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# Diversified cropping systems with limited carbon accrual but increased nitrogen supply

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Diversified cropping systems offer a chance to mitigate environmental impacts of conventional agriculture, but effects on soil organic carbon (SOC) sequestration and nitrogen (N) dynamics remain debated. We integrated a 20-year field experiment and laboratory measurements with three stable-isotope-enabled mechanistic models to examine SOC stocks and decomposition in a conventional corn–soybean system and two more diversified systems including small grains, legumes and manure inputs, in addition to corn and soybean. Contrary to the prevalent hypothesis that diversified systems increase SOC, we found no differences in 0.3 m topsoil or 1 m profile SOC and N stocks. Diversified systems markedly increased N mineralization rates and decomposition of older SOC from previous corn inputs. Models revealed that increased C decomposition with residence times of months to years counteracted higher C inputs but increased N supply. Our findings highlight a critical trade-off between C storage and N supply in these diversified systems, demonstrating that key climate benefits may arise from decreased N fertilizer use, not SOC sequestration.

Agricultural practices thought to increase soil organic carbon (SOC) have been widely promoted to mitigate greenhouse gas emissions and regenerate soil health[1–3]. Diversified cropping systems that increase the number and functional diversity of crop species in rotation offer opportunities to reduce multiple environmental impacts while sustaining food production[4,5]. In particular, by reducing fallow periods with perennial crops, integrating legumes and forages, and adding manure from integrated crop–livestock production, diversified systems may decrease reliance on synthetic nitrogen (N) fertilizer inputs. These practices can decrease N loss as nitrate, a major water pollutant, and nitrous oxide, a potent greenhouse gas[5–7]. In addition to well-documented benefits for soil health and nutrient availability, diversified cropping systems may also increase SOC due to greater plant carbon (C) inputs, especially from roots[8–10]. Accordingly, a widely used protocol for voluntary C offset programmes lists "increased diversity of plant species cultivated in regular cycles" as a beneficial practice for SOC storage projects[11]. Nevertheless, increases in SOC storage and associated impacts on soil fertility in diversified versus conventional cropping systems remain contentious.

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For example, a recent global synthesis showed that including perennial crops in rotations may increase SOC in topsoil (<20 cm) but not subsoil[12], whereas other experiments showed that diversified crop rotations had inconsistent or no impacts on SOC in topsoil (<30 cm)[13,14] or to 90 cm depth[15]. Although nearly half of SOC may be stored below 30 cm (ref. 16), few agricultural studies measured the top metre[12], casting doubt on broad conclusions about impacts of crop diversification on SOC. On one hand, frequent incorporation of legumes, manure inputs and greater root production may increase SOC[17–19]. On the other hand, roots may also accelerate SOC decomposition of previously protected C, causing SOC loss[20]. One study[13] suggested that despite greater C inputs, increased decomposition could explain a lack of SOC change in diversified versus conventional cropping systems, but this hypothesis could not be directly tested in that study and remains unresolved.

Moreover, less work has examined the long-term impacts of diversified cropping systems on N alongside SOC, despite their close coupling due to the fact that most soil N is stored with C in organic matter[21]. Diversified cropping systems can increase total N (TN)[22] and/or N cycling rates[23,24]. Increased N cycling may result from increased organic matter decomposition, leading to a trade-off between N availability and SOC storage[25]. Therefore, even without SOC accrual, increases in decomposition could potentially increase crop N availability and environmental sustainability by decreasing N fertilizer requirements.

Understanding mechanisms that may alter agricultural C and N cycling following management changes remains a critical challenge. Mechanistic ecosystem models play an important role in bridging theory and measurements[26], and are increasingly used to support greenhouse gas emissions inventories, government policy incentives and private-sector ecosystem services markets. Prevailing soil C models mainly include conceptual pools where C is decomposed and transferred among pools according to first-order kinetics, and may also include explicit representation of microbial- and enzyme-mediated decomposition[27]. However, the challenge of directly validating model predictions against empirical measurements presents a key barrier to model improvement and confidence for upscaling[28]. Models may be highly sensitive to specific parameters, possibly leading to notable over- or under-estimation of management impacts on SOC sequestration[29]. For example, without parameterization the DeNitrification-DeComposition (DNDC) and Rothamsted Carbon (RothC) models overestimated surface SOC storage after fertilizer management changes[30]. We thus need rigorous model–data comparisons to evaluate model structure and parameterization.

C stable isotopes ($\delta^{13}C$) provide an additional but underused empirical constraint for process-based models. Stable isotopes are natural tracers for C cycling processes that yield information on sources of C pools and fluxes[31]. Cropping systems that alternate between $C_4$ plants (for example, corn) and $C_3$ plants (for example, small grains and legumes) are valuable for $\delta^{13}C$ measurements and modelling. This is because the two types of plants have notably different $\delta^{13}C$ values—around −12‰ for $C_4$ plants and −26‰ for $C_3$ plants—due to the way they process C during photosynthesis[32]. However, natural abundance $\delta^{13}C$ has rarely been used for model–data integration and assessment in agriculture[33].

Here we integrate field and lab incubation measurements with three soil biogeochemistry models with different structures and mechanistic complexities to assess benefits and possible trade-offs in soil C and N cycling in diversified and conventional cropping systems, and mechanisms driving observed changes. We leverage a long-term (20-year) Iowa, USA, field experiment comparing a conventional, 2-year corn–soybean rotation with 3- and 4-year extended rotations (corn–soybean–small grain + red clover, or corn–soybean–small grain + alfalfa–alfalfa; Supplementary Fig. 1) that also received manure inputs, where each phase of the three rotations was grown during each year in replicated plots. We quantify SOC and TN stocks to 1 m and incubate additional intact 1 m soil cores in the lab to measure soil carbon dioxide ($CO_2$) fluxes, their $\delta^{13}C$ values and net N mineralization. The lab measurements are integrated with three C isotope-enabled models to test underlying mechanisms by which diversified cropping systems might alter decomposition, and to assess the response of C pools with different residence times (that is, active versus passive C, particulate organic C versus mineral-associated organic C). Models include the Agricultural version of the Integrated Biosphere Simulator (Agro-IBIS) soil biogeochemistry submodule, the organic C decomposition module (CN-SIM) from the Dynamic Land Ecosystem Model (DLEM) and

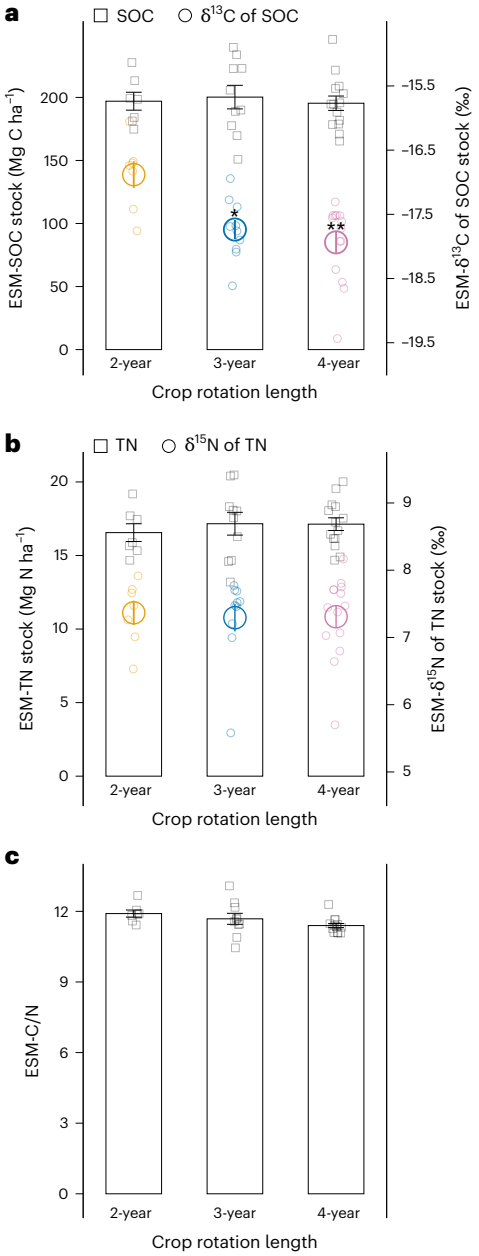


**Fig. 1 | SOC, TN and C/N among cropping systems. a–c**, ESM-based SOC and its weighted C isotope value (circles) (**a**), ESM-based TN and its weighted N isotope value (circles) (**b**), and ESM-based SOC/TN ratios (**c**). 2-year, 2-year corn–soybean rotation; 3-year, 3-year corn–soybean–small grain/red clover rotation; 4 year, 4-year corn–soybean–small grain/alfalfa–alfalfa rotation. The values were calculated by ESM approach, which used 0–1 m soil mass at 2-year rotation as a reference. The error bars indicate standard errors ($n$ = 8, 12 and 16 for 2-, 3- and 4-year rotations, respectively), with the centre denoting the mean value of the replicates. *$P$ = 0.011, **$P$ = 0.007 in one-way ANOVA followed by Dunnett's test. The circle within the bar plot represents isotopic values: yellow for 2-year, blue for 3-year and pink for 4-year rotations.

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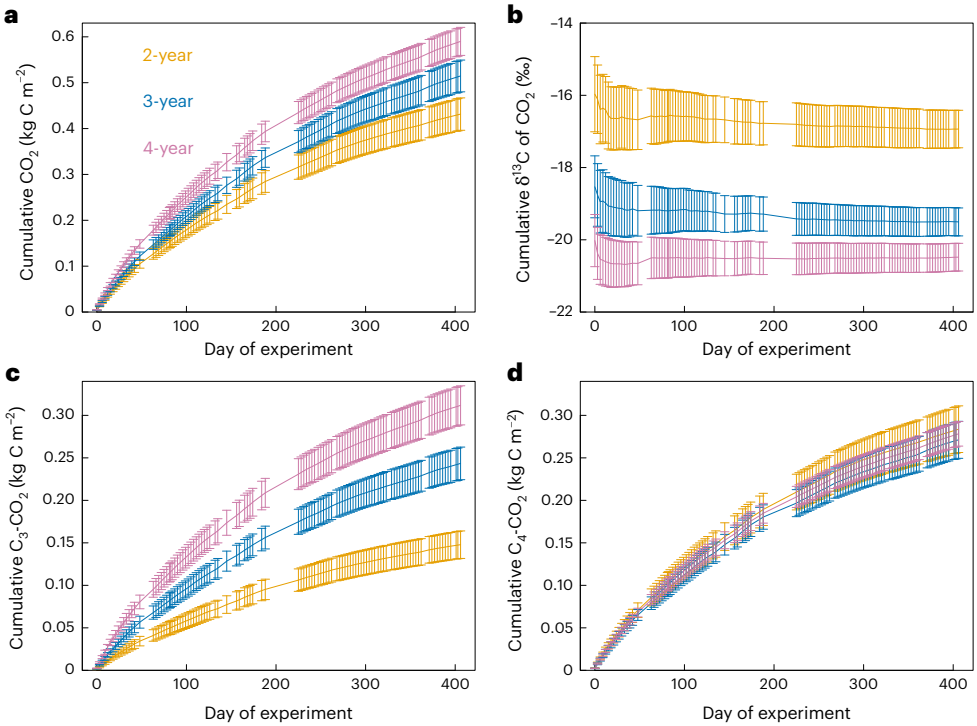


**Fig. 2 | Soil C decomposition among cropping systems.** Lab observations of cumulative C decomposition from soil cores (1 m depth) collected from a 2-year corn–soybean rotation (2-year), a 3-year corn–soybean–small grain/red clover rotation (3-year) and a 4-year corn–soybean–small grain/alfalfa–alfalfa rotation (4-year). **a**,**b**, Cumulative $CO_2$ production (**a**) and cumulative $\delta^{13}C$ of $CO_2$ production (**b**). **c**,**d**, Cumulative decomposition of $C_3$-derived C (**c**) and $C_4$-derived C (**d**) respired as $CO_2$. The error bars indicate standard errors of the cumulative values from replicates at each measurement date ($n = 8$, 12 and 16 for 2-, 3- and 4-year rotations, respectively), with the centre denoting the mean value of the replicates.

the Microbial-ENzyme Decomposition (MEND) model; each respective model includes progressively greater mechanistic complexity of microbial processes (Supplementary Fig. 2 and Supplementary Table 1). We hypothesize that (1) the diversified cropping systems would not increase the SOC stock versus conventional cropping systems due to enhanced organic matter decomposition; (2) the diversified cropping systems would increase the decomposition of older organic matter pools; and (3) the diversified cropping systems would promote soil N supply via accelerated net N mineralization.

## Results

### Observed SOC and N stocks

SOC and TN stocks calculated by equivalent soil mass (ESM), using 0–1 m mineral soil mass in the 2-year rotation as a reference, were similar among 2-, 3- and 4-year rotations, whereas the $\delta^{13}C$ values of SOC and soil C/N decreased with rotation length. The SOC and TN stocks, averaged across the three different rotations, were 197 ± 4 Mg C $ha^{-1}$ (mean ± s.e.) and 17.0 ± 0.3 Mg N $ha^{-1}$ at 0–1 m depth (Fig. 1), and 119 ± 3 Mg C $ha^{-1}$ and 10.2 ± 0.2 Mg N $ha^{-1}$ at 0–0.3 m depth, respectively (Supplementary Fig. 3). However, the $\delta^{13}C$ of SOC significantly decreased from −16.9 ± 0.2‰ for 2-year to −17.7 ± 0.1‰ and −17.9 ± 0.2‰ for 3-year and 4-year rotations ($P < 0.01$ for simple linear regression), and soil C/N also decreased from 11.9 ± 0.2 to 11.7 ± 0.2 and 11.4 ± 0.1 for the 3- and 4-year rotations, respectively ($P < 0.05$ for simple linear regression; Fig. 1). A similar pattern was observed for 0–0.3 m soils (Supplementary Fig. 3).

### Soil C decomposition and net N mineralization

A 13.5-month lab incubation of intact soil cores showed that longer rotations increased cumulative $CO_2$ production, with higher values in the 4-year (0.59 ± 0.03 kg C $m^{-2}$) than the 2-year rotation (0.43 ± 0.04 kg C $m^{-2}$, $P < 0.01$; Fig. 2a) and intermediate values in the 3-year rotation (0.51 ± 0.03 kg C $m^{-2}$). Cumulative $\delta^{13}C$ values of $CO_2$ decreased with rotation length (−16.9‰, −19.5‰ and −20.5‰ in the 2-, 3- and 4-year rotations, respectively; $P < 0.01$; Fig. 2b). We quantified the source ($C_3$- versus $C_4$-derived C) of decomposition using a two-source mixing model. Longer rotations had greater decomposition of $C_3$ plants (0.24 ± 0.01 and 0.31 ± 0.01 kg C $m^{-2}$ over 13.5 months in the 3- and 4-year rotations, respectively) than the 2-year rotation (0.15 ± 0.00 kg C $m^{-2}$; $P < 0.05$ for 3-year and $P < 0.01$ for 4-year), as expected given that $C_3$ crops were grown more frequently, and that manure added to the diversified rotations was derived mainly from $C_3$ sources (−21.7 ± 0.1‰; Supplementary Table 2). Strikingly, all rotations had similar decomposition of $C_4$ plants (0.28 ± 0.01 kg C $m^{-2}$; Fig. 2c,d), even though diversified rotations had lower inputs of $C_4$ plants because corn was grown less frequently (once every 3 or 4 years, rather than every other year), even after accounting for manure addition (Supplementary Table 2). We found similar patterns in magnitude of $C_3$-derived versus $C_4$-derived $CO_2$ when comparing only the corn phase among the rotations (Supplementary Fig. 4). Thus, $\delta^{13}C$ and $CO_2$ flux data from the laboratory incubation demonstrated that the diversified rotations altered the source of decomposition relative to the conventional rotation.

Similar to $CO_2$, net N mineralization to 1 m depth increased with rotation length (Fig. 3; $P < 0.01$) after 13.5 months of incubation. Net N mineralization increased by 69% and 71% in the 3- and 4-year rotations, respectively, compared with the 2-year rotation (210 kg N $ha^{-1}$). Longer rotations also increased net N mineralization ($P < 0.01$) when comparing results from the corn phase of each rotation (Supplementary Fig. 5).

### Cropping system effects on calibrated model parameters

We used the $CO_2$ flux and its $\delta^{13}C$ value from lab incubation for modelling and focused on simulations from the isotope-calibrated model driven by best-estimate initial C values. Additional scenarios and discussions of input uncertainty and parameterization strategies are provided in the Supplementary Information and Supplementary Table 3.

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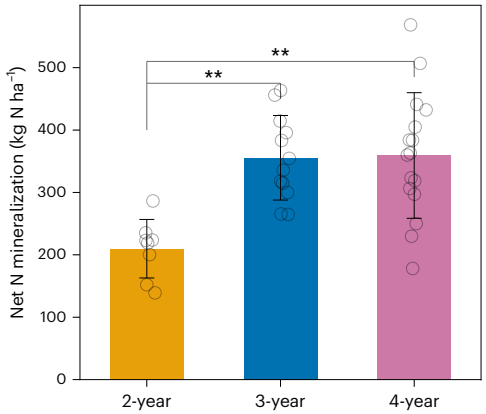


**Fig. 3 | Soil net N mineralization among cropping systems.** Lab observations of net N mineralization after a 13.5-month incubation from soil cores (1 m depth) collected from a 2-year corn–soybean rotation (2-year), a 3-year corn–soybean–small grain/red clover rotation (3-year) and a 4-year corn–soybean–small grain/alfalfa–alfalfa rotation (4-year). The error bars indicate standard errors ($n$ = 8, 12 and 16 for 2-, 3- and 4-year rotations, respectively), with the centre denoting the mean value of the replicates. **$P = 2.2 \times 10^{-5}$ and $5.9 \times 10^{-5}$ in one-way ANOVA followed by Dunnett's test for 2-year versus 3-year and 2-year versus 4-year comparisons, respectively.

All three models simulated soil C cycling using multiple conceptual pools with different turnover rates, including faster-, intermediate- and slower-cycling pools (Supplementary Table 1). We emphasize that the C pools and fluxes in the different models are not directly comparable but provide complementary insights into soil organic matter dynamics. Across models, longer rotations significantly ($P < 0.05$ for Agro-IBIS and CN-SIM) enhanced decomposition of C pools with residence times ranging from months to years (Supplementary Fig. 6), consistent with lab measurements of C decomposition. Specifically, compared with the 2-year rotation, longer rotations significantly increased the decay rate of non-protected organic C in Agro-IBIS ($k_{nb}$; $P < 0.05$) and the decay rate of particulate organic C in CN-SIM ($k_{poc}$; $P < 0.05$; Supplementary Fig. 6). In MEND, longer rotations tended to decrease intrinsic microbial C use efficiency ($Y_g$; $P = 0.08$ and $P < 0.05$ for the 3- and 4-year rotations, respectively; Supplementary Fig. 6), thereby increasing $CO_2$ release. Parameter changes during the corn phase of each rotation generally aligned with trends in all phases, although a few parameters, such as $V_g$ (maximum specific microbial growth rate) in MEND, exhibited smaller changes (Supplementary Fig. 7a).

### Simulated C fluxes from individual C pools

Corresponding to model parameter changes, cumulative $CO_2$ production from fast-cycling C pools was significantly affected by rotation length (Fig. 4) in model simulations. In Agro-IBIS, longer rotations (3-year and 4-year) had higher cumulative $CO_2$ loss from non-protected active C ($P < 0.05$) than the 2-year rotation (Fig. 4). CN-SIM revealed that longer rotations increased $CO_2$ production from particulate C ($P < 0.05$). MEND identified that higher cumulative $CO_2$ production in longer rotations was mainly attributed to increased microbial growth and maintenance (microbial C, $P = 0.18$ and $P < 0.05$ for 3- and 4-year rotations; Fig. 4). Our findings were robust even considering uncertainties in model inputs (Supplementary Fig. 8) and when considering only the corn phases of each rotation (Supplementary Fig. 9).

### Model-simulated net N mineralization

Overall, calibrated Agro-IBIS and CN-SIM models simulated the observed increase in net N mineralization in the lab incubation in 3- and 4-year rotations compared with the 2-year rotation, even though no N data were used for calibration (Supplementary Fig. 10). Specifically, compared with the 2-year rotation, Agro-IBIS estimated an increase in net N mineralization by 70 and 90 kg N ha$^{-1}$ over the incubation period (13.5 months) for 3- and 4-year rotations, respectively (Supplementary Fig. 10), as compared with measured increases of 145 and 150 kg N ha$^{-1}$. CN-SIM estimated an increase in net N mineralization by 64 and 122 kg N ha$^{-1}$ for 3- and 4-year rotations, respectively (Supplementary Fig. 10). MEND was not used for N simulations because it required additional unmeasured parameters; the other models estimated N mineralization based on C fluxes and substrate C/N.

## Discussion

Our data–model integration revealed a trade-off between SOC accrual and mineral N supply in diversified cropping systems. Supporting our first hypothesis, field measurements showed that systems including small grains, additional legumes and manure imput did not increase the topsoil (0–0.3 m) or whole soil profile (0–1 m) SOC stock compared with a conventional corn–soybean rotation (Fig. 1). Increased C decomposition under these diversified cropping systems indicated by soil core incubation (Fig. 2) can explain the lack of SOC increase despite increased plant C inputs (Supplementary Table 2)[15]. Diversified rotations received lower C inputs from $C_4$ plants than the conventional rotation because corn was grown every third or fourth year rather than every other year, even considering $C_4$-derived C addition from manure. However, $C_4$-derived C loss was similar among all rotations (Fig. 2), indicating that the diversified rotations increased the decomposition of older $C_4$-derived C from previous inputs of $C_4$ plants. The model simulations similarly indicated that longer rotations increased C decomposition from some of the fast-cycling C pools with residence time of months to years (Fig. 4). The differential impacts on C pools by diversified cropping systems led to shifts towards relatively more $C_3$-derived and N-rich organic matter than the conventional cropping system, as reflected by the weighted $\delta^{13}C$ of SOC stock and C/N ratio. Diversified cropping systems did not accrue more SOC than the conventional cropping system, and they significantly increased N availability due to stimulated decomposition (Fig. 3), consistent with our third hypothesis.

### Diversified cropping systems stimulated SOC decomposition

Synthesis studies have suggested that diversified cropping systems, a key component of regenerative agriculture, may enhance SOC accrual through manure application instead of synthetic fertilizers[34] and increased belowground C inputs[9,12]. However, we found that manure input and increased root production at our site[15,17] did not lead to higher SOC because of simultaneous increases in decomposition. Model simulation results offer insights into potential mechanisms underlying increased C decomposition, which was mainly attributed to relatively fast-cycling pools with residence times of months to years.

Several factors associated with root biomass probably account for altered patterns of C decomposition and loss. First, greater root biomass with a lower C/N ratio in the diversified rotations (Supplementary Information) stimulated C decomposition from fast-cycling C pools, which are thought to mostly represent new crop residue and manure inputs that are easily accessible to microorganisms (that is, non-protected active C pool in Agro-IBIS, particulate C in CN-SIM and microbial C in MEND; Fig. 4). Moreover, greater root biomass and/or exudate production in diversified rotations can also liberate older organic compounds associated with minerals[35]. The sustained decomposition of corn-derived C from previous phases of the diversified crop rotations (months to several years old) may explain our observation of comparable $C_4$-derived $CO_2$ loss in the diversified rotations despite lower $C_4$-derived C inputs and the concomitant increase in decomposition of C pools with ages of months to years observed across the various models. Together, diversified cropping systems had distinct impacts on various C pools but resulted in an overall increase in C decomposition to offset the higher plant C inputs from both belowground root biomass and manure.

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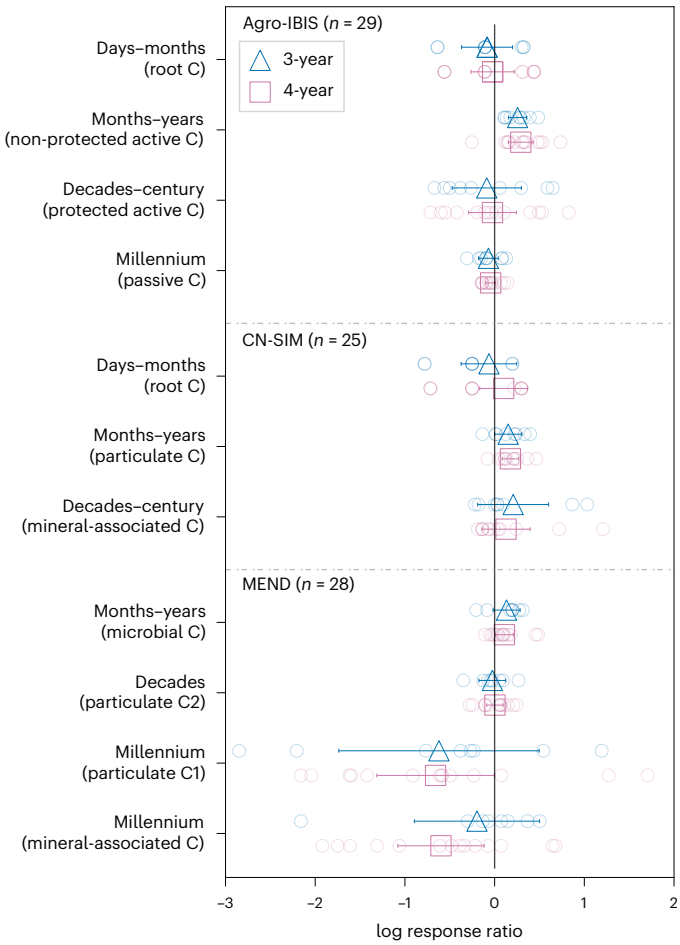


**Fig. 4 | Simulated C fluxes from different C pools among cropping systems.** Effects of the diversified cropping systems (3-year and 4-year crop rotations) on decomposition rates of different C pools relative to a conventional corn–soybean rotation (2-year rotation), as simulated using three mechanistic models calibrated with laboratory $CO_2$ fluxes and $\delta^{13}C$ of $CO_2$. The log response ratio is the natural logarithm of the value in the 3- or 4-year rotations divided by the value in the 2-year rotation. Simulation results were obtained using the best-estimate values for initial C pools based on previous measurements. The scenario with plausible lower-bound values for initial C pools is represented in the Supplementary Information. The error bars represent 95% confidence intervals. Dark symbols are the mean log response ratios, and partially transparent points show the individual values for each replicate plot. The *n* values ($n = 29$, 25 and 28 for the Agro-IBIS, CN-SIM and MEND, respectively) listed after the name of the model in each panel represent the numbers of plots that achieved satisfactory performance (determination $R^2 > 0.6$ for $CO_2$ and RMSE < 2‰ for the $\delta^{13}C$ value) in model simulation. The *y* axis represents the residence time of C pools in different models, with the pool names as defined by the original model developers listed in brackets.

The Marsden diversification experiment was conducted on a C-rich Mollisol, and whether we would expect similar findings (that is, increased C inputs balanced by increased C decomposition) on soils with lower SOC remains unclear. However, our results are consistent with another long-term cropping systems diversification study (the Wisconsin Integrated Cropping Systems Trial) conducted on a Mollisol with approximately 30% lower 0–30 cm SOC stock than measured at our site[13]. At that site, SOC did not differ among diversified crop–livestock systems with legumes and manure fertility and conventional grain cropping systems[36]. Nevertheless, the role of soil properties in shaping the response of SOC to changes in C inputs remains a pressing research question.

## Diversified cropping systems increased net N mineralization

Despite no SOC gain, these diversified cropping systems increased crop N supply by accelerating net N mineralization (Fig. 3), consistent with our third hypothesis. Manure application and/or legume incorporation are known to increase N mineralization[37], resulting from high N content in manure, legume N fixation and lower residue C/N ratio[38,39]. Longer rotations had significantly greater net N mineralization rates (Fig. 3) without an increase in total soil N (Fig. 1). The similarity in soil N stocks among the three cropping systems indicated that legume and manure N sources in the diversified rotations compensated for decreased synthetic N fertilizer inputs, sustaining high crop productivity[40]. This interpretation was further supported by partial N balances which suggested that N inputs were approximately balanced by N removal in harvested crops for all three rotations, acknowledging large uncertainty in these estimates (Supplementary Table 4). Given that total soil N did not increase, observed increases in net N mineralization cannot be due to increased substrate availability (that is, organic N). Instead, we interpreted this finding as a logical consequence of our first hypothesis and tight coupling of C and N cycling[41]: increased plant C inputs stimulated microorganisms and accelerated the depolymerization of organic matter to release N, thus promoting net N mineralization; that is, higher plant N demand in the diversified cropping systems was sustained by increasing soil organic matter decomposition.

Our results coincide with prior work conducted at our study site that found an increase in native protease activity, a pivotal factor influencing supply of plant-available N from soil organic matter[23]. Higher synthetic fertilizer usage inhibits internal soil organic N cycling, probably by disincentivizing microbial processing of proteins, oligopeptides and amino acids to obtain bioavailable N from organic sources, resulting in diminished rates of depolymerization[42,43]. Removing this constraint on microbial N mining may contribute to the effectiveness of substituting synthetic N fertilizers with composted manure, which may decompose rapidly in diversified systems with high microbial biomass.

Overall, our measurements and model simulations indicate the importance of increased N supply capability from diversified cropping systems (Fig. 4 and Supplementary Fig. 5). Model simulations consistently reproduced observed trends in net N mineralization among rotations (Supplementary Fig. 10), although modelled absolute values of net N mineralization rate were higher, probably resulting from the absence of gaseous N loss processes in these models as implemented in our study. Accelerated decomposition of N-rich organic matter under diversified cropping systems, as indicated by our lab incubation and model simulations, may have greatly contributed to the enhanced net N mineralization. Together, our results suggest a mechanism whereby diversified cropping systems could facilitate plant N acquisition by releasing N from decomposition of fast-cycling organic matter pools with residence times of months to years.

## Implications for agricultural sustainability

Other indicators besides SOC, such as soil N supply and non-$CO_2$ greenhouse gases, should be considered when evaluating agroecosystem climate impacts. Environmental benefits of reduced N fertilizer usage in diversified cropping systems are significant in many aspects. Our incubation experiment showed that incorporating small grains, clover or alfalfa, and manure into a corn–soybean rotation significantly increased N supply by >140 kg N $ha^{-1}$ during the 13.5-month incubation, explaining reduced need for synthetic N application (171, 60 and 52 kg N $ha^{-1}$ applied in 2-, 3- and 4-year rotations, respectively) during the corn phase. We hypothesize that the greatest climate benefits from diversified cropping systems may result from a decrease in $N_2O$ emissions following decreased synthetic N inputs[44]. Using the Intergovernmental Panel on Climate Change (2019) emission factors of 1.6% for N fertilizer and 0.6% for manure N emitted as $N_2O$ (tier 1 in humid climates)[45], we estimated ~60–70% reduction in $CO_2$-equivalent greenhouse gas emissions of $N_2O$ in the diversified cropping systems,

calculated using a global warming potential of 265 over a 100-year timescale (Supplementary Table 5). These estimates are consistent with model estimations reported by another study[44] for the same site. These values, however, are probably conservative, given that measured $N_2O$ emissions factors for our study region can be much higher than these default values[46]. We cannot clearly separate the roles of small grains, legumes and manure in driving increased N turnover in these integrated crop–livestock systems. However, other work aligns with our findings, showing that diversified systems without manure can also decrease N fertilizer requirements[7].

### Implications for C markets

Our study has several implications to inform C markets. First, we need to better consider the N aspect in C market accounting. Our study revealed that increasing crop diversity within conventional rotation systems was insufficient for SOC sequestration but yielded substantial benefits in N supply, leading to lower synthetic N fertilizer requirements[40] and possibly reducing $N_2O$ emissions. Second, mechanistic models (for example, DayCent) are currently used to estimate SOC gains and losses to inform C markets[47], and we found that applying existing models to new agricultural practices could lead to unreliable results when parameters were not recalibrated. The application of isotope-enabled models, calibrated with natural abundance C isotopic measurements, provided insights into the response of different C pools. We used $\delta^{13}C$ as an extra constraint to track the sources of emitted $CO_2$, particularly to differentiate $C_3$- or $C_4$-derived C in corn production phase (Supplementary Fig. 11), thereby reducing model uncertainty from parameter equifinality (equally good result with multiple parameter sets) and achieving more convergent distributions of decay parameters (Supplementary Fig. 12). Despite being more expensive than conventional elemental analysis, isotope ratio measurements are more sensitive and precise than absolute concentrations[48], and can record biological changes that may not be apparent in mass pools or fluxes alone[31]. Applying parameters from the conventional corn–soybean system to diversified systems greatly underestimated SOC loss (by 9–13% during the corn phase and 20–29% during the soybean phase over 13.5 months; Supplementary Fig. 13). Therefore, further tests of how different management practices influence model parameters appear necessary to effectively use models to inform C markets.

We integrated field and laboratory measurements from a 20-year experiment with mechanistic models to comprehensively study SOC and N stocks, C decomposition and N mineralization, and the response of different C sources/pools under different crop rotations. Field measurements demonstrated that diversified cropping systems (3- and 4-year rotations) did not increase SOC but maintained similar N stocks to a 2-year conventional corn–soybean rotation despite lower synthetic N fertilizer input. Notably, organic matter in diversified systems had a greater $C_3$ plant contribution, as indicated by the $\delta^{13}C$ values, and a lower C/N ratio. Despite limited accrual of SOC, diversified cropping systems significantly enhanced N availability due to stimulated decomposition from older $C_4$-derived organic C revealed by lab incubation and from fast-cycling soil C pools with residence times spanning months to years indicated by model simulations. Together, our findings suggested a trade-off between C accrual and N supply in diversified cropping systems and highlighted that their benefit for climate change mitigation probably results from decreased synthetic N fertilizer inputs and associated reductions in $N_2O$ emissions, but not from enhanced SOC sequestration.

## Methods

### Soil sampling

Soils were sampled in autumn 2021 from a long-term agroecosystem diversification experiment established in 2002 at Iowa State University's Marsden Farm near Boone, Iowa, USA (42° 01′ N, 93° 47′ W). During 2002–2021, the mean annual precipitation and temperature were 993 mm and 9.05 °C, respectively. Dominant soils include Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls), Nicollet loam (fine-loamy, mixed, superactive, mesic, Aquic Hapludolls) and Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquolls). The site had been managed for at least 20 years with a corn–soybean rotation receiving conventional fertilizer and herbicide inputs before the establishment of the experiment.

There were three different rotation systems: a 2-year corn–soybean rotation, a 3-year corn–soybean–small grain/red clover rotation and a 4-year corn–soybean–small grain/alfalfa–alfalfa rotation (Supplementary Fig. 1). All systems were managed with tillage. In the 3-year rotation, the small grain was triticale from 2003 to 2005 and oat after 2006, and red clover was used as a green manure (non-harvested); the system is hereafter denoted as the 3-year corn–soybean–small grain/red clover rotation. In the 4-year rotation, the small grain was the same as for the 3-year rotation and alfalfa was harvested for hay; the system is hereafter denoted as the 4-year corn–soybean–small grain/alfalfa–alfalfa rotation. Each crop phase of each rotation system was present every year in four replicate blocks, with a plot size of 18 m x 84 m. The 36 plots were arranged in a randomized complete block design. The 2-year corn–soybean rotation was representative of conventional cropping systems in the Midwest United States. The 3-year and 4-year extended rotations represent diversified farming systems integrated with animal production to partially replace synthetic N fertilizer (Supplementary Table 5 and Supplementary Fig. 1). Overall, N inputs remained consistent within rotations from 2005 to 2021. Over the period from 2005 to 2021, the 2-year rotation received an average annual rate of 106 kg N $ha^{-1}$ synthetic N before corn planting (pre-planting), whereas the 3- and 4-year rotations received no synthetic N fertilizer before corn planting. Synthetic N fertilizer application (side-dress) occurred when corn reached the V5/V6 stage, as guided by the late-spring soil nitrate test[49], with average rates of 65, 60 and 52 kg N $ha^{-1}$ for 2-, 3- and 4-year rotations, respectively. Composted cattle manure was applied in the previous autumn preceding corn production in the 3-year and 4-year rotations at an average rate of 105 kg N $ha^{-1}$ to replace pre-plant synthetic N application. More detailed information about site descriptions, crop yields, N fertilization and weed management are reported elsewhere[15,50].

Following harvest in 2021, we took four 3.8 cm diameter cores from four cardinal locations in each plot to a depth of 100 cm using a hydraulic soil probe. One intact soil core from each plot was randomly selected for lab incubation, as described below. The remaining three soil cores from each plot were divided into 0–15 cm, 15–30 cm, 30–60 cm and 60–100 cm depth increments, weighed, subsampled for moisture measurements at 105 °C for 24 h and then composited to form one sample per depth and per plot. Soil bulk density was determined by the dry weight and volume of each soil core after accounting for the presence of roots and stones (if any). The composite samples were air dried and passed through a 2 mm sieve, a subsample of which was finely ground for measurement of SOC, TN, $\delta^{13}C$ values and $\delta^{15}N$ values.

### Soil chemical analysis

Soil C and N and their $\delta^{13}C$ and $\delta^{15}N$ values were measured by an elemental analyser/continuous flow isotope ratio mass spectrometry at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley, California, USA. SOC was determined by subtracting carbonate C (Supplementary Information and Supplementary Table 6) from total soil C. The $\delta^{13}C$ value of SOC was calculated by a two-source mixing model as described in the Supplementary Information. We quantified SOC stock to 1 m by the ESM method as it can provide more reliable stock comparisons than the fixed depth (FD) approach[51–53]. We applied cubic spline interpolating functions and mineral soil masses to calculate ESM-based SOC stocks using the R script developed by ref. 51. The mineral soil mass of 0–1 m in the 2-year rotation was selected as the reference mass. For comparison, we also used the FD method

to calculate the SOC stock to 1 m by summing up the product of SOC concentration and bulk density for each depth increment (Supplementary Table 7). We found that the FD-based SOC stocks to 1 m tended to be lower than the ESM-based SOC stocks ($P = 0.08$ for the paired $t$-test across all the three rotations; Supplementary Fig. 14), primarily due to the 3-year rotation ($P = 0.08$); however, FD-based SOC stocks also remained unchanged across different rotations. To calculate the $\delta^{13}C$ value of SOC stock on an ESM basis, we first determined the $^{13}C$ stocks in each segment, then followed by the same procedure with ESM-based SOC stocks to calculate ESM-based $^{13}C$ stocks, and finally converted ESM-based $^{13}C$ stocks to ESM-based $\delta^{13}C$ values by reversing the equations used for calculating $^{13}C$ stocks[51]. We calculated ESM-based N stocks and their $\delta^{15}N$ values, similar to the calculation for ESM-based SOC stocks and $\delta^{13}C$ values.

Net N mineralization was measured by the differences in inorganic N (ammonium + nitrate) between the initial composited soil samples and the samples after incubation. The composited and air-dried soil subsamples were extracted with 2 M KCl in a 1:5 soil:solution mass ratio, shaken for 1 h and then centrifuged for 10 min at 10,000*g*. The extraction was measured by microplate colorimetry for ammonium ($NH_4^+$-N), and by second-derivative spectroscopy for nitrate ($NO_3^-$-N)[54]. The intact soil cores from the lab incubation (described below) were divided into the same soil depths and subsampled for measuring $NH_4^+$-N and $NO_3^-$-N using the same methods as described above. Net N mineralization was calculated as the difference in inorganic N before and after ~1 year incubation.

### Laboratory incubation

One intact soil core from each plot was incubated in the lab at constant moisture and temperature to monitor soil $CO_2$ production and its $\delta^{13}C$ value over 13.5 months (406 days). Soil moisture in each soil core was adjusted to field capacity by gradually adding water until extra water drained out of the core. The soil cores were sealed with plastic lids equipped with two stainless steel Swagelok fittings and tubing for air flushing and automated headspace gas sampling using a dynamic chamber approach[55]. Briefly, each soil core was flushed with air at a constant rate (60 ml $min^{-1}$), and an outlet from the core diverted gas to a tunable diode laser for measurement of $CO_2$ concentration and its $\delta^{13}C$ value when sampling and vented gas to the laboratory when not sampling, as described in ref. 55.

We measured soil respiration and its $\delta^{13}C$ value every 3 days on average. Soil respiration rate was determined as follows:

$$\text{Flux} = \frac{(C_o - C_i) \times \text{Flow}}{\text{Area}}$$

Here, $C_o$ and $C_i$ are the model fractions of $CO_2$ measured at the tube outlet and inlet, respectively. The inlet $CO_2$ was the equilibrated fresh air from the building ventilation system in two sequential 200 l containers, and was measured from blank cores (no soil present). Flow is the volumetric flow of air through the core. Area is the surface area of the soil core with a 3.8 cm diameter.

We used a two-source mixing model to determine the per cent contribution of $C_3$- and $C_4$-derived C to total soil respiration:

$$P_{C_3} = 100 \times \frac{\delta^{13}C_{\text{respiration}} - \delta^{13}C_{C_4}}{\delta^{13}C_{C_3} - \delta^{13}C_{C_4}}$$

$$P_{C_4} = 100 - P_{C_3}$$

Here, $P_{C_3}$ and $P_{C_4}$ are the per cent contribution of $C_3$- and $C_4$-derived C to total soil respiration, respectively, and $\delta^{13}C_{C_3}$ and $\delta^{13}C_{C_4}$ represent isotopic end-members for $C_3$ and $C_4$ biomass. We used −30‰ and −10‰ as the $C_3$ and $C_4$ end-member values, respectively, which are consistent with extreme observed end-members of $C_3$ and $C_4$ leaf issues[56], and encompass the majority of observed variation in the $\delta^{13}C$ of soil respiration. The fluxes of $C_3$- and $C_4$-derived respiration were calculated as the product of their respective percentage contribution and total soil respiration.

### Data–model assimilation

To examine underlying mechanisms explaining C mineralization under the different crop rotation systems, we integrated experimental data with three different mechanistic C models (Supplementary Fig. 2): the Agro-IBIS soil biogeochemistry submodule with a first-order kinetic scheme; the CN-SIM from the DLEM using Michaelis–Menten kinetics for $CO_2$ flux; and the MEND model with dependence of soil C fluxes on microbial and enzymatic physiology (Supplementary Table 1). Comparisons among models with different C pools and complexities enabled us to explore the consistent and/or complementary mechanisms contributing to the observed patterns of C mineralization under different rotation systems.

In brief, the first-order C model from the Agro-IBIS soil biogeochemistry subroutine was derived from the Verberne/MOTOR organic matter turnover model[57]. In this model, SOC consists of three major fractions (Supplementary Fig. 2): microbial biomass C, active organic C (non-protected plus protected fractions) and old inactive (passive) C components; the litter inputs are divided into three fractions (metabolic material, structural material and lignified material) based on root C/N ratio[58]. C decomposition and transformations between pools are assumed to be first-order reactions. Soil C model from CN-SIM includes particulate organic C (POC), mineral-associated organic C (MOC) and two microbial functional groups (micro-r and micro-K, representing different nutrient strategy responses to substrate quality); the root inputs are divided into two fractions (easily decomposable and hardly decomposable material) based on root C/N ratio. Microbial respiration follows Michaelis–Menten kinetics[59]. The MEND model includes POC (further divided into POC1 and POC2), MOC, dissolved organic C (DOC), adsorbed DOC (active layer of MOC adsorbing and desorbing DOC), microbial biomass (active and dormant microorganisms), and enzyme pools (POC-degrading and MOC-degrading enzymes)[60,61]. Soil C decomposition and DOC uptake by microorganisms follow the Michaelis–Menten equation. Model equations for each C pool, transformation fluxes between C pools and model parameters from the three models are listed in Supplementary Tables 8–14. The initial values of C pools and their $\delta^{13}C$ concentrations are described in the Supplementary Information.

To test the importance of incorporating C isotopes into the models, we first used only the measured $CO_2$ dataset for data assimilation (no-isotope-calibrated model), and then used both the measured $CO_2$ and the $\delta^{13}C$ datasets for data assimilation (isotope-calibrated model). We compared model performance between the no-isotope-calibrated model versus isotope-calibrated model by examining the model simulation for both $CO_2$ and the $\delta^{13}C$ values in each plot. In the Agro-IBIS soil biogeochemistry subroutine, $\delta^{13}C$ values are added to the model to track their changes for each C pool. The equations governing $\delta^{13}C$ values of each C pool and $CO_2$ flux are based on the principles of mass balance and the product rule of calculus. More details on the equations and the related parameters are provided in Supplementary Tables 8 and 9. In CN-SIM and MEND, a dimension containing the $^{13}C$ for each C pool is added, where all C-related processes are applied. The $^{13}C/^{12}C$ ratio is then used to calculate $\delta^{13}C$ of each C pool (see more details in Supplementary Tables 10–14).

We conducted a variance-based global Sobol sensitivity analysis[62] to quantify the relative importance of each parameter to model $CO_2$ fluxes and its $\delta^{13}C$ values. In the Agro-IBIS soil biogeochemistry subroutine, three parameters, including microbial decomposition rates of root structural C ($k_{rs}$), non-protected ($k_{nb}$) and protected C ($k_{pb}$), were identified as important parameters and then calibrated

using high-frequency time-series measurements of instantaneous $CO_2$ production rates and their $\delta^{13}C$ values from the 13.5-month soil incubation. The $CO_2$ production rate measured in the lab represented the total $CO_2$ production during microbial decomposition, while the measured $\delta^{13}C$ values of instantaneous $CO_2$ production corresponded to the mass-weighted $\delta^{13}C$ value of $CO_2$ production from each pool. The other model parameters were fixed by using the default values in the Agro-IBIS soil biogeochemistry subroutine in parameterization (details in Supplementary Table 9). In the CN-SIM, three important decomposition parameters were identified as important parameters, including decomposition rates of easily decomposable litterfall C ($k_{re}$), particulate organic C ($k_{poc}$) and mineral-associated organic C ($k_{moc}$), and then used for model parameterization (details in Supplementary Table 11). In the MEND model, five calibrated parameters included the initial active fraction of microorganisms ($r0$), one parameter regulating enzyme production of MOC ($f_{PEM}$), two parameters controlling microbial growth and maintenance ($V_g$ and $V_m$), the intrinsic C use efficiency ($Y_g$) at reference temperature (20 °C) and the other model parameters were fixed based on previous studies (details in Supplementary Table 14)[60,61,63]. The influential three parameters in the Agro-IBIS soil C model and CN-SIM soil C module were estimated by data assimilation using Bayesian probabilistic inversion and a Markov chain Monte Carlo technique[64]. In the MEND model, the shuffled complex evolution algorithm[65,66] was used to search the optimal model parameters that minimize the total objective function computed as the weighted average of multiple objectives. A detailed description of the data assimilation procedure for each model can be found in the Supplementary Information.

Model fitness was evaluated by the coefficient of determination ($R^2$) for $CO_2$ and root mean square error (RMSE) for $\delta^{13}C$ values of $CO_2$ as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(\mathrm{Sim}_i - \mathrm{Obs}_i\right)^2}{\sum_{i=1}^{n}\left(\mathrm{Sim}_i - \overline{\mathrm{Obs}}\right)^2},$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{n} \times \sum_{i=1}^{n}\left(\mathrm{Sim}_i - \mathrm{Obs}_i\right)^2},$$

where $i$ represents the index number of data points, $\mathrm{Sim}_i$ is the model simulation, $\mathrm{Obs}_i$ is the corresponding observation and $n$ is the total number of data points. The $R^2$ value indicates how well model simulations match the observations, with higher $R^2$ value denoting better model fitness. The RMSE, a non-negative value, is a measure of accuracy that computes model simulation errors; the closer the RMSE value approaches 0, the better the model fits the data. The criteria for satisfactory model performance were $R^2 > 0.6$ for $CO_2$ (ref. 66) and RMSE < 2 for $\delta^{13}C$ values of $CO_2$. We excluded plots where model simulations did not meet the criteria for satisfactory performance to enable further analysis of the model parameters and $CO_2$ response across different rotation types or during the corn growing phase (Supplementary Figs. 7 and 9). The overall modelling workflow is summarized in Supplementary Fig. 15. The calibrated parameters from data assimilation and time-series data–model comparisons are shown in Supplementary Figs. 16–19.

To test the influence of initial values on model simulation, we varied the initial root inputs and the pool size of initial POC (equivalent to non-protected plus protected C pool in Agro-IBIS) for evaluating the impact of model input uncertainty in the isotope-enabled models. We selected the most parsimonious sets of initial values by comparing the model performances among the scenarios with different initial values (Supplementary Information), which were used for further model synthesis. In the selected most parsimonious scenarios, we calculated $CO_2$ production from the root structural C, non-protected and protected slow C in the Agro-IBIS model; $CO_2$ production from easily decomposable root C, particulate and mineral-associated organic C in the CN-SIM model; and C fluxes from POC1, POC2, MOC and microbial growth and maintenance in the MEND model.

To compare the effects of different year rotations on the model parameters and $CO_2$ production from the C pools defined in the three soil C models, we calculated the natural logarithm of response ratios that were used as an indicator of effect size from 3-year and 4-year rotations relative to the control (2-year rotation). The effects of rotations for a variable were considered to be significant if the 95% confidence intervals did not overlap with zero. The analysis of data from corn plots followed the same protocol as for all phases of the rotations, specifically by selecting only corn plots for inclusion in the analysis.

### Data analysis

For SOC and TN stocks, $\delta^{13}C$ value of SOC stock, $\delta^{15}N$ value of TN stock, cumulative total $CO_2$ production, and cumulative $C_3$- and $C_4$-derived $CO_2$ production, we averaged the variables from the plots with the same year rotation to test differences among different year rotations ($n$ = 8, 12 and 16 for 2-, 3- and 4-year rotations, respectively). A normality test was conducted to ascertain whether the data conform to a normal distribution. Analysis of variance (ANOVA) followed by Dunnett's test was used to compare these variables in the 4-year and 3-year rotations with the control (2-year rotation). All statistical analyses were performed using the SciPy 1.14.1 package in Python 3.8 and R statistical software version 4.2.1.

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

## Data availability

The data that support the findings of this study are available in the Environmental Data Initiative at https://doi.org/10.6073/pasta/7f1dac6bf350221c54d1eb349ca89bbf (ref. 67). Source data for all the figures in the main text are provided with this paper.

## Code availability

Code (R and Python scripts) used to generate the figures are deposited to the Environmental Data Initiative at https://doi.org/10.6073/pasta/7f1dac6bf350221c54d1eb349ca89bbf (ref. 67).

## References

1. Paustian, K. et al. Climate-smart soils. *Nature* **532**, 49–57 (2016).
2. Minasny, B. et al. Soil carbon 4 per mille. *Geoderma* **292**, 59–86 (2017).
3. Oldfield, E. E. et al. Crediting agricultural soil carbon sequestration. *Science* **375**, 1222–1225 (2022).
4. Bowles, T. M. et al. Long-term evidence shows that crop-rotation diversification increases agricultural resilience to adverse growing conditions in North America. *One Earth* **2**, 284–293 (2020).
5. Tamburini, G. et al. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Sci. Adv.* **6**, eaba1715 (2020).
6. Lehman, R. M., Osborne, S. L. & Duke, S. E. Diversified no-till crop rotation reduces nitrous oxide emissions, increases soybean yields, and promotes soil carbon accrual. *Soil Sci. Soc. Am. J.* **81**, 76–83 (2017).
7. Yang, X. et al. Diversifying crop rotation increases food production, reduces net greenhouse gas emissions and improves soil health. *Nat. Commun.* **15**, 198 (2024).
8. Luo, Z., Wang, E. & Sun, O. J. Soil carbon change and its responses to agricultural practices in Australian agro-ecosystems: a review and synthesis. *Geoderma* **155**, 211–223 (2010).
9. McDaniel, M. D., Tiemann, L. K. & Grandy, A. S. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecol. Appl.* **24**, 560–570 (2014).

10. King, A. E. & Blesh, J. Crop rotations for increased soil carbon: perenniality as a guiding principle. *Ecol. Appl.* **28**, 249–261 (2018).
11. *U.S. Soil Enrichment Protocol Version 1.1* (Climate Action Reserve, 2022).
12. Prairie, A. M., King, A. E. & Cotrufo, M. F. Restoring particulate and mineral-associated organic carbon through regenerative agriculture. *Proc. Natl Acad. Sci. USA* **120**, e2217481120 (2023).
13. Rui, Y. 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. USA* **119**, e2118931119 (2022).
14. Baldwin-Kordick, R. et al. Comprehensive impacts of diversified cropping on soil health and sustainability. *Agroecol. Sustain. Food Syst.* **46**, 331–363 (2022).
15. Poffenbarger, H. J. et al. Whole-profile soil organic matter content, composition, and stability under cropping systems that differ in belowground inputs. *Agric. Ecosyst. Environ.* **291**, 106810 (2020).
16. Balesdent, J. et al. Atmosphere–soil carbon transfer as a function of soil depth. *Nature* **559**, 599–602 (2018).
17. Lazicki, P. A., Liebman, M. & Wander, M. M. Root parameters show how management alters resource distribution and soil quality in conventional and low-input cropping systems in central Iowa. *PLoS ONE* **11**, e0164209 (2016).
18. Drinkwater, L. E., Wagoner, P. & Sarrantonio, M. Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature* **396**, 262–265 (1998).
19. Poeplau, C. & Don, A. Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. *Agric. Ecosyst. Environ.* **200**, 33–41 (2015).
20. Dijkstra, F. A., Zhu, B. & Cheng, W. Root effects on soil organic carbon: a double-edged sword. *New Phytol.* **230**, 60–65 (2021).
21. Ros, G. H., Hanegraaf, M. C., Hoffland, E. & van Riemsdijk, W. H. Predicting soil N mineralization: relevance of organic matter fractions and soil properties. *Soil Biol. Biochem.* **43**, 1714–1722 (2011).
22. Smith, A., Snapp, S., Dimes, J., Gwenambira, C. & Chikowo, R. Doubled-up legume rotations improve soil fertility and maintain productivity under variable conditions in maize-based cropping systems in Malawi. *Agric. Syst.* **145**, 139–149 (2016).
23. King, A. E. & Hofmockel, K. S. Diversified cropping systems support greater microbial cycling and retention of carbon and nitrogen. *Agric. Ecosyst. Environ.* **240**, 66–76 (2017).
24. Osterholz, W. R., Liebman, M. & Castellano, M. J. Can soil nitrogen dynamics explain the yield benefit of crop diversification? *Field Crops Res.* **219**, 33–42 (2018).
25. Janzen, H. H. The soil carbon dilemma: shall we hoard it or use it? *Soil Biol. Biochem.* **38**, 419–424 (2006).
26. Paustian, K. et al. Quantifying carbon for agricultural soil management: from the current status toward a global soil information system. *Carbon Manag.* **10**, 567–587 (2019).
27. Chandel, A. K., Jiang, L. & Luo, Y. Microbial models for simulating soil carbon dynamics: a review. *J. Geophys. Res. Biogeosci.* **128**, e2023JG007436 (2023).
28. Le Noë, J. et al. Soil organic carbon models need independent time-series validation for reliable prediction. *Commun. Earth Environ.* **4**, 158 (2023).
29. Ogle, S. M., Breidt, F. J., Easter, M., Williams, S. & Paustian, K. An empirically based approach for estimating uncertainty associated with modelling carbon sequestration in soils. *Ecol. Modell.* **205**, 453–463 (2007).
30. Singh, P. & Benbi, D. K. Modeling soil organic carbon with DNDC and RothC models in different wheat-based cropping systems in north-western India. *Commun. Soil Sci. Plant Anal.* **51**, 1184–1203 (2020).
31. Ehleringer, J. R., Nina, B. & Lawrence, B. F. Carbon isotope ratios in belowground carbon cycle processes. *Ecol. Appl.* **10**, 412–422 (2000).
32. Farquhar, G. D., Ehleringer, J. R. & Hubick, K. T. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 503–537 (1989).
33. Camino-Serrano, M. et al. Including stable carbon isotopes to evaluate the dynamics of soil carbon in the land-surface model ORCHIDEE. *J. Adv. Model. Earth Syst.* **11**, 3650–3669 (2019).
34. Gross, A. & Glaser, B. Meta-analysis on how manure application changes soil organic carbon storage. *Sci. Rep.* **11**, 5516 (2021).
35. Keiluweit, M. et al. Mineral protection of soil carbon counteracted by root exudates. *Nat. Clim. Change* **5**, 588–595 (2015).
36. Dietz, C. L., Jackson, R. D., Ruark, M. D. & Sanford, G. R. Soil carbon maintained by perennial grasslands over 30 years but lost in field crop systems in a temperate Mollisol. *Commun. Earth Environ.* **5**, 360 (2024).
37. Stagnari, F., Maggio, A., Galieni, A. & Pisante, M. Multiple benefits of legumes for agriculture sustainability: an overview. *Chem. Biol. Technol. Agric.* **4**, 2 (2017).
38. Thilakarathna, M. S., McElroy, M. S., Chapagain, T., Papadopoulos, Y. A. & Raizada, M. N. Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agron. Sustain. Dev.* **36**, 58 (2016).
39. Martin, T. & Sprunger, C. D. Belowground dynamics influence nitrogen cycling and crop productivity in diversified corn systems. *Front. Sustain. Food Syst.* **5**, 705577 (2021).
40. Hunt, N. D., Hill, J. D. & Liebman, M. Cropping system diversity effects on nutrient discharge, soil erosion, and agronomic performance. *Environ. Sci. Technol.* **53**, 1344–1352 (2019).
41. Tomer, M. D. & Liebman, M. Nutrients in soil water under three rotational cropping systems, Iowa, USA. *Agric. Ecosyst. Environ.* **186**, 105–114 (2014).
42. Noll, L., Zhang, S., Zheng, Q., Hu, Y. & Wanek, W. Wide-spread limitation of soil organic nitrogen transformations by substrate availability and not by extracellular enzyme content. *Soil Biol. Biochem.* **133**, 37–49 (2019).
43. Breza, L. C. et al. Complex crop rotations improve organic nitrogen cycling. *Soil Biol. Biochem.* **177**, 108911 (2023).
44. Hunt, N. D., Liebman, M., Thakrar, S. K. & Hill, J. D. Fossil energy use, climate change impacts, and air quality-related human health damages of conventional and diversified cropping systems in Iowa, USA. *Environ. Sci. Technol.* **54**, 11002–11014 (2020).
45. Hergoualc'h, K. et al. in *2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories* (eds Calvo Buendia, E. et al.) 11.1–11.48 (IPCC, 2019).
46. Lawrence, N. C., Tenesaca, C. G., VanLoocke, A. & Hall, S. J. Nitrous oxide emissions from agricultural soils challenge climate sustainability in the US Corn Belt. *Proc. Natl Acad. Sci. USA* **118**, e2112108118 (2021).
47. Mathers, C. et al. Validating DayCent-CR for cropland soil carbon offset reporting at a national scale. *Geoderma* **438**, 116647 (2023).
48. Fry, B. (ed.) in *Stable Isotope Ecology* 40–75 (Springer, 2006).
49. Liebman, M. et al. Agronomic and economic performance characteristics of conventional and low-external-input cropping systems in the central Corn Belt. *Agron. J.* **100**, 600–610 (2008).
50. Hunt, N. D., Hill, J. D. & Liebman, M. Reducing freshwater toxicity while maintaining weed control, profits, and productivity: effects of increased crop rotation diversity and reduced herbicide usage. *Environ. Sci. Technol.* **51**, 1707–1717 (2017).
51. von Haden, A. C., Yang, W. H. & DeLucia, E. H. Soils' dirty little secret: depth-based comparisons can be inadequate for quantifying changes in soil organic carbon and other mineral soil properties. *Glob. Change Biol.* **26**, 3759–3770 (2020).
52. Fowler, A. F., Basso, B., Millar, N. & Brinton, W. F. A simple soil mass correction for a more accurate determination of soil carbon stock changes. *Sci. Rep.* **13**, 2242 (2023).

53. Raffeld, A. M. et al. The importance of accounting method and sampling depth to estimate changes in soil carbon stocks. *Carbon Balance Manag.* **19**, 2 (2024).
54. Crumpton, W. G., Isenhart, T. M. & Mitchell, P. D. Nitrate and organic N analyses with second-derivative spectroscopy. *Limnol. Oceanogr.* **37**, 907–913 (1992).
55. Ye, C. & Hall, S. J. Mechanisms underlying limited soil carbon gains in perennial and cover-cropped bioenergy systems revealed by stable isotopes. *Glob. Change Biol. Bioenergy* **12**, 101–117 (2020).
56. Cerling, T. E. et al. Global vegetation change through the Miocene/Pliocene boundary. *Nature* **389**, 153–158 (1997).
57. Verberne, E. L. J., Hassink, J., de Willigen, P., Groot, J. J. R. & van Veen, J. A. Modelling organic matter dynamics in different soils. *Neth. J. Agric. Sci.* **38**, 221–238 (1990).
58. Whitmore, A. P. et al. Simulating trends in soil organic carbon in long-term experiments using the Verbeme/MOTOR model. *Geoderma* **81**, 137–151 (1997).
59. Yi, B. et al. Resolving the influence of lignin on soil organic matter decomposition with mechanistic models and continental-scale data. *Glob. Change Biol.* **29**, 5968–5980 (2023).
60. Wang, G. et al. Soil moisture drives microbial controls on carbon decomposition in two subtropical forests. *Soil Biol. Biochem.* **130**, 185–194 (2019).
61. Jian, S. et al. Multi-year incubation experiments boost confidence in model projections of long-term soil carbon dynamics. *Nat. Commun.* **11**, 5864 (2020).
62. Sobol', I. M. Global sensitivity indices for nonlinear mathematical models and their Monte Carlo estimates. *Math. Comput. Simul.* **55**, 271–280 (2001).
63. Wang, G., Post, W. M. & Mayes, M. A. Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses. *Ecol. Appl.* **23**, 255–272 (2013).
64. Xu, T., White, L., Hui, D. & Luo, Y. Probabilistic inversion of a terrestrial ecosystem model: analysis of uncertainty in parameter estimation and model prediction. *Glob. Biogeochem. Cycles* **20**, GB2007 (2006).
65. Duan, Q., Sorooshian, S. & Gupta, V. Effective and efficient global optimization for conceptual rainfall-runoff models. *Water Resour. Res.* **28**, 1015–1031 (1992).
66. Wang, G. et al. Microbial dormancy improves development and experimental validation of ecosystem model. *ISME J.* **9**, 226–237 (2015).
67. Yi, B. et al. *Carbon Decomposition and Nitrogen Mineralization in Marsden Agroecosystem Diversification Experiment, Iowa, 2021* (Environmental Data Initiative, 2024); https://doi.org/10.6073/pasta/7f1dac6bf350221c54d1eb349ca89bbf


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## Author contributions

S.J.H. conceived this study. M.L. initiated and maintained the field experiment. W.H. and S.J.H. conducted the experimental work. B.Y. and W.H. conducted the modelling simulation and analysed the data with S.J.H. M.W. collected soil samples. W.H., B.Y. and S.J.H. wrote the manuscript. M.L., M.W., M.D.M., C.L., A.V., S.A., B.P., S.J., H.J.P., G.W. and Y. L. provided suggestions for substantial revisions.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41893-024-01495-4.

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| ☐ | ☒ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☐ | ☒ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | In this study, data collection was performed exclusively through laboratory incubations and field measurements, without the use of external software. |
| Data analysis | In this study, Markov chain Monte Carlo simulations were conducted using the DREAM 1.0 software package, with all statistical analyses were performed using the SciPy 1.14.1 package in Python 3.8 and R statistical software version 4.2.1 |

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Data availability: The data that support the findings of this study are available in the Environmental Data Initiative with the identifier https://doi.org/10.6073/pasta/71cbefa68d69571534f05a747d7891a8. Source data for all the figures in the main text are provided with the paper.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | N/A |
| Population characteristics | N/A |
| Recruitment | N/A |
| Ethics oversight | N/A |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Study description | This study leveraged a long-term (20-year) Iowa, USA field experiment comparing a conventional, two-year corn-soybean rotation with three- and four-year extended rotations (corn-soybean-small grain + red clover, or corn-soybean-small grain + alfalfa-alfalfa) that also received manure inputs, where each phase of the three rotations was grown during each year in four replicated blocks (a plot size of 18 m x 84 m). We quantified SOC and total N stocks to 1 m, and incubated additional intact 1-m soil cores in the lab to measure soil carbon dioxide ($CO_2$) fluxes and δ13C values and net N mineralization. The measurements were integrated with three C isotope-enabled models to test underlying mechanisms by which diversified cropping systems might alter decomposition. |
| Research sample | Soil samples were collected in fall 2021 from 0 to 100 cm depth across 36 field sites featuring different cropping systems: a 2-year corn-soybean rotation (8 field plots), a 3-year corn-soybean-small grain/red clover rotation (12 field plots), and a 4-year corn-soybean-small grain/alfalfa-alfalfa rotation (16 field plots). |
| Sampling strategy | Following harvest in 2021, we took four 3.8-cm diameter cores from four cardinal locations in each plot to a depth of 100 cm using a hydraulic soil probe. One intact soil core from each plot was randomly selected for lab incubation. The remaining three soil cores from each plot were divided into 0–15 cm, 15–30 cm, 30–60 cm, and 60–100 cm depth increments and then composited to form one sample per depth per plot for soil chemical analysis. |
| Data collection | Wenjuan Huang collected data on the measurements of soil chemical properties. Soil organic C (SOC) was determined by subtracting carbonate C from total soil C. The δ13C value of SOC was calculated by a two-source mixing model. Soil N and its δ15N value were also measured by an elemental analyzer/continuous flow isotope ratio mass spectrometry at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley, CA, USA. We calculated N stock and its mass-weighted δ15N value, similar to the calculation for SOC.<br>Net N mineralization was measured by the differences in inorganic N (ammonium + nitrate) between the initial composited soil samples and the samples after incubation. The composited and air-dried soil subsamples were extracted with 2 M KCl in a 1:5 soil: solution mass ratio, shaken for 1 hr, and then centrifuged for 10 min at 10,000 g. The extraction was measured by microplate colorimetry for ammonium (NH4+-N), and by second-derivative spectroscopy for nitrate (NO3--N) 53. The intact soil cores from the lab incubation were divided into the same soil depths and subsampled for measuring NH4+-N and NO3--N using the same methods as described above. Net N mineralization was calculated as the difference in inorganic N before and after ~1-year incubation.<br>Wenjuan Huang also conducted the lab incubation to measure soil $CO_2$ production and its carbon isotope ratios (δ13C). One intact soil core from each plot was incubated in the lab at constant moisture and temperature to monitor soil $CO_2$ production and its δ13C value every three days on average over 406 days. Soil moisture in each soil core was adjusted to field capacity by gradually adding water before extra water ran off the core. The soil cores were sealed with plastic lids equipped with two stainless steel Swagelok fittings and tubing for air flushing and automated headspace gas sampling using a dynamic chamber approach. Briefly, each soil core was flushed with air at a constant rate (60 ml/min), and an outlet from the core diverted gas to a tunable diode laser for measurement of $CO_2$ concentration and its δ13C value when sampling and vented gas to the laboratory when not sampling.<br><br>Bo Yi and Wenjuan Huang conducted the data-model assimilation in the three mechanistic C models. The three models included the Agricultural version of the Integrated Biosphere Simulator (Agro-IBIS) soil biogeochemistry submodule, the organic carbon decomposition module (CN-SIM) from the Dynamic Land Ecosystem Model (DLEM), and the Microbial-ENzyme Decomposition (MEND) model. |
| Timing and spatial scale | Soil samples were collected near Boone, Iowa, USA (42°01'N, 93°47'W) in fall 2021.<br>Gas samples were taken 106 times (at three-day intervals on average) over 406 days from 2022 to 2023 at Iowa State University, and |

| | |
|---|---|
| | measurements from day 188 to day 255 were missed due to equipment failure.<br>Model were conducted over a 406-day period based on laboratory incubation settings. Two models, CN-SIM and Agro-IBIS, operated on a daily timestep, while MEND utilized a hourly timestep. |
| Data exclusions | No laboratory incubation data were excluded from our analysis.<br>Model simulations on some soil samples did not achieve satisfactory performance and were excluded based on the criteria of a determination coefficient ($R^2$) greater than 0.6 for CO2 and a root mean square error (RMSE) less than 2‰ for the δ13C value. |
| Reproducibility | The findings can be reproducible by following the above description of sampling strategy, data collection, timing, and spatial scale. |
| Randomization | The 36 plots in these three different rotation systems were arranged in a randomized complete block design. In each plot, we took four 3.8-cm diameter cores from four cardinal locations to a depth of 100 cm using a hydraulic soil probe. One intact soil core from each plot was randomly selected for lab incubation. The remaining three soil cores from each plot were divided into 0–15 cm, 15–30 cm, 30–60 cm, and 60–100 cm depth increments for soil chemical analysis. |
| Blinding | Blinding is not applicable to our study since our focus is on soil. |

Did the study involve field work? ☒ Yes ☐ No

## Field work, collection and transport

| | |
|---|---|
| Field conditions | Soils were sampled in Marsden Farm near Boone, Iowa. During 2002–2021, the mean annual precipitation and temperature were 993 mm and 9.05 °C , respectively. |
| Location | Iowa State University's Marsden Farm near Boone, Iowa, USA (42°01'N, 93°47'W). |
| Access & import/export | We get permission to collect soil samples from the Research Team from Iowa State University's Marsden Farm, and they are contributors for this manuscript. |
| Disturbance | Some soil holes were left in the field; we attempted to mix the surrounding soil to prevent further erosion. These holes will be refilled during subsequent cultivation. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
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| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

### Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |