Rui et al. (1) reported that perennial pasture with rotational grazing significantly increased soil organic carbon (SOC) stock at 0 to 30 cm by 15 to 28% compared to conventional continuous annual cropping systems in a 29-y field experiment in the north-central United States. They applaud their efforts in managing such a valuable long-term experiment as well as revealing the intriguing potentials to enhance SOC stock by promoting land conversion from annuals to perennials with optimized management. However, we think that additional efforts should be taken to interpret their results soundly.

A reanalysis of their data shows that cropping systems significantly affected SOC and mineral-associated organic matter (MAOM)-C stocks in the topsoil (0 to 15 cm) but not in the subsoil (15 to 30 cm; Fig. 1). Thus, the increased SOC stock under the pasture and the associated contribution from MAOM-C in Rui et al. (1) could be detected only for the topsoil, where plant C inputs are the highest.

Moreover, 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 (Fig. 2). Altogether, our results highlight that not only SOC stock changes but also the underlying mechanisms may differ between the topsoil and the subsoil. Therefore, processes affecting the buildup of SOC may differ greatly with soil depth.

Indeed, depth-dependent responses of SOC stock to land use and management have been reported (2, 3). Based on 19-y experimental observations in California, Tautges et al. (4) showed that addition of winter cover crops to a conventionally managed system increased SOC stock by 4% at 0 to 30 cm but decreased it by 11% at 30 to 200 cm, resulting in net losses of SOC stock at 0- to 200-cm depth. Thus, Rui et al. (1) may overestimate the SOC stock benefits when they merely reported results at 0- to 30-cm depth.

Apart from the depth-dependent response, there are four other uncertainties. First, the lack of measurements at the start of the experiment may induce some biases even in replicated trials (5). For example, increases in SOC stock are larger when the initial SOC is lower (6). Second, decades or even longer periods are required to document significant changes in SOC stock after land conversions (6, 7). Therefore, the historical patterns may contain equally important information as the latest observations. Third, equivalent mass SOC stock rather than SOC stock at fixed depth should be reported considering land conversions (5). Finally, there are some intrinsic challenges when integrating long-term observations with a single measurement of soil microbial variables (8), especially when considering the diurnal and seasonal variations of the studied microbial variables.

We compliment Rui et al. (1) for presenting results from the long-term experiment that provides valuable insights on SOC dynamics, particularly related to novel microbial and enzyme mechanisms (9, 10). Such insights offer the...
basis for developing climate-smart agriculture, and we concur with the authors on the needs for unraveling the underlying mechanisms associated with enhanced SOC accumulation in perennial pastures.

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1. 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).
2. J. Balesdent et al., Atmosphere-soil carbon transfer as a function of soil depth. Nature 559, 599–602 (2018).
3. M. W. I. Schmidt et al., Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56 (2011).
4. N. E. Tautges et al., Deep soil inventories reveal that impacts of cover crops and compost on soil carbon sequestration differ in surface and subsurface soils. Glob. Change Biol. 25, 3753–3766 (2019).
5. C. Poeplau, M. A. Bolinder, T. Katterer, Towards an unbiased method for quantifying treatment effects on soil carbon in long-term experiments considering initial within-field variation. Geoderma 267, 41–47 (2016).
6. P. Poulton, J. Johnston, A. MacDonald, R. White, D. Powlson, Major limitations to achieving “4 per 1000” increases in soil organic carbon stock in temperate regions: Evidence from long-term experiments at Rothamsted Research, United Kingdom. Glob. Change Biol. 24, 2563-2584 (2018).
7. P. Smith et al., How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. Glob. Change Biol. 26, 219-241 (2020).
8. J. Chen, R. L. Simsabaugh, Linking microbial functional gene abundance and soil extracellular enzyme activity: Implications for soil carbon dynamics. Glob. Change Biol. 27, 1322-1325 (2021).
9. J. Chen et al., A key enzyme microbial enzyme for nitrogen control of soil carbon storage. Sci. Adv. 4, eaq1689 (2018).
10. J. Chen et al., Soil carbon loss with warming: New evidence from carbon-degrading enzymes. Glob. Change Biol 26, 1944-1952 (2020).

Fig. 2. Correlations between land conversion-induced changes of the studied variables. POM-C, particulate-associated organic carbon. MAOM-C, mineral-associated organic carbon. MBC, microbial biomass carbon. CUE, microbial carbon use efficiency. AS, amino sugars. BG, β-glucosidase. AG, α-glucosidase. CBH, β-cellobiohydrolase. NAG, N-acetyl-β-glucosaminidase. PPO, polyphenol oxidase. PER, peroxidase. Significant differences were evaluated at ***p < 0.001, **p < 0.01, and *p < 0.05.