PNAS LETTER

Reply to Chen et al.: Soil organic carbon stocks and persistence of surface 30 cm of Mollisols

Yichao Rui[a,b,1], Randall D. Jackson[c], M. Francesca Cotrufo[d], Gregg R. Sanford[c], Brian J. Spiesman[e], Leonardo Deiss[f], Steven W. Culman[f], Chao Liang[g], and Matthew D. Ruark[a]

Chen et al. (1) provide valuable feedback on our paper (2), suggesting that our work overestimated soil organic carbon (SOC) benefits of certain systems because we lacked data below 30-cm soil depth. In our paper, we acknowledged the uncertainties associated with deep soil C stocks, making no claim that our observations applied to deeper soils. On the second page of our paper we stated "although trends at deeper depths might differ from the surface layers." While we investigated the mechanisms associated with SOC changes in the surface 30 cm, future studies should explore the potential for SOC change under alternative management in deeper soil layers.

Chen et al. (1) argue that cropping systems only significantly affected SOC and mineral-associated organic matter (MAOM)-C stocks at 0- to 15-cm but not at 15- to 30-cm depth. Our results showed that when these two depth increments were considered separately with ANOVA, the main treatment effects of cropping systems on SOC and MAOM-C stocks were significant ($P < 0.001$ and $P = 0.013$ for SOC and MAOM-C, respectively), while the interaction between cropping systems and depth (system × depth) were not ($P = 0.80$ and $P = 0.26$ for SOC and MAOM-C, respectively). Therefore, our main treatment effects represented the overall effects of cropping systems on SOC and MAOM-C stocks across these two depths. Similar trends were found when SOC and MAOM-C concentrations were analyzed with ANOVA, suggesting that cropping systems had significant effects across two depths, but with no system × depth interaction (table S2 of ref. 2). We acknowledged that results may vary with deeper soils.

Chen et al. (1) argue that "SOC stock is positively correlated with the content of amino sugars solely in the topsoil, but it is negatively correlated with *N*-acetyl-β-D-glucosaminidase and polyphenol oxidase activities in the subsoil." Table S5 of ref. 2 showed the correlations between MAOM-C concentrations, not SOC stock, with soil microbial attributes. We believe using SOC or MAOM-C stock can help with soil C accounting purposes, but to unravel mechanistic relationships with soil microbial attributes it would be better to use SOC or MAOM-C concentrations. In addition, rather than exploring simple correlations among the soil variables, we conducted piecewise structural equation modeling to examine the direct and indirect relationships between soil C (particulate organic matter C and MAOM-C) and soil microbial traits. We believe this approach provided a mechanistic framework that incorporated microbial attributes into the understanding of management effects on soil C stocks and persistence.

Chen et al. (1) suggest that uncertainties in the initial SOC among treatments undermine our analyses. We clearly described this experiment as a randomized complete block design with initial SOC levels and 20-y SOC analysis reported by Sanford et al. (3). This was also acknowledged in a PNAS commentary published by Amundson (4): "The Pandora's Box of Soil Carbon." All background information is publicly available.

Author affiliations: [a]Department of Soil Science, University of Wisconsin–Madison, Madison, WI 53706; [b]Research Department, Rodale Institute, Kutztown, PA 19530; [c]Department of Agronomy, University of Wisconsin–Madison, Madison, WI 53706; [d]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523; [e]Department of Entomology, Kansas State University, Manhattan, KS 66506; [f]School of Environment and Natural Resources, The Ohio State University, Wooster, OH 44691; and [g]Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

Author contributions: Y.R., R.D.J., M.F.C., G.R.S., B.J.S., L.D., S.W.C., C.L., and M.D.R. wrote the paper.

The authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: yichaorui@gmail.com.

Published June 23, 2022.

1. J. Chen, Y. Luo, T. Kätterer, J. E. Olesen, Depth-dependent responses of soil organic carbon stock under annual and perennial cropping systems. *Proc. Natl. Acad. Sci. U.S.A.* **119**, 10.1073/pnas.2203486119 (2022).
2. Y. Rui *et al.*, Persistent soil carbon enhanced in Mollisols by well-managed grasslands but not annual grain or dairy forage cropping systems. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2118931119 (2022).
3. G. R. Sanford *et al.*, Soil carbon lost from Mollisols of the North Central U.S.A. with 20 years of agricultural best management practices. *Agric. Ecosyst. Environ.* **162**, 68–76 (2012).
4. R. Amundson, The Pandora's box of soil carbon. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2201077119 (2022).