating fungi diminishes under simulated Cenozoic atmospheric CO$_2$ decline" by J. Quirk et al.

J. Quirk (corresponding author)

j.quirk@sheffield.ac.uk

Reviewers' comments (***R.C.***) are in *blue italics*, author comments (**A.C.**) are below.

Reviewer 1: Dana Royer

R.C. *Quirk and colleagues present here experimental and modeling results that quantify the effect of atmospheric CO2 on plant- and mycorrhizal-mediated chemical weathering. This topic is of broad significance to biogeochemistry, and especially the long-term dynamics of carbon and nutrient cycling. The presented results break substantial new ground, particularly with regards to quantifying the role of CO2 and mycorrhizae on chemical weathering. This paper should serve as an important reference for years to come.*

*The authors note in their introduction that long-term carbon cycle models like GEOCARB include in their parameterization a CO2 fertilization effect on chemical weathering. The authors now have, for the first time, a quantification of this effect. Given this, it would be useful to cross-check with the (previously untested) parameterization of GEOCARB. In GEOCARB: fBB=(1+0.087*GCM*log(RCO2)-0.087*Ws*(t/570)+0.087*GEOG)*(2*RCO2/(1+RCO2))ˆFERT where: fBB is the effect of CO2 on plant-assisted weathering for silicates and carbonates at time (t) to the present-day; GCM is related to climate sensitivity (deltaT2X), where deltaT2X = GCM*ln(2); t is time in Myrs ago; GEOG is the change in land mean surface temperature at time (t) relative to the present-day due only to changes in paleogeography (K); and FERT is the fraction of vegetation whose growth is stimulated by elevated CO2; it is related to enhanced chemical weathering by the Michaelis-Menton expression [2RCO2/(1+RCO2)]ˆFERT.*

Holding all parameters other than CO2 constant, and assuming the standard value of 0.4 for FERT, how close does the GEOCARB response (i.e., [2RCO2/(1+RCO2)]ˆ0.4) match the measured response by the authors?

A.C. We thank the reviewer for his encouraging response to our manuscript. With regard to his main point, assuming that the CO$_2$ fertilization effect (a Michaelis-Menten function which we will call "MM" here) is the primary effect of plants on the long-term carbon cycle as modelled in GEOCARBSULF then:

MM=(2*RCO2/(1+RCO2))^FERT

where RCO2 is CO$_2$(ppm)/280ppm (as in GEOCARBSULF) and FERT is set to 0.4. This function is meant to capture the effect of increased NPP on weathering, assuming 35% of vegetation responds to higher CO$_2$ (as assumed in GEOCARBSULF).

Following the suggestion of the reviewer to investigate how well our data match the assumed $[CO_2]_a$ fertilization value of 0.4 , we have normalized our weathering rate data for each of the three tree species so that they match the "MM" function at 200 ppm (see Fig. R1). Fitting FERT function curves to these data reveals the standard 0.4 FERT value of the GEOCARB models is too low for all three species in our study, with a FERT value of 1.4 best fitting the *Sequoia* data, and a value of 2.5 fitting the *Betula* data. We were unable to find a good fit to the data for *Acer.* Figure 3 of Taylor et al., 2011 (*Am J Sci* (311), 369-403) also suggests that the "MM" function in GEOCARB is too flat. On the basis of our small number of species grown under laboratory conditions we are cautious about making strong inferences from these observations which clearly will be interesting to follow up in future studies. We acknowledge the discord between the models and our results in the revised manuscript.

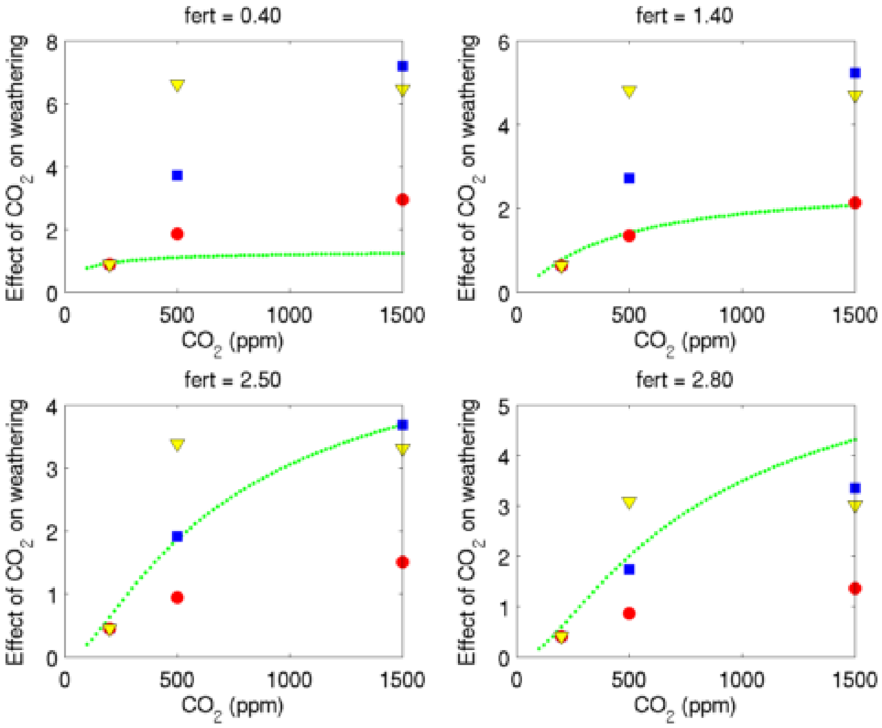

Fig. R1. Circles are Sequoia, triangles are Acer, and squares are Betula. The line represents "MM" tuned to each value of FERT.

The GEOCARB models also have a function expressing the weathering efficiency of the dominant vegetation (called fE). In GEOCARB models, fE is 1 for modern angiosperm-dominated vegetation. For gymnosperm-dominated vegetation fE is 0.875. These values were derived from field studies comparing EM pines with EM angiosperms (eg Moulton et al 2000 *Am J Sci* (300), 539-570). The data presented in our Figure 5 do not support this angiosperm-gymnosperm distinction because *Acer* and *Sequoia* (our arbuscular mycorrhizal (AM) species) have similar weathering fluxes, while *Betula* (our ectomycorrhizal (EM) species) has much higher weathering fluxes. Yet we cannot simply replace "angiosperm" with "EM tree", because the ratio between *Acer* (or *Sequoia*) and *Betula* (EM) weathering fluxes is between 0.020 and 0.039. Our experiments are focussed on mycorrhizal hyphae-mineral interactions by using root-excluding mesh compartments. Our findings demonstrate that weathering by vegetation associating with AM or EM fungi cannot be assumed to respond to atmospheric $[CO_2]_a$ changes in the same way, and highlight the need for researchers working in the field of plant-driven mineral weathering to carefully consider both the vegetation types and their mycorrhizal associations (e.g. Quirk et al. 2013 *Biol. Letts.* (8) 1006-1011; Koele et al. 2014 *Soil Biology and Biochemistry* (69) 63-70).

R.C. *Two minor comments:*

It would be helpful to cite this paper in the introduction: Andrews, J., and Schlesinger, W., 2001, Soil CO2 dynamics, acidification, and chemical wethering in a temperate forest with experimental CO2 enrichment: Global Biogeochemical Cycles, v. 15, p. 149-162.

A.C. We have incorporated this reference into the revised manuscript.

R.C. *On p.3 , line 17: need a reference for this statement.*

A.C. Review papers by Taylor et al., 2009 (*Geobiology* (7) 171-191) and Lambers et al., 2009 (*Plant Soil* (321) 83-115) discuss the weight of evidence for EM weathering processes, and the lack of that for AM fungi. These have been inserted into the revised manuscript.

Reviewer 2: Jason Price

R.C. *General Comments*

This paper provides a very thorough and detailed experimental design addressing the mechanisms of biological weathering as a function of atmospheric CO2. This laboratory investigation provides an excellent justification for the need for such studies based on previous global-scale modeling work. Mechanistic studies such as this are very capable of illuminating specific processes operating at the scale of mycorrhizal root symbionts. Such specific bio-mineral interactions are lost at larger scale investigations.

The authors are to be commended for developing such a meticulous and unequivocal investigation, especially in choosing a range of [CO2]a concentrations reflecting that of the last 35 Ma. I also like how the authors have been able to correlate weathering mineral surface features with fungal hyphae, refuting previous reports of certain features being purely of abiotic origin.

A.C. We thank the reviewer for his positive appraisal of our manuscript.

R.C. *Specific Comments*

(1) Early in the Abstract, and elsewhere, the importance of terrestrial Ca-Mg-silicate chemical weathering as being responsible for the sequestration of atmospheric CO2 in marine carbonate bedrock is emphasized. This is an important point and I'm pleased it is explicitly stated by the authors. However, the biological weathering of muscovite, a K-silicate mineral, is then addressed.

Wouldn't biotite have been the more applicable mica to include? There is also a very large literature on biotite weathering, including both experimental and field-based studies to which the results of this study could be compared.

A.C. We have conducted other tree-mycorrhiza weathering studies with biotite, muscovite and phlogopite as representative types of primary phyllosilicates, and found clear evidence of fungal-trenching of all three of these classes of silicate. For the VSI work we need near-atomically smooth minerals in order to detect the surface changes and in the present study focussed on muscovite as one of these model minerals as it is easiest to handle, with the lowest rate of delamination and loss of the uppermost layer of the mineral, which represents the pre-incubation characterised surface.

***R.C.** (2) Following item (1), the inclusion of basalt in the study is very appropriate. However, basalt can exhibit a relatively complex mineralogy with solid-solutions for olivine and pyroxene, and variable proportions of glass which can also have varying compositions. Previous research has shown the glass to be most reactive material in basalt. Can any additional specific information be provided regarding specific phases in the basalt that are weathering?*

A.C. Our focus on basalt rests on the fact that its weathering accounts globally for 30-35% of the atmospheric CO_2 flux consumed by silicate weathering, whilst it only comprises ~8.4% of continental crust silicate minerals (Dessert et al. 2003 *Chem Geol* (202) 257-273). We have characterised our basalt using XRD analysis and this showed that its mineralogical composition is consistent with olivine-poor tholeittic flood basalt. The major Ca- and Mg-bearing phases are plagioclase (An50), augite and orthopyroxene (see Table 5, Taylor et al 2011, *Am J Sci* (311), 369-403). We acknowledge that dissolution of highly reactive interstitial glass may contribute to the weathering flux, at least during the early stages of weathering of our crushed grains. In our recently published study of weathering by trees in the field (Quirk et al. 2012, *Biol. Letts.* (8) 1006-1011), we analysed rates of calcium dissolution from the same basalt as used in the present study. Quirk et al., 2012 used a sequential extraction method to quantify weathering from the carbonate and silicate phases of the basalt. Analysis of Ca:Sr ratios from each of the extracted phases indicated that the carbonate and silicates were well-distinguished by the extraction procedure.

***R.C.** (3) The results of this study certainly support global carbon cycle models, even though nanoscale processes operating in soils are addressed. An inherent assumption of this paper is that soils developed on Ca-Mg-silicate bedrock contain significant quantities of weathering minerals. In saprolitic landscapes which dominate the Earth's surface the soils may be relatively thin (<30 cm of a 5-10+ m-thick regolith) and composed of highly leached material containing relatively few reactive minerals. The most reactive minerals would have been chemically weathered/dissolved at or near the saprolite-bedrock interface, presumably dominated by inorganic processes. If true, then any biological weathering in the soil would exert relatively little influence on solute budgets at the watershed-/landscape-scale.*

A.C. Our studies and our conclusions do not rest on making assumptions about soil depth. The three tree species we studied here grow in temperate-boreal regions in which intensively weathered surface layers underlain by deep saprolite rarely occur. This links to our earlier point about the disproportionate importance of weathering in basaltic watersheds and areas of active orogenesis –

some regions and lithologies contribute disproportionately to global land-to-ocean calcium and magnesium fluxes – and the regions with very deeply weathered soil now have relatively low fluxes of Ca and Mg as a result of long-term depletion of these elements. We do not scale up to a global system given the constraints of the experimental conditions (i.e. lack of a representative soil column). The over-arching hypothesis motivating our work is that evolutionary innovations of land plants and their mycorrhizal associates drive intensification of biological weathering rates and cumulative element fluxes over millions of years. This includes the evolution of deeper rooting systems able to interact with a greater depth of saprolite to expand the critical zone and intensify lithosphere-biosphere-atmosphere element cycling.

R.C. *(4) Following item (3) above, Section 2.7 (Canopy transpiration) contains numerous assumptions that allow the hyposphere results of this study to be scaled up to a global weathering model. Assumptions include stoichiometries of nutrient uptake to support NPP, respiration and GPP rates based on simple multipliers, calculation of organic acid exudation by EM hyphae using GPP, dimensions of the hyphosphere, etc. Furthermore, the geometric surface area of the basalt is used, with any estimate of mineral "reactive" surface area always being fraught with errors. I realize references are provided in some cases, but the potential sources of error here are very large and only propagate with further calculations. If the authors are confident in these values, then more justification is needed. As written, making such a large jump in scale may be met with a great deal of skepticism by the readers.*

A.C. It is important to point out that we do not use the model to up-scale to global weathering rates. We have adapted the framework of the existing model to assess weathered element fluxes from the hyphospheres of our different tree-mycorrhiza partnerships. Bear in mind that the contents of the mesh bags were physically isolated from direct interaction with tree roots, meaning the fungal networks inside the bags were the primary conduits for the delivery of plant-fixed carbon-energy driving weathering. The series of assumptions we make in order to derive element fluxes from the hyphosphere of each tree are unavoidable, but we are confident they have sound physiological underpinning and are appropriate for the scale at which we multiply (i.e. up to the level of the hyphosphere). The differences in element fluxes from the hyphospheres within our mesh bags are primarily driven by the measured hyphal network size and mycorrhizal type. These assumptions are well-established and have been discussed previously (Taylor et al. 2011, *Am J Sci* (311), 369-403). We have made it explicit that we do not use the model to up-scale our results to the global scale in the revised manuscript.

R.C. *(5) I realize this may be beyond the scope of the present study, but after completing such a rigorous and thorough study is it possible to relate the findings to present-day/near-future climate change? For example, there is some evidence in Fig. 1 that the influence of [CO2]a on biological weathering my reach a steady-state above ~500 ppm.*

A.C. This is an interesting point. Our experimental design was optimized to assess responses of biological weathering rates to $[CO_2]_a$ concentrations of the deep past. The response of mycorrhizal network size and weathering rates do appear to exhibit non-linearity with $[CO_2]_a$ increases from half those of today (200 ppm) to very high concentrations of 3.75 times those of today (1500 ppm), but we would not feel confident in commenting on the influence that more modest, near-future rises in $[CO_2]_a$ towards 600-700 ppm may have on biological weathering and its potential to buffer against further $[CO_2]_a$ increases.

***R.C.** Technical Corrections*

(1) In section 15781, line 7, the phrase "…35 Ma ago…" is used. Including the word "ago" is redundant as "Ma" reflects time before present.

A.C. We do this for consistency. Here, we take Ma to represent the time unit "mega-annum" (millions of years) as opposed to "million years ago". This ensures consistency of meaning when we use Ma to refer to the "past 400 Ma", for example.

Reviewer 3: Roger Finlay

***R.C.** This paper deals with the general theory that symbiotic mycorrhizal fungal hyphae, associated with different plant hosts, are able to use plant-derived carbon to weather mineral surfaces, mobilizing nutrients that become available to their plant hosts. The theory is not totally new in itself and has been put forward by a number of authors with varying degrees of experimental support. The specific advance made by this study is the use of vertical scanning interferometry imaging of nanoscale surface topography of muscovite and basalt to investigate physical interactions of these minerals with different mycorrhizal fungi growing from plant hosts exposed to different atmospheric concentrations of CO2. The data presented show decreased amounts of surface modification at low (200 ppm) levels of CO2, corresponding to the minimum Cenozoic level, compared with the surface alteration seen at maximum CO2 levels of 1500 ppm. Surface/physical alteration of minerals by fungi has been shown before in previous publications (Gazze et al 2012, Saccone et al. 2011, Bonneville et al 2009) but the comparison of treatments of different types of mycorrhizal symbiosis at different CO2 levels provides novel information. Integration of these results into process based biotic weathering models suggests there may be a feedback regulation between reduced levels of CO2 and reduced flux of calcium and magnesium from silicates. The ultimate conclusion is that trees and fungi may have had a stabilizing influence in stabilising CO2 levels during the past 24 million years. The data provided, methodology used and statistical analysis carried out appear satisfactory.*

A.C. We appreciate the reviewer's comments, useful summary overview of the achievements of the work and its relationship to previous studies.

***R.C.** The lack of "direct confirmatory evidence" in previous studies is mentioned by the authors on several occasions. Some of the results provided here are also not "direct" in the sense that no direct connections between the plants and the supposed mycorrhizal fungi are shown. Only superficial morphological features are discussed and no DNA-based analysis of the fungi is provided. There is also no "direct" confirmation of the mycorrhizal status of the experimental plants used in this study. This could easily have been remedied by microscopic visualization/ quantification of internal/external fungal structures associated with the root systems. Such demonstrations would greatly improve the robustness of the conclusions made and might even have supported the differences in hyphal length.*

A.C. In the abstract lines 15-16 we mention previous work that has attributed features on minerals, like feldspar, excavated from mineral soil horizons beneath EM forests to trenching by EM fungi. We argue that these assertions are made "with little confirmatory evidence" because the resistance of the minerals used by necessity require long periods of time to weather, certainly to become trenched by foraging hyphae. This limits the ability to attribute likely mechanisms to trench formation. To our knowledge, no studies have physically characterized minerals prior to their interactions with soil microorganisms and then again afterwards as a direct comparison of pre-post exposure to the weathering environment inside root-excluding hyphal in-growth bags, as we have done here. The fungal trenching of minerals in our study clearly establishes the ability of these organisms to physically alter minerals. The hyphae-shaped trench features and residual fungal biomass we have observed microscopically has established that fungi forming clamp connections (i.e. basidiomycetes) and fungi that lack these but form terminal spores morphologically similar to AM fungi are the agents of weathering. We agree that the identify of these fungi is not unequivocally proven but given the experimental conditions in soils of relatively low organic matter content in which mycorrhizal plants were growing, it seems most likely that the effects we see are the result of mycorrhiza-driven weathering. To measure hyphal lengths the extracted hyphae are mounted on gridded membranes and examined under a light microscope. The samples from AM host plants are stained with trypan blue which gives a distinctive staining of AM-like hyphae, and the samples from EM hosts are dominated by fungi with clamp connections, some of which have distinctive pigmentation similar to the tomentelloid group of fungi.

The mycorrhizal colonization of roots was confirmed but not formally quantified for each test species at the end of the experiment- and the trees were well-colonized by mycorrhiza. We had inoculated the plants with roots of the same species collected from the field (Westonbirt Arboretum) to ensure we had host-specific fungal inoculum. We have made this clear in the revised manuscript.

***R.C.** However the comparisons between different systems, including controls with no plants, support the conclusion that plant-associated mycorrhizal fungi may be involved. Although the "background" values of non-mycorrhizal fungal hyphae are subtracted it would be interesting see how large (or small) these are.*

A.C. We apologise for this lack of detail. The hyphal lengths per unit mass of dry basalt grains in plant free treatments, that were subtracted from those measured in treatments with plants, were substantially lower than those in mycorrhizal plant treatments – especially at the higher $[CO_2]_a$ concentrations. This supports the view that most of the hyphal lengths seen at the two highest $[CO_2]_a$ values with trees are due to mycorrhiza. The plant-free control values are as follows:

200 ppm 0.59 (0.08) m g^{-1}; 500 ppm 0.68 (0.07) m g^{-1}; 1200 ppm 0.61 (0.10) m g^{-1} figures in parentheses are standard error. One-way analysis of variance testing for the effect of $[CO_2]_a$ showed no response ($F_{2,6} = 0.27$; $P = 0.77$).

We have incorporated these results into the revised manuscript.

The paper is largely well written, clear to follow and appropriate references are provided. The text should be carefully revised to take account of the comments above and special attention should be *paid to terminology used in different places. For example -it would be good to explain what is meant by "hyphal strands" in the context of AM fungi. Are these linear hyphal aggregates as formed by ectomycorrhizal fungi?*

A.C. We appreciate the inadvertent confusion this could cause. We will revise the manuscript to ensure consistency of terminology. Hyphal strands in this context refer to the fragmented pieces of extraradical mycelium retained on filter papers following their sonication from basalt grains into suspension. We have included a note clarifying the lack of aggregated hyphal structures within the basalt-filled mesh bags in both AM and EM treatments.