General Response to Referees' Comments

Three anonymous referees have provided generally positive comments on our manuscript, with some constructive critiques that we plan to address as explained below (responses are written in *italic*; modified text in quotation marks). The three main comments are as follows:

- Reviewer #1: ambiguous definition of C-use efficiency (CUE) for organisms and communities. The reviewer correctly argues that CUE should be defined as 1-respiration rate/uptake rate. This means that biological products such as exudates should be accounted for in the CUE definition, differently from gross growth or biomass production efficiency in which net biomass increments only are accounted for. We will revise our definition according to the reviewer's suggestion.
- Reviewer #2: lacking discussion on human impacts on ecosystem scale efficiencies. This is an excellent suggestion as it prompted us to generalize the definition of C storage efficiency (CSE). We will include anthropogenic C fluxes in our definition of CSE and comment on the role of ecosystem management as a driver of change for CSE.
- Reviewer #3: lack of clarity in some definitions. We will revise the text to clarify ambiguous definitions, and change Table 3 to indicate which components of the C cycle represent inputs and outputs in each of the systems/scales considered.

Detailed responses are reported in the individual rebuttal letters.

Anonymous Referee #1

Manzoni et al reviewed and synthesized patterns in carbon use efficiency (CUE) across scales. This is a large effort that can help reconciling previously identified differences in CUE. The authors go into the details of the different definitions that have been used and clarify some of the misunderstandings in the past. I think this could become an important contribution to the field, as differences in definitions and equations for CUE have been mostly ignored and confusion exists on what CUE should reflect. However, I do not fully agree on the presented definitions and think the manuscript still fails to fully resolve discrepancies. The current manuscript does not accurately represented what CUE is, where the term originates from and how it has been used in the past. As the manuscript reads now, I find it a missed opportunity to resolve the confusion that is associated with this topic.

We agree that the manuscript can be improved along the lines suggested below and we appreciate the reviewer's efforts to exemplify his/her concerns. This is a very helpful evaluation that led to a more rigorous definition of CUE vs. BPE terms.

About the definitions:
From a plant perspective, theory indicates that CUE = NPP/GPP, with NPP = the synthesis of organic compounds = GPP – R. Hence, CUE = 1-R/GPP. (NPP = net primary production; GPP = gross primary production, R = respiration). This corresponds more or less to equation 3 (CUE = 1-outputs/inputs) used by Manzoni et al. However, Manzoni et al consider egestion (EG) and exudation (EX, including symbionts) as part of the outputs, and not as part of NPP. Consequently, the CUE considered here is actually biomass production efficiency (biomass production/inputs = BPE) instead of CUE. Both CUE and BPE are meaningful terms – CUE focusses on the C cycle, while BPE targets the biomass that is produced. In the past, both terms have been rarely distinguished though and they have also not been used consistently.

We agree that this is a critical point. In the original manuscript, we had noted this discrepancy between BPE and CUE and pointed to their different interpretations. The two definitions differ if egestion and exudation are important components, as in the case of animals (mainly egestion) and plants (mainly exudation). Whether the distinction matters for microbial community is debatable, as estimates of exudation rates in natural communities are not available. While we agree fully on the importance of the distinction, the literature does not agree on how to call these different efficiencies across disciplines. For example, classical ecological stoichiometry texts (Sterner and Elser 2002) use the term 'gross growth efficiency' (GGE) instead of 'biomass production efficiency' (BPE), which has become common in papers focusing on stand-scale carbon balances. The same term CUE indicates – depending on the source – different efficiencies (including exudation or not), creating some confusion. Therefore, an unambiguous definition of CUE and an explanation on how it differs from GGE (=BPE) are indeed much needed.

Correct use can be critical, however, as CUE and BPE may respond differently to environmental changes. For example, an increase in BPE could be associated with un-altered CUE if the partitioning to EX is the sole responsible of the change in BPE (i.e. R unchanged). Such understanding becomes important for example when comparing models with observations. Model evaluation assuming observed BPE = modelled CUE (=1-R/GPP) can lead to serious flaws, as illustrated by the following hypothetical example. Assuming modeled CUE should equal observed BPE, a (hypothetical) decrease of BPE with increasing CO2 concentration would suggest and increase in R/GPP whereas in reality the decrease in BPE may be solely due to an increase of EX while R/GPP, and hence true CUE, remain unaltered. In this hypothetical example, adjusting the model to reflect observed BPE in modeled CUE would lead to an overestimation of the response of CUE and R to elevated CO2.

This is a very useful example, and we fully agree that there is a risk of misinterpretation. We will revise the text to clarify this issue, as described below.

The above problem related to the assumption that BPE = CUE is more prominent at some levels (e.g. vegetation) than at others (e.g. bacteria). Hence, differences among levels may in part be due to differences in the definition used. This is somewhat acknowledged by the authors, but it would be much clearer and more accurate if BPE and CUE were clearly distinguished throughout the manuscript and if it was made clear in the figures and tables where BPE is calculated, where CUE is calculated and perhaps also where BPE∼CUE.

We will implement a clearer definition of both – CUE and BPE. For 'historical' reasons, we will use the term GGE (Sterner and Elser 2002), but making sure to underline that BPE is a completely equivalent term. Regarding CUE, we will adopt the suggested definition of 1-respiration/uptake – that is, CUE is a measure of C conversion to organic compounds that will eventually become new biomass or will be exuded (plants, microbes). When changing this definition, we will clarify that it differs from the one proposed earlier by Manzoni et al. (2012), but that it is preferred for consistency with more recent work, especially in the areas of plant physiology and in stand-scale C cycling studies. Using the notation of Sterner and Elser (2002), CUE is thus defined as:

CUE = (G + EX)/U = G/U + EX/U = GGE + EX/U.

Using this relation allows explaining how CUE~GGE (or equivalently CUE~BPE) only when EX<<U. Given the proliferation of terms with different meaning, we will also add a comparison table in the Supplementary Information, in addition to the current Tables 2 and 3:

Definition	*Context*	*Originally used terms*	*Source*
CUE_A = 1-O/I	*Soil microbial communities*	*Ecosystem-scale efficiency of microbial biomass synthesis and recycling of necromass/exudates (CUE_E)*	*(Eq. 2 in Geyer et al. 2016)*
GGE = G/U	*Animals and microorganisms*	*Gross growth efficiency (GGE)*	*(Sterner and Elser 2002)*
	Microbial communities	*Carbon use efficiency (CUE)*	*(Eq. 2 in Manzoni et al. 2012)*
	Soil microbial communities	*Community-scale efficiency of microbial biomass synthesis (CUE_C)*	*(Eq. 1 in Geyer et al. 2016)*
	Individual plants	*Carbon use efficiency (CUE)*	*(Gifford 1995)*
	Plant communities	*Biomass production efficiency (BPE)*	*(Campioli et al. 2015)*
CUE = 1-R/U	*Soil microbial communities*	*Community-scale efficiency of microbial biomass synthesis when EX≈0 (also denoted as CUE_C)*	*(Figure 3 in Geyer et al. 2016)*
	Plant communities	*Carbon use efficiency (CUE = NPP/GPP)*	*(Cannell and Thornley 2000)*

There are also many more other definitions of growth/uptake ratios for both heterotrophs and autotrophs, but their interpretation depends on the measurements done to estimate growth and uptake rates (see Table 2 in the main text). In the large majority of cases, the exudation rate and its role in differentiating between GGE and CUE is not mentioned.

Moreover, with the definition CUE = 1-R/U, C losses from organisms and ecosystems are treated in the same way –in both cases CUE is defined as the amount of C that is fixed in organic form and not respired. Exudation and leaching (for organisms and ecosystems, respectively) are subtracted from this quantity and lead to the definition of GGE or net ecosystem C balance (NECB). This perspective will be also presented in the revised manuscript.

To summarize, in response to this important remark, we will amend the text on the definition of organism- and community-level CUE as follows (we will also update the Supplementary Information along the same lines):

"We now define CUE at the organism level as the ratio between the rate of production of biomass and products (G+EX), and the rate of C uptake (U),

$$CUE = \frac{G+EX}{U} = 1 - \frac{EG+R}{U}. \qquad (5)$$

As a result, the mass balance equation can be rewritten as,

$$\frac{dC}{dt} = CUE \times U - EX - T. \qquad (6)$$

With this definition, CUE represents the fraction of C taken up allocated to biosynthesis (biomass and products that will be eventually exuded), but excluding respired and egested C, which do not contribute to biosynthesis. Including exudates such as enzymes and polymeric compounds in the CUE definition is motivated by the clear fitness advantage these products have for the organism. Moreover, C storage compounds and osmolytes are also regarded as 'biomass', as they would be measured as cellular material.

This definition is consistent with previous work on plant C budgets (Thornley and Cannell 2000), but it differs from definitions often used for soil microorganisms where only biomass synthesis is considered and CUE = GGE (Manzoni et al. 2012, Geyer et al. 2016). It is thus important to emphasize that CUE is higher than GGE. The difference between GGE and CUE is relevant when EX is large, as in the case of organic C exchanges between roots and plant symbionts (Hobbie 2006, Ekblad et al. 2013). For microbial communities, the entity of the extracellular enzyme and polysaccharide synthesis is unknown but presumably small compared to the other rates involved, at least in soils (Schimel and Weintraub 2003), making the distinction less important in practice (for further discussions in this context, see Geyer et al. 2016)."

Specific comments: l.26 and l.50: I don't think biomass production/C uptake is the consensus definition of CUE (see above). Intro: I suggest to review the history of the definitions for CUE more elaborately. Where was it first used, what was the exact definition, how have definitions been applied in different fields...

In most fields, CUE is defined as a biomass production over uptake, but we agree that in the case of plants, CUE is defined as NPP/GPP. The term CUE was first used by plant physiologists in the early 1990's based on a search with ISI Web of Science. We cite for example one of those early papers, in which CUE is actually defined as net biomass production over gross photosynthesis (Gifford 1995). That initial definition is thus not consistent with the one proposed by the reviewer. However, the same author changed the definition in his more recent work to CUE = NPP/GPP (Gifford 2003). Notably, the term "carbon use efficiency" was also used in the same period and discipline (plant physiology), but with a completely different meaning – the net C gain in terms of plant biomass per unit C spent to support mycorrhizal associations (Tinker et al. 1994). Therefore, even within a specific discipline, there is no consensus on the use of these terms.

We will provide a short historical overview of the efficiencies of C conversion, to give readers an idea of how terms have evolved, but we will not cover the historical angle comprehensively, as it would require a lengthy presentation of terms for all the disciplines involved. Table 2 in the main text already contains key publications (with early papers from the 1970's), and we will add further key references such as the seminal paper by Monod (1949). The interested reader can follow the evolution of the terminology starting from those references. However, we will take the opportunity offered by the historical context for introducing the main difficulties in defining and interpreting CUE, and change the second paragraph of the Introduction as follows:

"For biological systems (organs, individual organisms, or even entire communities), CUE is defined as the ratio between the amount of C allocated to biosynthesis (new biomass and biological products, including e.g., exudates) and the amount of C taken up. While the term CUE was proposed in the mid-1990s in the context of plant C balances (Gifford 1995), other terms – e.g., 'growth yield' – referring to the efficiency of substrate conversion into biomass had been in use since the early 1900 (Monod 1949). Now, efficiency definitions are proliferating across many disciplines in biology, ecology, and Earth sciences. While some of these definitions are comparable (and all are deceptively simple), subtle differences often emerge, partly due to conceptual and methodological advances that allow quantification of previously ignored C exchanges. These differences and in general the evolution of CUE definitions make interpretation of results difficult and complicate cross-disciplinary comparisons.

The main difficulty is to unambiguously define what represents growth or C storage, and reconcile conceptual definitions with empirical estimates (Clark et al. 2001, Chapin et al. 2006, Geyer et al. 2016)…"

l. 160: clearly define the difference between uptake and assimilation to help the reader in following the different equations

We will change the sentence below Eq. 4 as "where U is the uptake rate, U-EG is the assimilation rate (i.e., A in Fig. 1), and G is the net growth rate in C units".

l.175: define overflow respiration. Ion uptake respiration is not mentioned. Is it considered part of growth respiration? See for example Lambers et al 1983, Physiologia Plantarum, 58: 556-563.

Quoting the more recent review by Cannell and Thornley (2000), "Ion uptake and phloem loading have elements of both growth and maintenance and all forms of wastage respiration are neither"(p. 49). We have indeed separated wastage (overflow) respiration from the other components, but we would prefer not to delve into the discussion on whether ion uptake should be included in growth or maintenance respiration. This issue is important in practice for modelling purposes, but we feel it is outside our goals in this contribution. However, we will clarify that overflow respiration includes wastage respiration and any C respired to compensate stoichiometric imbalances.

l.200: replace 'reduces' with 'can be simplified to'.
l.222: add 'and to EX' after 'exports to other parts of the plant'.
l.295: I suggest to replace 'lower estimates of CUE' by 'an underestimation of CUE'.
l.444: 'for a given uptake rate' seems more logical than 'for a given respiration rate'.

Thanks for these comments – we will implement the suggested changes

I think the authors missed some relevant publications. Cotrufo et al 2013 (Global Change Biology 19, 988-995) discuss the influence of substrate quality on microbial substrate use efficiency (another alternative for CUE), and consequences for soil C storage. This framework deserves at least a mention.

We will add the suggested reference and include a comment on recent results of positive correlation between microbial CUE and soil C storage (Kallenbach et al. 2016) – consistent with the framework proposed by Cotrufo et al. (2013).

Campioli et al 2015 (Nature Geoscience 8, 843-846) provide an update of Luyssaert et al 2007 and Vicca et al 2012 (both cited in the manuscript), and include also other vegetation types than forests. Data are provided in the supplementary files. I suggest considering including these data, or at least refer to them.

The dataset by Campioli et al. (2015) provides a new interpretation of nutrient availability effects on forest BPE, which we will also present in the revised manuscript. After checking the dataset itself, it appears to be less useful than the Luyssaert et al. (2007) dataset for the purpose of comparing plant community and ecosystem CUE because it focuses on GPP and biomass production. Therefore, we do not plan to change the analysis made for Figures 6 and 7 in the main text. Please see also the response to Reviewer 2 regarding the role of human activities on ecosystem CUE and CSE – in the new Discussion paragraph, we will cite the paper by Campioli et al. (2015).

Table 2: Cannell and Thornley 2000 actually used the definition CUE = 1- Ra/GPP.

That is correct. We will move the citation to Cannell and Thornley (2000) to the correct position in the table, next to DeLucia et al. (2007), and cite here Gifford (1995) instead.

DeLucia et al 2007 used data on biomass production/GPP but termed it NPP/GPP (hence ignoring other NPP components such as exudates and symbionts). This is part of the confusion and I suggest the authors take the opportunity to clarify this.

We will add a clarification to this regard in the Supplementary information (Section 1.3):

“As shown in Eq. (4) in the main text, plant community CUE should be calculated by including all these C fluxes in the numerator. When only net biomass increments are available, the terms gross growth efficiency (GGE) or biomass production efficiency (BPE) are more accurate (as in Vicca et al. 2012, Campioli et al. 2015).”

Fig. 2: CUEplant is defined as NPP/GPP, but NPP is undefined. In line with my earlier comments, I suggest to clearly define NPP.

We will more clearly define NPP in the main text and in the SI (Section 1.3), but would prefer not to add more definitions in the caption, in which we already refer to Chapin et al. (2006), where NPP is also defined.

Figs. 6 and 7: clarify where the data originate from (refer to SI)

We will refer to the SI by adding: “Data sources are described in the Supplementary Information.”

References

Campioli, M., S. Vicca, S. Luyssaert, J. Bilcke, E. Ceschia, F. S. Chapin Iii, P. Ciais, M. Fernandez-Martinez, Y. Malhi, M. Obersteiner, D. Olefeldt, D. Papale, S. L. Piao, J. Penuelas, P. F. Sullivan, X. Wang, T. Zenone, and I. A. Janssens. 2015. Biomass production efficiency controlled by management in temperate and boreal ecosystems. Nature Geosci **8**:843-846.

Cannell, M. G. R., and J. H. M. Thornley. 2000. Modelling the components of plant respiration: Some guiding principles. Annals of Botany **85**:45-54.

Chapin, F. S., G. M. Woodwell, J. T. Randerson, E. B. Rastetter, G. M. Lovett, D. D. Baldocchi, D. A. Clark, M. E. Harmon, D. S. Schimel, R. Valentini, C. Wirth, J. D. Aber, J. J. Cole, M. L. Goulden, J. W. Harden, M. Heimann, R. W. Howarth, P. A. Matson, A. D. McGuire, J. M. Melillo, H. A. Mooney, J. C. Neff, R. A. Houghton, M. L. Pace, M. G. Ryan, S. W. Running, O. E. Sala, W. H. Schlesinger, and E. D. Schulze. 2006. Reconciling carbon-cycle concepts, terminology, and methods. Ecosystems **9**:1041-1050.

Clark, D. A., S. Brown, D. W. Kicklighter, J. Q. Chambers, J. R. Thomlinson, and J. Ni. 2001. Measuring net primary production in forests: Concepts and field methods. Ecological Applications **11**:356-370.

Ekblad, A., H. Wallander, D. L. Godbold, C. Cruz, D. Johnson, P. Baldrian, R. G. Bjork, D. Epron, B. Kieliszewska-Rokicka, R. Kjoller, H. Kraigher, E. Matzner, J. Neumann, and C. Plassard. 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. Plant and Soil **366**:1-27.

Geyer, K. M., E. Kyker-Snowman, A. S. Grandy, and S. D. Frey. 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry **127**:173-188.

Gifford, R. M. 1995. Whole plant respiration and photosynthesis of wheat under increased CO2 concentration and temperature: Long-term vs short-term distinctions for modelling. Global Change Biology **1**:385-396.

Gifford, R. M. 2003. Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. Functional Plant Biology **30**:171-186.

Hobbie, E. A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology **87**:563-569.

Kallenbach, C. M., S. D. Frey, and A. S. Grandy. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature communications **7**:13630.

Manzoni, S., P. G. Taylor, A. Richter, A. Porporato, and G. I. Ågren. 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist **196**:79-91.

Monod, J. 1949. The growth of bacterial cultures. Annual Review of Microbiology **3**:371-394.

Schimel, J. P., and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry **35**:549-563.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry. The biology of elements from molecules to the biosphere. Princeton University Press, Princeton and Oxford.

Thornley, J. H. M., and M. G. R. Cannell. 2000. Modelling the components of plant respiration: Representation and realism. Annals of Botany **85**:55-67.

Tinker, P. B., D. M. Durall, and M. D. Jones. 1994. Carbon use efficiency in mycorrhizas - Theory and sample calculations. New Phytologist **128**:115-122.

Vicca, S., S. Luyssaert, J. Penuelas, M. Campioli, F. S. Chapin, III, P. Ciais, A. Heinemeyer, P. Hogberg, W. L. Kutsch, B. E. Law, Y. Malhi, D. Papale, S. L. Piao, M. Reichstein, E. D. Schulze, and I. A. Janssens. 2012. Fertile forests produce biomass more efficiently. Ecology Letters **15**:520-526.