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Biogeosciences Discussions

Interactive comment on "A novel isotope pool dilution approach to quantify gross rates of key abiotic and biological processes in the soil phosphorus cycle" *by* Wolfgang Wanek et al.

Anonymous Referee #2

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General comments With great interest, I have read the bg discussion paper by Wanek et al. entitled "A novel isotope pool dilution approach to quantify gross rates of key abiotic and biological processes in the soil phosphorus cycle". The paper aims to improve the isotope pool dilution approach to assess gross organic P mineralization rates that has been proposed by various authors (Kirkham and Bartholomew, 1954; Di et al. 2000; Randhawa et al. 2005, Kellogg et al. 2006). In principle, the study seems well executed. However, I would like to raise a couple of points that are rather critical and could be improved. My main comment is that the claimed novelty of the method lies in the introduction of an abiotic control. However, the adequacy of autoclaved soils is questionable. Therefore, further experiments would be needed to fully prove the

validity of the measured rates. A second worry is that the description of the experiment is not too clear. You probably need to add a scheme showing the procedure, and maybe detail also the recommended procedure at the end of the manuscript. Thirdly, I am disappointed that the suggested method was not compared to existing protocols, whether this concerns the use of water as an extractant, the sequential extraction of microbial P, or the comparison to the Po mineralization method by Oehl et al. For this, you could contact Klaus Jarosch who is at present using the method, or Astrid Oberson. For sure, they will be willing to collaborate and compare the two approaches on a few soils. If you include some additions of quickly mineralized compounds such as RNA, you should be able to evaluate both methods thoroughly. Finally, you do not mention a possibility to derive net organic P mineralization rates. Could you please address this in the discussion? In conclusion, the manuscript still needs some major improvements before it can be accepted.

Specific comments Line 10: this first sentence is a rather bold statement, or wouldn't net rates of soil processes suffice for modelling purposes? Line 56: IEX – this is a new abbreviation, at least I don't think I have seen it before. Most of the studies categorized as IEX studies used isotopic exchange kinetics (IEK) techniques. I wonder if the abbreviation is needed or if it would not be easier to speak of isotope exchange techniques throughout the text. The second abbreviation (ID) is rather confusing due to the similarity to IPD and is not needed at all, I think. Line 94-99: this whole argument is highly questionable. An incomplete extraction of a homogeneous pool is not a problem, because the specific activity in the pool is captured and enters the calculations. Line 104: need (not needs) Line 113: it appears to be quite risky to recommend only two time points, given that you state that the rates should be constant during the measurement period. Line 147: Determination of total soil P (and total soil Pi) by extraction of ignited and non-ignited soils with 0.5 M H2SO4 is not valid for highly weathered tropical soils (see Condron, L.M., Moir, J.O., Tiessen, H., Stewart, J.W.B., 1990. Critical evaluation of methods for determining total organic phosphorus in tropical soils. Soil Science Society of America Journal 54, 1261-1266.) Line 153 onwards: in my

experience, autoclaving is very effective to sterilize soils. But re-colonization has to be avoided by all means, since there is ample substrate available and no competition. To me, it is not entirely clear which measures were taken to keep abiotic controls sterile throughout the experiment. Line 211: "the procedure as outlined above": please avoid these unclear references – above I see a long description of the isobutanol procedure. Is this what you mean? Line 213: how can you do time point 0 h? Line 217: why do you use different volumes of 0.5 M NaHCO3 for different soils? Line 218: there is a problem with this sequential extraction, as you state yourself later in the manuscript: a second extraction in the absence of chloroform would release P... It is okay to test things during method development, but then you need to describe the recommended procedure at the end of the manuscript – if you have tested the method thoroughly and ideally compared it to existing protocols. Line 221: it would be better to detail the calculations that were made. Line 230: abiotic immobilization – at which time? I suppose you mean at a given time point, but it would help the reader that you write this (or give a proper equation). Line 234: ok, this is a rapid assessment, but is it precise? Is abiotic immobilization not affected by autoclaving? Line 241: "had to be corrected" – are you still referring to previous work, or do you want to announce that gross rates ... have to be corrected for... ? Line 243: you need to state here that this calculation occurs under the assumption that abiotic influx is unaffected by autoclaving, i.e. abiotic influx in sterilized and live soils is similar. Line 289: an increase in available Pi by 1.6-1.9 will affect tracer behavior. E.g. Table 3 and Table 5 in Bünemann et al. 2007 give IEK values indicative of fast and slow Pi sorption as well as concentration of Pi in soil solution and E1min as an estimate of isotopically exchangeable = available P. Importantly, these autoclaved soils were incubated for several weeks before doing these assessment, thus, the first P flush after autoclaving has partly been resorbed by the soil. At the very least, you need to state clearly for how long autoclaved soils were kept until the experiments were performed, or if you once checked for sterility at the end of the experiments. Line 295-308: please state if this was done in live soils only, or if abiotic controls were included. line 312: Please state the number of replicates from which the detection limit

was calculated. Line 334-336: it would be useful to refer to Fig. 4 already after this sentence. Line 339-343: these observations are completely in line with isotope dilution theory (e.g. Fardeau, Frossard). Line 363: it would be very interesting to compare this net 33 P immobilization by microbes with calculations based on fumigation-extraction techniques of 33P-labeled soils. Line 371-375: how is this possible? I think you need to word more carefully here, since you are presenting results of an approach (sequential extraction) which you later state is probably not useful. Line 416: please indicate where Bünemann, 2015 suggested that the IPD approach necessitates constant pool sizes (see end of section 2.6 reading "the (erroneous) assumption of a constant pool Q in the Di approach is problematic") Line 425-428: I don't think the result was that clear with respect to E values. Line 469-472: differences between field replicates have completely different underlying reasons such as gradients or heterogeneity in the field, or fertilization effects, and thus this argumentation is not helping at all here. Line 473: how do you account for it if you calculate a difference between abiotic and live soils? Line 488: depends on what you mean by "closely"... Line 500: I am not sure what you mean with "is included with water-extractable P", at least in Bünemann et al. 2007 33P activity in microbial biomass was determined by difference between fumigated and non-fumigated samples. Line 610: yes, I would have expected to find this in the present manuscript. Consider maybe adding small amounts of organic P substrates that are known to mineralize quickly in soils and assess the rate changes using either method. Line 617: do you have evidence for organic P availability? I did not see it... Line 655: can gross Pi sorption rates exceed gross Pi desorption rates 2-fold without a significant change in pool sizes? Did you see the intermediate draw down of available Pi mentioned in line 658? Line 669-670: this well known and not a new finding (as you make it sound). Table 1 column IEX/ID: measure P pool: water-extractable Pi, sometimes including microbial P – yes, true, but microbial P is not determined for calculation of gross Po mineralization Table 2: why are the soils not named 1-6, or which soil is soil 1? Please state methods for total organic P and soil Pi in a footnote under the table. Figure 3: can you indicated temperate and tropical soils in a and b as well? Figure

4c: how can the specific activity be greater in live than in sterile soils? Figure 5: which temperate and which tropical soil were used here?; add “measured in sterile soils” after (sorption, A, C) and “measured in live soils” after (microbial uptake, B, D). Figure 7: explain the symbols (for tropical and temperate soils) in a legend or in the caption.

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