Cover letter

Dear Dr. Subke,

Included in this submission is our revised manuscript (bg-2017-338) entitled, "*Stable isotopic constraints on global soil organic carbon turnover*". We would like to thank both the editorial team and the reviewers for the time and effort they have put into assessing the previous version of this manuscript. Based the highly constructive critiques we received from the referees, we have made a number of changes to the manuscript that we feel have improved its quality immensely.

In the revised manuscript, we revised our dataset and corrected the errors according to soil profiles from savanna ecosystems and tropical forests. We added 20 new soil profiles into the revised dataset from 8 peer-reviewed articles, which resulted in 177 separate soil profiles in the revised dataset. We also added the detailed information of the data source (spatial information of global SOC and WorldClim) in the revision and discussed more on the inherent uncertainties associated with those estimated k values. The detailed reply to each comments from the reviewers was attached below.

We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comment you or the reviewers may have.

Sincerely,

Edith Bai

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Point by point response to reviewer

Reviewer 1

A. SUMMARY OF THE REVIEW The topic is fully relevent for publication in Biogeoscience. The study confirms the correlations between ¹³C enrichment down the depth in soil profiles and environmental variables, that bave been already reported by several authors on smaller datasets. The title makes sense. But the manuscript requires

significant revisions for two major reasons. - The first concern the consistency of the

dataset itself, i.e., some erroneous attribution of data to biomes, omission of true tropical savannas profiles and profiles that reveal paleo C4-vegetations. The contribution of past C4 vegetation (delta ¹³C -10 to 13‰ in such profiles is misunderstood. This leads to (i) non-representative values of beta and (ii) overestimation of temperature dependence of ¹³C enrichments.

We agree that there are some erroneous attributions of data to biomes, omission of true tropical savannas profiles and profiles that reveal paleo C4-vegetations. We revised the dataset and corrected the erroneous attributions of data to biomes. We deleted the tropical savannas profiles and those profiles that reveal paleo C4-vegetations.

The second point concerns interpretation beyond this bias. The discussion tends to maximize the importance of kinetic fractionation by microbes as an explanation of beta's variance, but without providing results supporting it. I suggest reducing the section of discussion on this point. These comments are detailed in point "C. Extended review".

Thank you for your suggestion. We have reduced the discussion part on effect of kinetic fractionation on beta's variation and discussed the uncertainties of this method.

Finally, the "Stable isotopic constraints on global soil organic carbon turnover " is there, but has to be recalculated after database correction. Interpretation of the correlations in terms of processes should be minored. I suggest to correct the database by restrict it to pure C3 ecosystems, and furthermore make the proposition to merge this database with another one, therefore doubling the number of observations (see B. below).

B. ONE UNUSUAL PROPOSITION TO IMPROVE THIS STUDY. In line with the philosophy of Biogeoscience Discussion, which stimulates interactive and cooperative research more than competitive research, I make the proposition to provide 155 additional profiles worldwide. In the frame of the COST (European COoperation in Science and Technology) action SIBAE (Stable Isotopes in Biosphere-Atmosphere-Earth System Research; 2009-2013), a group of 10 scientists (10 institutions) has built and analyzed an exactly similar database of 196 World 13C/C profiles and beta values under pure C3 ecosystems. 43 are common to yours, 99 in other peer-reviewed articles, and 56 in non peer reviewed literature (Figure 1). This dataset shows significant multiple regressions with climate and clay, which are similar to those presented in the present manuscript BGD 2017-338, but with less predictive value. This less predictive value is in accordance with the above-mentioned biases, with more profiles exhibiting less negative beta values (including some positive) and more varied environments. If the authors of MS BG2017-338 accept this proposition, a final dataset merging the present dataset (after correction) with SIBAE's one, would provide a stronger view of the stable isotopic constraints on global

soil organic carbon turnover.

We revised the dataset and restricted it to pure C3 ecosystems. We deleted the soil profiles in tropical savanna and tropical forests which are marked by ancient C4 vegetations (see below). To compensate, we added 20 new soil profiles into the revised dataset from 8 peer-reviewed articles, which resulted in 177 separate soil profiles in the revised dataset-these studies were not available or missed when we first started gathering our data set. The addition of these additional data did not alter our summary conclusions, yet added robustness to our overall global findings. We thank the reviewer's suggestion to add 20 more profiles from an unpublished source; however, because our analysis is focused on synthesis of the published literature, we would not add this dataset, but would like to share our full dataset once it is published.

1. Consistency of the database.

Almost all so-called "tropical savannas" profiles in the database refer to afforestation or tree encroachments in C4 savannas. In published "real" tropical savannas profiles, delta13C decreases with depth, with values close to -12 -15 ‰ in surface and lower than - 20‰ in deep layers because of the presence of millenary-old forest-derived carbon. These have therefore POSITIVE beta values (of course non log transformable).

If not log transformed, they would draw the "tropical beta" toward the opposite direction. In the so-called "tropical savannas" (profiles # 85, 86, 87, 106-112), reforestation leads to a strong gradient from C3 signature in the top, and predominant C4 signature below. These systems were precisely chosen by the authors to analyse C dynamics through 13C signature change, and are not representative of world savannas. In some of the cited papers, profiles with positive beta were omitted. Interpretations of highest ln(-beta) in this "expansive and dynamic biome" (line 149) are therefore based on forest expansion data! As a result Figure 3 is wrong: either real savannas should be included (positive beta) or tropical savannas and these C4 to C3 conversions should be removed from the database. The latter is my suggestion.

We agree with reviewer comments and the "tropical savannas" profiles with number 9, 85, 86, 87, 93, 94, 106-112, 140 and 141 were removed from the revised dataset.

Beyond the case of "savannas", several profiles under tropical forests are marked by ancient C4 vegetations (profile numbers 78, 82-84, 115-121). They have been studied for this reason and are therefore not representative of world tropical forests. In both cases, beta is not linked to C turnover, but paleoclimate, as in many regions of the world. Almost all profiles with beta < -5 in the database are concerned. This overestimation of tropical ln(-beta) strongly affects Figure 4, the correlation with MAT or MAP (Figure 5), and Table 1, i.e., the main results.

We agree. The profiles of 78, 82-84, 115-121 were deleted from the revised dataset.

"C. Extended review"

2. Discussion of the relationships between beta and other variables (beyond paleo C4 vegetation) The discussion might sometimes be confusing. A "kinetic isotope fractionation" associated to biodegradation process (decay) would not directly imply a dependence on the rate (speed) of decay, i.e., the turnover rate. In the Rayleigh distillation equation, beta is typically independent on the rate. A partial explanation of the variance in beta by the turnover rate rely on complex

processes (e.g., Acton, Garten, Schlesinger), and should not neglect other sources of 13C variations, such as the change in plant isotopic composition with time, post-photosynthetic fractionation in plants, bioturbation, isotope composition of nitrogenous compounds, etc., which can also be involved in the correlation of beta with carbon turnover rate. On the contrary, the discussion tends to minimize these processes (lines 215 to 234). Furthermore, the magnitude of a kinetic fractionation by heterotrophic respiration in soils is still debated (e.g., Breecker et al., 2015). Since the results provide no new demonstration, I suggest minoring this part of the discussion. Since the dataset includes turnover rate (k), some hypotheses of factors affecting the "turnover rate" (e.g., MAP > 3000 mm) might be discussed also on the basis of k, and not only ln(-beta). The apparent decrease of ln(-beta) under climates with MAP > 3000 mm is probably linked to overestimated -beta in moderately moist tropical areas (C4 zone).

Thank you so much for your suggestion. We added more discussion on the factors that affected soil 13C variation with depth beyond microbial fractionation. We also discussed the uncertainty when using beta value to study soil carbon cycling at the global scale.

3. Details. Precise when defining beta that you used the decimal logarithm ("log" is ambiguous). Table 1 and Figure 5 legend: indicate that N (%) and Clay (%) refer to topsoil. Table 1 Add one digit to the regressor of MAP. Figure S1 is after Acton et al. 2013

Thank you for catching those and the errors mentioned above were corrected in the revised manuscript.

Reviewer 2

The manuscript "Stable isotopic constraints on global soil organic carbon turnover" by Wang et al. presents an interesting approach of deriving information about SOC decomposition kinetics from stable carbon isotope information along the soil profile. For this, they derive a slope "beta" from the relationship of δ^{13} C values and SOC content of soil profiles across the globe, and then relate these "beta" values to calculated decomposition kinetic constants "k" (or more precisely their log-transformed negative values). They state that the highly significant linear relationship of the log-transformed variables can be used to derive SOC decomposition kinetics from δ^{13} C profiles of SOC. Furthermore, they relate these ln(-beta) values to four different parameters, i.e. MAT, MAP, soil clay and nitrogen content. For all four parameters they find significant relationships with ln(-beta). This approach is interesting and might be promising if proven to be reliable.

The weak part is the calculation of the kinetic decomposition constants with several secondary data sources and a fixed relationship between heterotrophic and total soil respiration, which might be too much of a simplification for this global approach, given the large range of ratios between heterotrophic and autotrophic respiration found for different ecosystems and conditions.

1) The kinetic decomposition constants k for the different soil profiles have been calculated by assuming steady-state conditions, i.e. SOC input and output are in equilibrium. While this assumption might hold true for many of the sites, there is no evidence provided that this really is the case.

2) The SOC stocks, which represent the denominator in equation 1, were extracted from the Global Organic Soil Carbon and Nitrogen (Zinke et al. 1998). There is no mention whether there was an exact match between the soil profiles used in the present study, or whether spatial approximations were made, and if yes, which criteria were used for these spatial approximations.

3) Heterotrophic soil respiration was calculated from total soil respiration by a fixed linear relationship adopted from Bond-Lamberty et al. (2004). Given the large variability of the fraction of Rh to total soil respiration (varying between 10% and 90% in vegetated ecosystems), this approach is highly questionable.

4) Also total soil respiration was not measured, but derived from a climate-driven regression model (Raich et al. 2002).

5) And finally, climate data were derived from WorldClim as a function of latitude and longitude (what about altitude?), whenever climate data were not available in the literature tapped in this study. Again, there is no mention whether there was an exact match between the locations of the present study, or whether spatial approximations were made, and if yes, which criteria were used for these spatial approximations.

Given all above-mentioned uncertainties concerning the calculation of the key variable of the study, i.e., the SOC decomposition rate constant k – which by the way is an apparent constant, as it is a composite of the decomposition of several SOC pools with different decomposability/recalcitrance – the reader would expect an extensive uncertainty analysis. However, not a single attempt was made to quantify those uncertainties, which certainly will amount to a large relative error due to multiple convolutions of single functions and error propagation. Also no mention is made of this crucial point in the discussion, and how this might affect the far-reaching conclusions drawn.

Authors' response: We would like to thank the reviewer for the time and effort of into reviewing our manuscript. We agree with the reviewer's comments that there are uncertainties associated with kinetic decomposition constant k – which have been discussed previously. However, to our knowledge, the approach we took is the best available for providing integrative soil carbon decomposition rates estimates along profiles. Numerous published modeling studies have used approaches involving multiple data sources and assumptions - similar to our approach. While we agree that the coupling of different data sources inherently injects uncertainties - and we have discussed the caveats in revision. Getting quantitative with the uncertainty is unfortunately not possible (as other studies have noted), for the following reasons. First, we mainly focus on soil 13C-based proxy and its variations with MAT and MAP in this manuscript. Hence, we compared our beta value with the kinetic decomposition constant k to explore correlation between these two factors, but we can't quantitatively assess the relationships at the global scale. This does not devalue the correlations we find across sites, though it does limit our quantitative assessment, pointing to an area for future research for the community. Thus, if useful, we could move the comparison between beta and k to the supporting information if this seems like the best approach.

Second, just because there are large uncertainties with the kinetic decomposition constant k, we believe the independent approach provided in this manuscript using carbon isotope variations along soil profiles is a promising approach. Indeed, it allows for larger-scale geographic exploration of soil carbon decomposition at the global scale in a way that differs fundamentally from current approach. We believe that this constraint can be used to help benchmark global models, which are lacking in their ability to generate global soil C patterns and responses to change.

We added the detailed information of the data source (spatial information of global SOC and WorldClim) in the revision and discussed the inherent uncertainties associated with those estimated k values. We added the following discussion in the revised manuscript.

"The SOC stock for each soil profile was extracted from a global soil organic carbon map (within 1 m depth), which was created by IGBP-DIS (1998) with a resolution of 0.5 by 0.5 degree; and the mean annual soil total respiration (Rs) was extracted from a long-term dataset with a resolution of 0.5 by 0.5 degree (Raich et al., 2002). We added the two dataset and the coordinate of soil profiles into ArcGIS (version 10.0, ESRI, Redlands, CA) to extract SOC stock and respiration of each profile using spatial Analysis tool."

"It is important to stress that our applied the steady-state assumptions in estimating turnover, which would not hold for all sites, especially highly disturbed of human altered environments, such as agricultural soils. Application of our results to ecosystems that are for from steady-state should be approached with caution. Further, we used multiple data sources to estimate soil carbon store and respiration for profiles in our dataset and then calculated the k values, which might add uncertainty to the final results. Finally the coordinates that we used to extract climate data for some soil profiles (15 soil profiles out of 177) from a world climate dataset may not exact match the location of soil profiles."

1 Marked-up manuscript version

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3 Stable isotopic constraints on global soil organic carbon turnover

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11 Abstract. Carbon dioxide release during soil organic carbon (SOC) turnover is a pivotal component of atmospheric CO₂ 12 concentrations and global climate change; however, reliably measuring SOC turnover rates at large spatial and temporal scales 13 remains challenging. Here we use a natural carbon isotope approach, defined as beta (β), which was quantified from the $\delta^{13}C$ of 14 vegetation and soil reported in the literature (177 separate soil profiles), to examine large-scale controls of climate, soil physical 15 properties and nutrients over patterns of SOC turnover across terrestrial biomes worldwide. We report a significant relationship 16 between β and calculated soil C turnover rates (k), which were estimated by dividing soil heterotrophic respiration rates by SOC 17 pools. $\ln(-\beta)$ exhibits a significant linear relationship with mean annual temperature, but a more complex polynomial relationship 18 with mean annual precipitation, implying strong-feedbacks of SOC turnover to climate changes. Soil nitrogen (N) and clay content 19 correlate strongly and positively with $\ln(-\beta)$, revealing the additional influence of nutrients and physical soil properties on SOC 20 decomposition rates. Furthermore, a strong ($R^2 = 0.76$; p < 0.001) linear relationship between ln(- β) and estimates of litter and root 21 decomposition rates suggests similar controls over rates of organic matter decay among the generalized soil C stocks. Overall, 22 these findings demonstrate the utility of soil δ^{13} C for independently benchmarking global models of soil C turnover and thereby 23 improving predictions of multiple global change influences over terrestrial C-climate feedback.

24 1 Introduction

25 Soil contains a large amount of organic carbon (C) and plays a crucial role in regulating Earth's C cycle and climate system 26 (Schmidt et al., 2011; Reichstein et al., 2013). Approximately 1500 Gt of soil organic carbon (SOC) is stored in the upper meter of global mineral soil (Scharlemann et al., 2014), which is equivalent to ~ 160 years-worth of current fossil fuel CO₂ emissions. 27 28 Disagreement exists, however, over the residence time of this vulnerable C stock and its relationship to factors of ongoing change, 29 particularly climate changes and widespread nitrogen pollution (Reay et al., 2008; Reichstein et al., 2013). Biogeochemical models 30 rely heavily on turnover rates of discrete SOC pools (active, intermediate, and recalcitrant) derived from lab incubation studies 31 (Davidson and Janssens, 2006;Xu et al., 2016). In practice, however, SOC pools fall along a continuum of characteristic turnover 32 times (from days to centuries; (Schmidt et al., 2011;Lehmann and Kleber, 2015)), in a given ecosystem site. Furthermore, lab-33 derived estimates of SOC turnover disrupts the sensitive balance between plant-soil-microbe interactions in ecosystems, adding 34 questions on the reliability of such techniques when applied to real-world conditions.

The rate of SOC turnover is an important parameter for process-based ecosystem models (Davidson and Janssens, 2006;Schimel et al., 1994) and those used to forecast the global carbon cycle and climate system in the future (Friedlingstein et al., 2006). Global biogeochemical models often use climatic factors such as precipitation and temperature to predict SOC turnover

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38 rates (Schimel et al., 1994; Nishina et al., 2014). While several studies reported positive relationships between temperature and 39 SOC turnover (Chen et al., 2013; Trumbore et al., 1996; Bird et al., 1996; Trumbore, 1993; Carvalhais et al., 2014), however, others 40 have called the generality of such relationships into question (Giardina and Ryan, 2000). This discrepancy could be due to 41 interactions among factors which are difficult to separate in the field, for example, among soil temperature, soil moisture and 42 nutrient controls over SOC decomposition (Davidson and Janssens, 2006). Nitrogen (N) in particular can affect SOC 43 decomposition by changing microbial community structure, microbial activity or both (Curiel et al., 2007). Incorporation of factors 44 besides climate is crucial for improving model performance and predicting the feedback-response of the terrestrial carbon cycle to 45 climate change (Nishina et al., 2014).

In addition, questions remain regarding whether the turnover of different C stocks behaves fundamentally similarly. For example, climate is considered to be a "master regulator" of leaf litter (Zhang et al., 2008), root (Gill and Jackson, 2000;Silver and Miya, 2001) and soil organic C pools (Davidson and Janssens, 2006). However, recent findings have pointed out that soil microbial community composition may play a more important role in litter decomposition rates than climate or litter quality (Bradford et al., 2016;Keiser and Bradford, 2017). Due to the different microbial communities among leaves, roots and soils, and different chemical composition of such pools, rates of C turnover have the potential to vary widely across generalized classes of C stocks.

52 Stable carbon isotope composition (δ^{13} C) provides relatively non-disruptive insights into the turnover of SOC (Garten et al., 53 2000; Accoe et al., 2002; Powers and Schlesinger, 2002; Bird et al., 1996). For sites with reasonably stable vegetation stocks, measures of vertical soil-profile δ^{13} C can provide constraints on SOC turnover rates in ecosystems (Acton et al., 2013;Garten et 54 al., 2000; Wynn et al., 2006). Soil δ^{13} C generally increases from shallow to deep mineral soils in relatively well-drained systems, 55 56 concomitant with decreasing SOC concentrations (Fig. S1). The vertical distribution of the δ^{13} C reflects microbial preferences for 57 ¹²C vs. ¹³C in decomposing substrates (Garten et al., 2000), which, in turn, increases the ¹³C/¹²C of residual organic C fractions 58 with a kinetic isotope effect defined by ε (Fig. S1). Therefore, SOC δ^{13} C tends to increase with depth along vertical soil profiles 59 until it reaches a maximum value at which point a steady-state is achieved (Kohl et al., 2015;Accoe et al., 2002;Brunn et al., 60 2014;Garten et al., 2000;Wynn et al., 2006;Brunn et al., 2016). These trends result in a negative linear relationship between the 61 log-transformed SOC concentration and soil δ^{13} C (Acton et al., 2013;Garten et al., 2000;Garten and Hanson, 2006;Powers and Schlesinger, 2002). The slope of the linear regression between soil δ^{13} C and the log-transformed SOC concentration is defined as 62 beta (β), which has been proposed as a proxy for SOC turnover rate in a select number of sites (Acton et al., 2013;Garten et al., 63 2000; Powers and Schlesinger, 2002). β has also been assessed in set of regional scale analyses (Acton et al., 2013; Brunn et al., 64 65 2014); however, whether β values can be used to constrain rates and controls on SOC turnover is yet to be explored at the global 66 scale. Furthermore, the Suess effect (the atmospheric isotopically depleted fossil fuel CO₂ lowering atmospheric δ^{13} C-CO₂) 67 (Boström et al., 2007; Wynn et al., 2006) and the mixing of different C sources (Acton et al., 2013; Diochon and Kellman, 2008; Wynn, 2007) may also influence the profile of soil δ^{13} C with depth. Identifying the relative influence of different factors in 68 69 vertical profiles of soil δ^{13} C is essential to applying this proxy to patterns of SOM turnover.

Here, we examine the efficacy of β as a proxy for SOC turnover rates by synthesizing soil profile data from sites around the world (Fig. 1). To understand the overall utility of C isotope composition for constraining SOC turnover rates, we explore the relationship between β and modeled SOC decomposition constant *k* and environmental factors, particularly climate, soil clay content and nutrient availability. We also compare the variation of β with that of root and litter turnover rates across latitude (thermal) gradient to examine whether and how the decomposition of generalized C pools varies as a function of likely controls.

76 2.1 Data compilation

77 Using the key words of 'carbon isotope & vertical profile', $\delta^{13}C$ & soil depth profile', or 'soil carbon turnover & stable isotope' 78 on the Web of Science source, we assembled a total of 150 soil profiles from 53 journal papers (Fig. 1; A list of the literature 79 sources is given in Table S1). Only soil profiles under pure C₃ vegetation without significant human disturbance were selected. For 80 each profile, we collected carbon isotope (δ^{13} C), organic carbon (SOC) and N concentration of leaf/litter and mineral soil layers at 81 different depths if the data is available, and more than four δ^{13} C values should be provided within the top 1 meter. Where data were 82 not available in tables, Data Thief software (http://www.datathief.org/) was used to acquire values from figures. We also noted the 83 experiment location (latitude and longitude), biome types, mean annual precipitation (MAP), and mean annual temperature (MAT) 84 for each soil profile. In cases where climate variables were not reported (15 soil profiles out of 177), we used the WorldClim data 85 (http://www.worldclim.com/), which have average monthly temperature and precipitation between 1970 to 2000 with a resolution 86 of $\sim 1 \text{ km}^2$ at the global scale, to reconstruct climate values based on latitude and longitude coordinates in ArcGIS version 10.0 87 using Spatial Analysis tool (ESRI, Redlands, CA).

88 In addition, a previous reported arid and semi-arid grassland transect along 3000 km with 27 sampling locations was added 89 into the dataset (Wang et al., 2017). Those sampling sites are dominated by C_3 plants and cover approximately 16° longitude 90 ranging from 104°52' E to 120°21' E and 10° latitude ranging from 40°41' N to 50°03' N. The MAP ranges from 90 mm to 420 91 mm and MAT ranges from -2 °C to +7 °C. At each location, five 1 m \times 1 m sub-plots (or one 5 m \times 5 m sub-plot in areas with 92 shrub as the dominating plants) were setup within a 50 m × 50 m plot. Twenty soil cores (0 - 100 cm) in each 1 m × 1 m sub-plot 93 were collected and divided into 0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm and 60-100 cm depth segments and bulked to form one 94 composite sample for each segment per sub-plot. Leaf samples of five dominating genera (Stipa, Leymus, Caragana, Reaumuria 95 and *Nitraria*) were sampled for carbon isotope analysis if these genera were present in the sub-plots.

96 In laboratory, leaf samples were washed with deionized water to remove dust particles and then dried at 65 °C for 48 h. Both 97 soil and leaf samples were ground in a ball mill and stored in a plastic bag. Soil carbonate was removed from soil samples using 98 0.5 M HCl. Organic carbon concentration and isotope composition of soil and leaf were carried out at the Stable Isotope Faculty 99 of University of California, Davis.

100 2.2 Beta calculation

A negative linear regression between the log_{10} -transformed SOC concentration and δ^{13} C for each soil depth profile was conducted (Fig. S1). The slope of the this linear regression is defined as beta (β) value (Acton et al., 2013;Garten et al., 2000;Powers and Schlesinger, 2002).

104 **2.3** Soil decomposition rate constant (*k*)

We applied the steady-state assumption in our study. While this is not always valid – especially in highly disturbed human altered environments such as agriculture – this is a reasonable approximation in natural ecosystems, where SOC turnover on decadal to century to millennial time-scales. The carbon decomposition rate constant (*k*) was estimated as the ratio between soil heterotrophic respiration (R_H) and soil organic carbon stock (SOC) (Sanderman et al., 2003): *k* = R_H / SOC. The SOC stock for each soil profile was extracted from a global soil organic carbon map (within 1 m depth), which was created by IGBP-DIS (1998) with a resolution of 0.5 by 0.5 degree; the mean annual soil total respiration (R_s) was extracted from a long-term dataset with a resolution of 0.5 by 0.5 degree (Raich et al., 2002). We added the two datasets and the coordinate of soil profiles in ArcGIS (version 10.0, ESRI, 112 <u>Redlands, CA) to extract SOC stock and respiration rates for each profile</u> using the Spatial Analysis tool. Then, we used the linear 113 relationship between soil respiration (R_s) and R_H to calculate R_H (Bond - Lamberty et al., 2004): ln (R_H) = 1.22 + 0.73 × ln (R_s).

114 2.4 Data analysis

Negative β value and decomposition rate constant *k* were log-transformed to perform statistical tests. Larger ln(- β) translated to faster SOC decomposition rates (Acton et al., 2013;Powers and Schlesinger, 2002). Soil ln(- β) was analyzed and <u>summarized</u> across different biome types. Soil ln(- β) was also compared with litter and root decomposition rate along latitude at the global scale. Two-variable regression analysis was first performed to explore the relationship between ln(- β) and ln(*k*), or ln(- β) and climate variables (MAP and MAT) as well as soil edaphic factors (N and clay content). Multiple regression analysis was then used to examine the relationship between ln(- β) and these variables (MAP, MAT, soil N and clay content). Akaike information criterion (AIC) was used to estimate the quality of model when increasing the number of parameters.

122 3 Results

123 3.1 Worldwide patterns of β

A total of <u>177</u> soil profiles from all continents other than Antarctica were encapsulated in our compiled dataset (Fig. 1). Carbon isotope composition (δ^{13} C) increased with soil depth in the majority of <u>examined</u> profiles and was strongly correlated with the logarithm of SOC (Fig. S1). ln(-β) was significantly positively related with site-based estimates of the soil C decomposition constant, ln(*k*) with R² = <u>0.34</u> (Fig. 2).

The values for ln (- β) ranged from -0.50 to 2.20 across sites (non-transformed β values ranged from -0.60 to <u>-7.41</u>, Table S1). Highest mean ln(- β) was observed in <u>tropical forests</u> (Fig. 3), followed by deserts, temperate forests, and temperate grasslands<u>with a mean value of 1.15, 0.70, 0.58 and 0.49</u>, respectively. MAP among those <u>four</u> biomes increased from desert < temperate grassland < temperate forest < tropical savanna < tropical forest and MAT increased from temperate grassland < temperate forest < < desert < tropical forest (<u>Fig. S2</u>).

Along the latitude gradient, $\ln(-\beta)$ decreased from the equator to poles, but was higher at 20-30° N compared to the 10-20° N latitudinal band (Fig. 4a). The mean decomposition rate of leaf-litter and root C displayed similar latitudinal patterns ($R^2 = 0.76$; p<0.001, Fig. 4b).

3.2 Controls on β across ecosystems

137 The ln(- β) and MAT displayed a strong, positive relationship across the global dataset (R² = 0.43; P < 0.001; Fig. 5a). Ln(- β) did 138 not show a simple linear correlation with MAP, but instead showed a polynomial relationship with a tipping point at MAP = 3000 139 mm (Fig. 5b). When MAP was less than 3000 mm, $\ln(-\beta)$ was positively correlated with MAP ($R^2 = 0.23$, P < 0.001); $\ln(-\beta)$ 140 decreased with the increasing of MAP in areas receiving > 3000 mm of MAP. A quadratic equation provided the best fit to the 141 relationship between ln(- β) and MAP for all sites (R² = 0.24, P < 0.001; Fig. 5b). Soil N explained 20% of the variations in ln(- β) (P < 0.001; Fig. 5c). Moreover, a quadratic equation best described the relationship between soil clay and ln(- β), with R² = 0.49 (P 142 143 < 0.001; Fig. 5d). AIC analysis showed that the full-factors model (i.e., MAT, MAP, soil N and clay) accounted for more of the 144 variation in $\ln(-\beta)$ than any other regression model in the global data set (Table 1).

145 4 Discussion

146 Our global data synthesis reveals significant relationships between $ln(-\beta)$ and the turnover of soil, litter and root C pools at

147 geographically broad scales (Fig. 2 and 4). These findings build on site-based observations and regional assessments (Accoe et al., 148 2002;Garten et al., 2000;Powers and Schlesinger, 2002;Brunn et al., 2014;Brunn et al., 2016), and suggest that C isotope 149 composition is a useful proxy for understanding generalized patterns of SOC turnover and the underlying controls over soil C 150 metabolism. That our results hold across all major soil C pools implies that SOC, root and litter turnover share common controls, 151 in particular those related to climate and soil nitrogen contents. These findings suggest that decomposition of belowground and 152 aboveground soil C may have similar responses to global climate change, such as global warming and increasing atmospheric N 153 deposition. Furthermore, our results highlight the potential of incorporating natural stable C isotopes in global biogeochemical and 154 Earth system models to constraint soil and litter decomposition rates that are vital to climate change forecasts.

155 Within the terrestrial biosphere, our findings point to highest mean $\ln(-\beta)$ in tropical forest ecosystems, which is consistent 156 with previous studies (Carvalhais et al., 2014). however, tropical rainforest ranked second to savannas in our global synthesis (Fig. 3). This difference might be due to data coverage, moisture or nutrient effects, or all of these. Our estimates of $\ln(\beta)$ were not area-157 158 weighted, nor did the data cover this diverse ecosystem exceptionally well; the small area of wet tropical sites in our analysis may 159 therefore not reflect the average conditions of tropical lowland forest. The tropical forest data were largely from sites in Costa Rica 160 and Brazil, with MAP equal to 4058 mm and 1359 mm, respectively. Our analysis thereby points to the need for more data from an array of tropical forest sites, given the substantial biogeochemical diversity within this globally important biome. At the other 161 162 extreme, our analysis suggests that slowest mean rates of SOC decomposition occur in temperate grassland (Fig. 3), consistent 163 with results from previous simulation modeling (Carvalhais et al., 2014:Schimel et al., 1994). Relatively slow decomposition rates 164 have been observed for plant litter decay in arid grassland sites (Zhang et al., 2008), and largely reflects strong moisture controls 165 on decomposition. In addition, microbial biomass and microbial activities are much lower in arid/semi-arid vs. mesic or humid 166 sites (Fierer et al., 2009), thus leading to low rates of SOC and litter decomposition.

167 <u>4.1 Climate and nutrient dependences of β</u>

168 The differences of β value among different biomes reflects several controlling variables – especially mean annual temperature, 169 mean annual precipitation, soil N contents, and clay content. Of particular importance are temperature-driven controls over β , in 170 which MAT explains 43% of the variation of ln(- β) in our global data compilation (Table 1; Fig. 5a). A recent meta-analysis, which 171 included 24 soil profiles across a range of cool temperate to tropical forest sites, reported similarly strong temperature-dependencies 172 of β (Acton et al., 2013). Our findings broaden this perspective to a global range of terrestrial biomes and climates, and indicate 173 that, with increasing MAT, SOC turnover is substantially accelerated. This result agrees with previous studies which have identified 174 temperature as the strongest regulator of soil C decomposition among all known controls (Carvalhais et al., 2014;Schimel et al., 175 1994), and is consistent with global C-climate feedback models, which project acclereated rates of CO₂ efflux from the land 176 biosphere with climate warming (Ciais et al., 2014).

177 Our study also points to significant relationships between β and precipitation-climates, which are more complex than those 178 observed for MAT. Rather, we find an inflection point in β in our global data set at MAP ~ 3000 mm (Fig. 5b). This relationship 179 reveals negative effects of moisture on change of soil δ^{13} C in very wet climates. Most of the C isotope data used to estimate β were 180 available in sites with MAP < 3000 mm, which collectively account for > 98% of the world's terrestrial ecosystem rainfall regimes. 181 Moreover, in extremely wet sites, it is likely that leaching of dissolved organic carbon (DOC) from soils to streams affects the relationship between decomposition and isotope effect expression (Powers and Schlesinger, 2002). Previous studies have shown 182 that the DOC increases with increasing soil depth (Kaiser et al., 2001). Because DOC is generally.¹³C enriched (Kaiser et al., 2001), 183 184 increasing DOC leaching into very wet sites would be expected to induce a larger change in soil δ^{13} C with depth, and hence, increasing In(-B). Thus, we may have overestimated the SOC turnover rate in areas with high DOC leaching; however, this cannot 185

explain the low ln(\beta) values in areas with MAP > 3000 mm. A cross-system compilation of the smaller though more dynamic litter pool shows a similar pattern of decreasing decay rates in regions with MAP > 3000 mm compared to drier sites (Zhang et al., 2008). In addition, Schuur (2001) showed that leaf and root decomposition rates declined significantly with increasing precipitation along a highly constrained rainfall sequence in Hawaiian forest sequence (from 2020 mm < MAP < 5050 mm), thereby resulting in slower rates of nutrient mineralization and declines in net primary production (NPP) in the wettest sites. The consistencies between our study and past work suggests that precipitation affects the decomposition of SOC and litter in similar ways, slowing decomposition rates when MAP is very high and anaerobic conditions dominate (i.e. MAP > 3000 mm; Schuur 2001).

In addition to climate, nutrients influence the magnitude of $\ln(-\beta)$ in our compilation, with SOC turnover rates generally increasing with soil N concentrations across ecosystem sites (Table 1, Fig. 5c). Although soil N has been suggested as an important control over SOC decomposition in previous work (Schimel et al., 1994), our study is one of the few to confirm the existence of such a relationship at the global scale. Positive correlations between litter decomposition rates and litter N contents during the early stages of decay have been reported previously (Berg, 2000). Past work has also suggested that high N availability enhances soil degrading enzyme activities (Fioretto et al., 2007).

Finally, our results suggest that soil physical factors, particularly soil clay content, plays a role in $\ln(-\beta)$ and soil organic C turnover (Fig. 5d), consistent with previous expectations (Schimel et al., 1994;Xu et al., 2016). In sites where clay content is < 50% (i.e., sandy soils), for example, $\ln(-\beta)$ increases with the soil clay content; however, when clay content is > 50% (loamy or clayey soils), no clear relationship between $\ln(-\beta)$ and clay content is observed (Fig. 5d). The change in this relationship could be explained by the higher "preservation capacity" of clayey soils (Vogel et al., 2014).

- 204 SOC turnover is an important parameter for process-based models and Earth system models (Schimel et al., 1994;Davidson 205 and Janssens, 2006), and models used to forecast the carbon cycle and climate system into the future (Friedlingstein et al., 2006). 206 Global biogeochemical models commonly use climatic factors as predictors of SOC turnover rates (Carvalhais et al., 2014). In 207 contrast, our results point to factors beyond climate singly, soil N content and soil texture, in altering organic C turnover across the 208 terrestrial biosphere. Taken together, for instance, our multiple regression analysis considering all factors (i.e., MAT, MAP, soil 209 N and clay) explains nearly 70% of variation of $\ln(-\beta)$ (R²= 0.66, P<0.001; Table 1), suggesting the high dependence of SOC 210 turnover on these factors. We therefore suggest the need for models that include all of these factors when forecasting global C 211 cycle response to change.
- 212 In addition, our findings suggest that the C isotope composition of the soil can help to improve global C model performance. 213 A common problem in global C research is finding consistent and sufficiently integrated metrics against which the performance of 214 different biogeochemical models can be quantitatively analyzed (Tian et al., 2015). The strong relationships we observe between 215 β and SOC turnover suggest that this natural-isotope proxy can be used to ground-truth large-scale patterns of model-simulated 216 soil C dynamics. Future work to collect and analyze C isotope data in vertical soil profiles, which is a relatively inexpensive process, 217 can further extend the regional coverage of β and help benchmark SOC turnover estimates among global model simulations. This 218 is important given the potential for SOC turnover to respond to multiple global changes and produce significant feedbacks on 219 climate at the global scale (Carvalhais et al., 2014;Lehmann and Kleber, 2015).

220 <u>4.2 Alternative controls over soil δ^{13} C with depth</u>

221 Several processes have been proposed to explain the widely observed pattern of increasing δ^{13} C from shallow to deep soils beyond

- 222 <u>those related to kinetic isotope fractionation during decomposition.</u> For example, atmospheric δ^{13} C-CO₂ has been decreasing since
- the industrial revolution owing to the combustion of ¹³C-depleted fossil fuels, which could lead to lower δ^{13} C in surface soils vs.
- deeper horizons (Friedli et al., 1987). However, the magnitude of this effect is small (i.e., 1.4 1.5‰) compared to the substantial

- variation of soil δ^{13} C along depth profiles ($\simeq 3.5\%$) in our dataset. Based on a 100-year-old soil archive (i.e. soil collected before extensive fossil fuel CO₂ emissions) and modern samples collected from a common site in the Russian steppe, Torn *et al.*(2002) <u>demonstrated that</u> δ^{13} C profiles of modern and pre-industrial soils were similar. <u>Thus, the δ^{13} C values of SOM in soil profiles that</u> can be attributed to the changes in the δ^{13} C of atmospheric CO₂ should be small.
- In addition, bioturbation and consequent mixing of C from different sources has the potential to alter soil δ^{13} C profiles (Acton et al., 2013;Ehleringer et al., 2000). The δ^{13} C of root material is generally higher than that of above ground biomass, such as leaves (Powers and Schlesinger, 2002), and so the δ^{13} C of SOC at the soil surface may be lower than deep soils. Because microbes, invertebrates, and other soil fauna are typically enriched in δ^{13} C compared to source-substrates, biological migration and physical mixing of soils may alter relationships between soil C concentrations and δ^{13} C (Wynn et al., 2006). Kohl et al. (2015) suggested that increased proportions of soil bacteria (¹³C enriched) relative to fungi (¹³C depleted) biomass might also contribute to increasing δ^{13} C with depth; however the process-based modeling predicts the opposite pattern (Acton et al., 2013).
- Moreover, in extremely wet sites, it is likely that leaching of dissolved organic carbon (DOC) from soils to streams affects the
 relationship between decomposition and isotope effect expression (Powers and Schlesinger, 2002). Previous studies have shown
 that DOC increases with increasing soil depth (Kaiser et al., 2001). Because DOC is generally ¹³C-enriched (Kaiser et al., 2001),
 increasing DOC leaching into very wet sites would be expected to induce a larger change in soil δ¹³C with depth, and hence,
 increasing ln(-β).

241 <u>4.3 Uncertainty on carbon isotope method</u>

It is important to stress that we applied steady-state assumptions in estimating turnover, which would not hold for all sites, especially highly disturbed or human altered environments, such as agricultural soils. Application of our results to ecosystems that are for from steady-state should be approached with caution. Further, we used multiple data sources to estimate soil carbon store and respiration for profiles in our dataset and then calculated the *k* values, which might add uncertainty to the comparison between $\ln(-\beta)$ and $\ln(k)$. Finally, the coordinates that we used to extract climate data for some soil profiles (15 soil profiles out of 177) from a world climate dataset may not match the exact locations of these soil profiles.

248 5 Conclusion

Our analysis provides a globally integrative tool for understanding variations of SOC turnover rate, which can be applied spatially based on estimates of factors such as climate and soil properties. Compared with other methods, utilization of C isotope composition ratios in soil profile provides an independent approach that does not rely on disruption of plant-soil-microbe interactions. It has the added benefit of integrating over longer time scales (decade to centuries), and thus provides a common measurement for model-based benchmarking and calibration schemes.

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Table 1: Multiple regressions between $ln(-\beta)$ and climate and other edaphic factors at global scale.

Variables	R^2	п	AIC
$\ln(-\beta) = \underline{0.046} \text{ MAT} - \underline{0.058} \text{ MAP} + \underline{0.225}$	<u>0.43</u> ***	<u>176</u>	167.30
$\ln(-\beta) = \underline{0.053} \text{ MAT} - \underline{0.137} \text{ MAP} + \underline{0.384} \text{ N} + \underline{0.150}$	<u>0.55</u> ***	<u>104</u>	<u>64.71</u>
$\ln(-\beta) = \underline{0.046} \text{ MAT} - \underline{0.222} \text{ MAP} + \underline{0.849} \text{ N} + \underline{0.006} \text{ Clay} + \underline{0.069}$	<u>0.66</u> ***	<u>70</u>	<u>28.95</u>

MAT: Mean Annual Temperature (°C); MAP: Mean Annual Precipitation (<u>m</u>); N: Soil nitrogen concentration -(%)<u>in topsoil</u>. Clay:

Soil clay concentration (%) in topsoil. *n* is the number of data, and R^2 is the coefficient of determination for the regression line. AIC: Akaike information criterion. ***represents significant at *p* less than 0.001.







Figure 2. Link between β value and modeled soil carbon turnover rate (k), which was estimated as the ratio between soil heterotrophic respiration and soil carbon stocks. Solid line is regression line and dashed lines denote 95% prediction interval.



Figure 3. Variations of mean β with biome types. Blue and red points present MAP and MAT for each biome, respectively.



Figure 4. Variation of mean β value across latitude (bar chart), litter decomposition rate *k* (yr⁻¹, red dots, Zhang et al., 2008) and root decomposition rate *k* (yr⁻¹, blue dots, Silver and Miya, 2001) at the global scale. The inner panel is the

400 regression between soil β value and litter and root decomposition rate *k*.



Figure 5. Beta varied with climate and edaphic factors. Relationships between ln(-β) and MAT (a), MAP (b), soil N
(c), and clay concentration (d) for global dataset. Solid line is regression line and dashed lines denote 95% prediction
interval.