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Soil pore architecture regulates carbon persistence via anaerobic microsites

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Abstract

Enhancing soil organic carbon (SOC) persistence in agricultural soils is a promising lever for climate mitigation, but realizing this potential depends on management practices that reliably promote SOC stabilization. Physical protection of organic compounds within soil aggregates is widely invoked to explain carbon stabilization under no-till farming, yet the role of pore architecture in regulating oxygen supply to microorganisms and its consequences for SOC turnover remains mechanistically unresolved. Here we show that no-till management promotes SOC persistence by selectively expanding a microbially accessible pore domain prone to oxygen limitation, thereby fostering anaerobic microsite development independently of soil texture without suppressing bulk carbon decomposition. Integrating pore network imaging, oxygen transport measurements, microbial functional gene quantification, and carbon mineralization experiments, we demonstrate that no-till specifically expands the 1–30 μm pore domain and elevates anaerobic microbial functional potential, while soil texture governs bulk oxygen supply and carbon turnover. These findings reframe physical protection as an oxygen-mediated, pore-scale mechanism responsive to agricultural management that complements mineral-associated carbon stabilization, with implications for soil carbon modeling under changing land management and climate.

Main

Soil organic carbon (SOC) reservoirs contain roughly twice the carbon found in the atmosphere¹, yet human agriculture has depleted this pool by an estimated 133 Pg C globally². Reclaiming this historical carbon debt through optimized agricultural management, including reduced and no-tillage practices, represents an important climate mitigation lever. Global croplands hold a theoretical technical potential to sequester 0.90–1.85 Pg C yr⁻¹³. However, the real-world efficacy of conservation tillage remains highly variable across soil textures, climates, and management

histories^{4–6}, reflecting an incomplete mechanistic understanding of how mechanical soil disruption alters the physical and biophysical microenvironments governing SOC stabilization and microbial turnover.

SOC stabilization is commonly attributed to physical protection through soil aggregation and organo-mineral associations, which limit microbial access to substrates and increase SOC residence time^{7–9}. However, these aggregate-based frameworks do not resolve the pore-scale transport pathways governing oxygen accessibility to microorganisms, despite growing recognition that oxygen limitation constrains SOC decomposition alongside substrate inaccessibility^{10,11}. Bridging this gap requires direct quantification of soil pore architecture and its consequences for oxygen, water, and substrate movement within the soil matrix.

Oxygen limitation can arise at the microscale within aggregates and water-filled pores even when bulk measurements indicate oxic conditions^{12–14}. These oxygen-restricted microsites support anaerobic metabolisms that alter decomposition pathways and may reduce mineralization efficiency relative to aerobic processes^{15–17}, underscoring the importance of spatial redox heterogeneity^{14,18}. Yet how soil structure governs oxygen accessibility under typical upland moisture conditions, and how this shapes microbial functioning and SOC dynamics, remains poorly resolved.

Texture and tillage are the primary controls on pore architecture in agricultural systems. Clay-rich soils contain more fine pores and stronger organo-mineral associations, while coarser soils contain larger, more gas-permeable pores^{19–21}. Tillage further reshapes pore networks: conventional tillage transiently increases large-pore abundance but destabilizes biopore connectivity, whereas no-till preserves aggregation and connectivity and often favors finer pores^{22–24}. Texture and tillage thus jointly regulate pore architecture and the physical constraints on oxygen diffusion and microbial activity.

Pore size and connectivity determine the functional role of soil structure: pores $>100\ \mu\text{m}$ aerate but are water-limited habitats; intermediate pores ($\sim 30\text{--}100\ \mu\text{m}$) balance oxygen, water, and substrate supply^{11,25–27}, pores $1\text{--}30\ \mu\text{m}$ remain microbially accessible but are prone to oxygen limitation; pores $<1\ \mu\text{m}$ are largely inaccessible to microbes and are associated with organo-mineral associations, a chemically distinct stabilization pathway^{11,26,28}. Empirical links between these pore-scale domains, microbial metabolic potential, and SOC dynamics in intact agricultural soils remain scarce. If structure governs pore-scale oxygen and carbon accessibility, fine or poorly connected pore networks should favor communities adapted to low oxygen, reflected in elevated denitrification, iron reduction, sulfate reduction, and methanogenesis gene abundances^{29,30}, while larger, better-connected networks should sustain aerobic mineralization, though whether this yields measurable differences in bulk SOC mineralization remains an open question.

Here, we quantify how soil structure regulates oxygen accessibility and microbial metabolic potential, and their relationship to SOC dynamics, using intact cores from a long-term field experiment containing naturally contrasting clay and loam soils within the same site, isolating texture and tillage effects from confounding climatic and management history. We integrate pore-scale imaging^{31–35}, gas transport measurements^{36,37}, microbial functional gene quantification, and incubations across defined matric potentials. We show that no-till selectively expands the $1\text{--}30\ \mu\text{m}$ pore domain, uniquely associated with elevated anaerobic metabolic potential, yet this does not translate into reduced bulk SOC mineralization. This indicates that pore-scale anoxic processes and whole-soil carbon turnover are more decoupled than current models assume, and that physical protection of SOC extends beyond soil aggregation to include oxygen-mediated, pore-scale controls responsive to management.

No-till and soil texture act through distinct pore-scale pathways

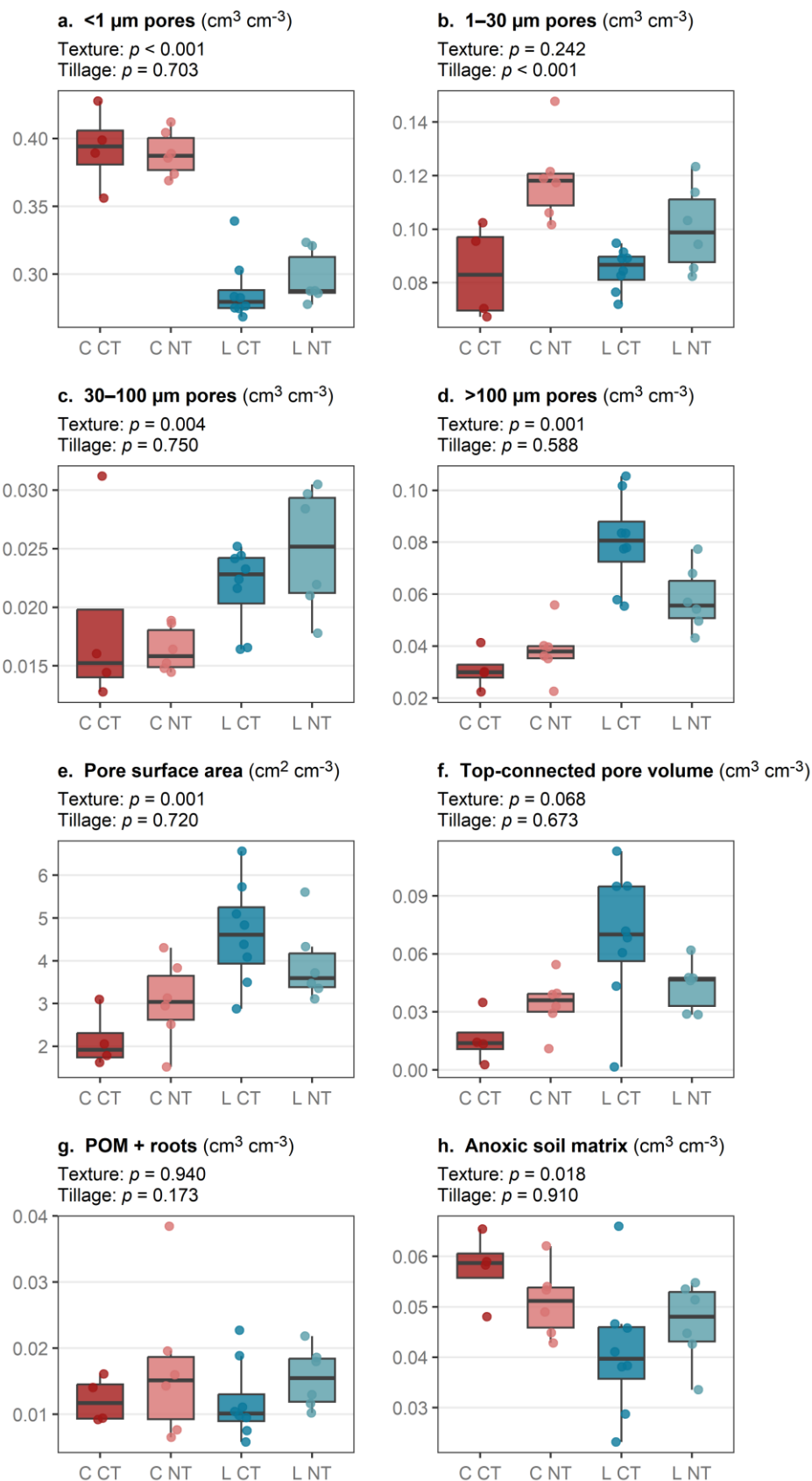
No-till management selectively increased the abundance of pores in the 1–30 μm range, the size class most accessible to microorganisms but prone to oxygen limitation (Fig. 1b), whereas soil texture was the primary determinant of three-dimensional pore architecture captured by X-ray imaging (Fig. 1e,h). This separation of controls was clear: tillage selectively modified the microbially accessible 1–30 μm pore fraction, while texture governed the macropore network and, through its association with clay content, the abundance of fine (<1 μm) pores (Fig. 1a) where organo-mineral associations are most prevalent.

Pores smaller than 1 μm were significantly more abundant in clay than loam soils ($p < 0.001$), while the 1–30 μm class was shaped exclusively by tillage, with no-till soils containing significantly more of these pores than conventionally tilled soils ($p < 0.001$), independently of texture (detailed pore size distributions and total porosity are provided in Supplementary Figs. 1 and 2). Larger pore classes were primarily texture-controlled: the 30–100 μm class was more abundant in loam than clay soils ($p = 0.004$), as was the >100 μm class ($p = 0.001$), with a significant texture $\times$ tillage interaction ($p = 0.021$) driven by greater macropore volume under conventional tillage in the loam soil only. Three-dimensional metrics from X-ray imaging corroborated texture as the dominant control on macropore architecture: imaged pore surface area was greater in loam than clay soils ($p = 0.001$; Fig. 1e), a similar tendency was observed for top-connected pore volume ($p = 0.068$; Fig. 1g) and for additional metrics (Supplementary Figs. 3 and 4). None of the CT-derived connectivity or topology metrics were affected by tillage (all $p > 0.05$, Supplementary Fig. 3), indicating that long-term tillage did not detectably alter macropore architecture at the ~ 100 μm resolution captured by imaging. Imaged particulate organic matter and root volumes did not differ among treatments (all $p > 0.173$; Fig. 1g), indicating that differences in imaged pore network properties reflect pore geometry rather than organic matter inputs. The potential for oxygen limitation, estimated as the soil matrix volume beyond 1 mm from venting pores, was greater in

clay than loam soils ($p = 0.018$; Fig. 1h), consistent with the finer, less-connected pore network of clay-rich soils, with no effect of tillage.

This structural separation indicates that no-till operates on soil architecture largely independently of texture, expanding the 1–30 μm pore class across both soil types and identifying this domain as a management-sensitive microhabitat linked to anaerobic metabolic potential and SOC stabilization, developed below. This pore domain is accessible to microorganisms but prone to oxygen limitation due to restricted gas diffusion¹¹, consistent with enhanced micro-aggregation and structural preservation under reduced mechanical disturbance^{7,23,38}. Critically, this tillage effect was not captured by X-ray imaging or bulk gas transport measurements (see below), both of which showed no significant tillage effect, underscoring the complementary value of water retention curves for resolving the functionally relevant fine pore fraction that coarser imaging resolutions miss. Soil texture, in turn, set the broader structural template within which management operated: clay soils' greater volume of fine pores -the domain where organo-mineral associations are most prevalent- combined with lower macroporosity, establish stronger diffusion limitation, consistent with prior work linking clay content to anoxic microsite development^{12,16,39}.

Fig. 1: Soil pore architecture across scales under contrasting soil texture and tillage management.



Pore volume fractions across pore size classes **(a)** $<1\ \mu\text{m}$ ($\text{cm}^3\ \text{cm}^{-3}$), **(b)** $1\text{--}30\ \mu\text{m}$ ($\text{cm}^3\ \text{cm}^{-3}$), **(c)** $30\text{--}100\ \mu\text{m}$ ($\text{cm}^3\ \text{cm}^{-3}$), and **(d)** $>100\ \mu\text{m}$ ($\text{cm}^3\ \text{cm}^{-3}$), and three-dimensional pore network metrics derived from X-ray images of intact soil cores collected after the incubation experiment, including **(e)** pore surface area ($\text{cm}^2\ \text{cm}^{-3}$), **(f)** top-connected pore volume ($\text{cm}^3\ \text{cm}^{-3}$), **(g)** particulate organic matter (POM) and roots ($\text{cm}^3\ \text{cm}^{-3}$), and **(h)** potential anoxic soil matrix ($\text{cm}^3\ \text{cm}^{-3}$). Pore size classes $<100\ \mu\text{m}$ were determined from soil water retention curves as equivalent capillary pore diameters using intact soil cores (set A), whereas the $>100\ \mu\text{m}$ pore size class and all three-dimensional pore metrics were derived from X-ray imaging of separate intact soil cores (set B) following the incubation experiment, representing geometrically resolved pore space (effective resolution $\sim 100\ \mu\text{m}$). Data are shown for clay (C) and loam (L) soils under conventional tillage (CT) and no-till (NT) management. Boxplots represent the median, interquartile range, and whiskers extending to $1.5 \times$ the interquartile range, with individual points indicating independent soil cores ($n = 4$ for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT). Statistical analyses were performed using linear mixed-effects models with texture, tillage, and their interaction as fixed factors and sampling plot as a random factor. *P* values shown above each panel correspond to the main effects of texture and tillage.

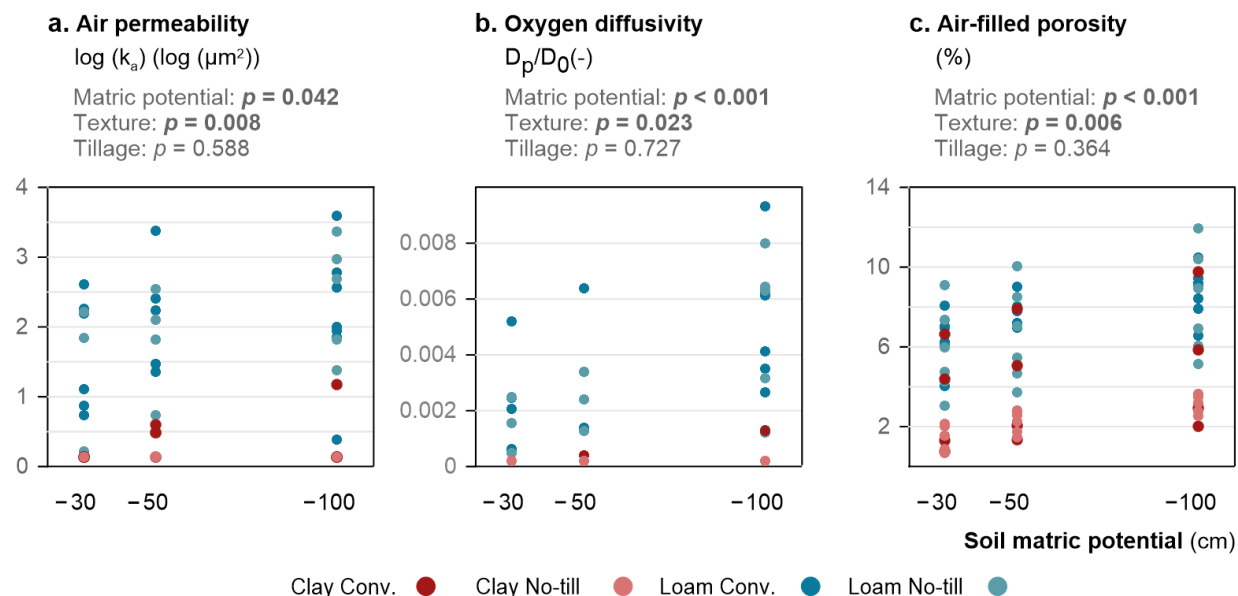
Gas transport confirms texture as the dominant control on oxygen supply

Soil texture, not tillage, dominated gas transport properties, with loam soils showing consistently greater air permeability and oxygen diffusivity than clay soils across all matric potentials (Fig. 2a,b); no significant effects of tillage or texture $\times$ tillage interactions were detected. Air-filled porosity increased with decreasing matric potential ($p < 0.001$) and was consistently greater in loam than clay soils ($p = 0.006$), with no effect of tillage ($p = 0.364$; Fig. 2c): loam soils exhibited substantially higher air-filled porosity ($\sim 6\text{--}8\%$) than clay soils ($\sim 2\text{--}5\%$) across all moisture conditions, with clay no-till consistently showing the lowest values.

These differences in air-filled porosity translated directly into bulk gas transport. Air permeability differed significantly between textures at all matric potentials ($p = 0.043$, 0.018 , and < 0.001 at -30 , -50 , and -100 cm, respectively), and oxygen diffusivity showed a similar pattern, with a significant texture effect at -100 cm ($p = 0.009$) and a marginal effect at -50 cm ($p = 0.087$). Both metrics increased with decreasing matric potential, consistent with progressive drainage of water-filled pores and expansion of the air-conducting network, an effect more pronounced in loam soils given their greater abundance of $30\text{--}100$ and >100 μm pores (Fig. 1c,d). In contrast, gas transport in clay soil was frequently near or below detection limits across all matric potentials, with oxygen diffusivity undetectable at -30 and -50 cm and very low at -100 cm under conventional tillage; all loam treatments showed measurable gas transport across the full moisture range.

Together, these results indicate that the fine pore-dominated architecture of clay soils strongly restricts bulk oxygen supply across all moisture conditions tested here, largely independent of tillage management. This texture-driven control is consistent with stronger diffusion limitation in fine-textured soils reported elsewhere^{12,16,39}, and with the greater volume of potentially anoxic soil matrix already observed in clay soils (Fig. 1h), together supporting texture as the primary control on the spatial extent of oxygen-limited domains at the pore scale^{14,17}. At -30 , -50 , and -100 cm matric potential, pores larger than approximately 100 , 60 , and 30 μm respectively are expected to be air-filled, directly linking moisture-dependent pore drainage to the oxygen supply available for microbial activity and, as shown below, to carbon turnover. That bulk gas transport showed no detectable tillage effect, in contrast to the clear tillage effect on the $1\text{--}30$ μm pore class (Fig. 1b), indicates that this management-sensitive pore domain operates below the resolution of bulk aeration measurements - a distinction the following section resolves through microbial functional gene abundances.

Fig. 2: Air permeability and oxygen diffusivity across texture, tillage and matric potentials.



a. Log air permeability (μm^2), **b.** Relative oxygen diffusion coefficient (D_p/D_0 (-)), and **c.** Air-filled porosity measured on intact soil cores at -30, -50, and -100 cm matrix potential. Measurements were performed on the same set of cores used for water retention curve analyses. Data are shown for clay (C) and loam (L) soils under conventional tillage (CT) and no-till (NT) management. For both panels, all points represent individual observations ($n = 4$ for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT). Statistical analyses were conducted using linear mixed-effects models with matric potential, texture, tillage and their interaction as fixed factors, and sampling plot as a random factor. P values shown above each panel correspond to the main effects of texture and tillage.

Anaerobic microbial potential tracks the tillage-sensitive 1–30 μm pore domain

Clay soils and no-till management consistently harbored greater anaerobic microbial functional potential than loam soils and conventionally tilled soils, with denitrification, iron reduction, and methanogenesis gene abundances following the same directional pattern across treatments (Fig. 3). Denitrification gene abundance was significantly affected by both texture and tillage for *nirK* (both $p < 0.001$) and by tillage for *nirS* ($p < 0.001$), with a marginal texture effect for *nirS* ($p =$

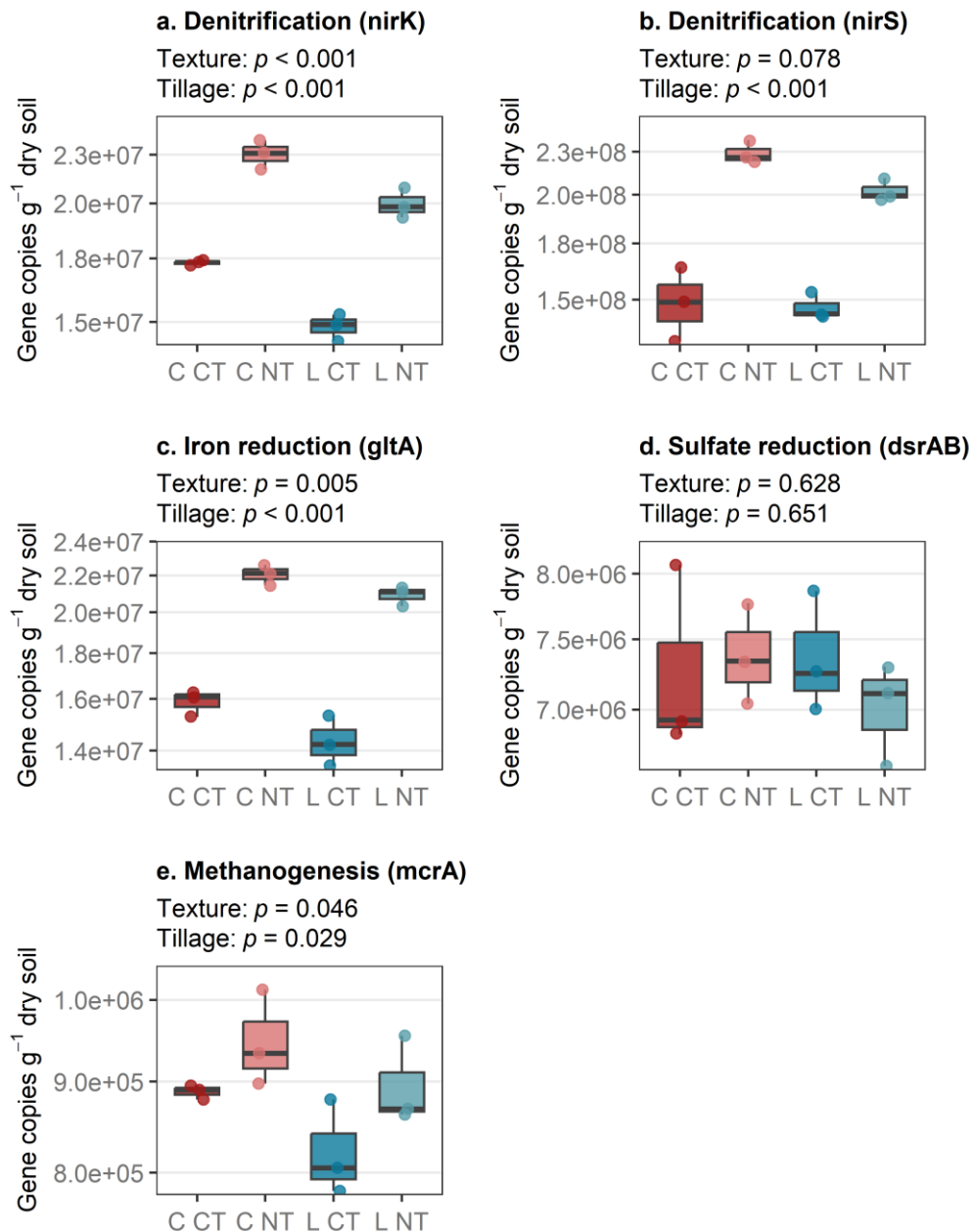
0.078). Iron reduction (*gltA*) and methanogenesis (*mcrA*) were also significantly influenced by both texture and tillage ($p \leq 0.046$), with consistently higher abundances in clay and no-till soils. Sulfate reduction (*dsrAB*) did not differ among treatments, and no significant texture $\times$ tillage interactions were detected for any gene.

Critically, these differences were strongly associated with the abundance of 1–30 μm pores -the pore class selectively expanded by no-till (Fig. 1b)- through significant linear relationships across all treatments: *nirS* ($R^2 = 0.697$, $p < 0.001$), *nirK* ($R^2 = 0.617$, $p = 0.002$), *gltA* ($R^2 = 0.716$, $p < 0.001$), and *mcrA* ($R^2 = 0.425$, $p = 0.022$; Supplementary Fig. 5). No relationship was found for *dsrAB* ($R^2 \approx 0$, $p = 0.974$), consistent with its absence of treatment effects, and likely reflecting that sulfate reduction is governed more by substrate availability than oxygen accessibility in these systems. None of the other pore size classes, namely the <1 , 30–100, or >100 μm , showed significant relationships with any anaerobic gene (all $p > 0.128$; Supplementary Figs. 6–8), indicating that anaerobic potential is not linked to mineral-associated micropores or aeration-dominated macropores, but specifically to the microbe-accessible, oxygen-limited 1–30 μm domain.

This pore-class specificity points to two independent structural pathways generating anaerobic potential: texture acting through bulk oxygen transport restriction (previous section), and no-till acting through selective expansion of the 1–30 μm microhabitat. Soils for gene quantification were sampled at similar water-filled pore space within each texture (clay: 84% vs. 85%; loam: 74% vs. 75%, conventional vs. no-till), indicating that elevated anaerobic gene abundances under no-till are not attributable to differences in bulk soil moisture but instead reflect this pore-scale reorganization. These findings align with proposals that microbial functional genes serve as integrative proxies for anoxic microsite prevalence in upland soils³⁰, and with reports that no-till effects on anaerobic gene abundance vary across sites while finer-textured soils generally support greater anaerobic potential⁴⁰; here, we identify selective expansion of the 1–30 μm pore class as

the likely structural mechanism underlying this response. The positive association between anaerobe abundance and SOC concentration observed here and elsewhere⁴⁰ likely reflects a bidirectional relationship, in which carbon availability promotes anoxic microsite development while oxygen-limited conditions simultaneously contribute to SOC persistence.

Fig. 3: Abundance of microbial functional genes associated with anaerobic metabolism.



241

242 Boxplots show gene copy numbers (per g dry soil) for **a.** *nirK*, **b** *nirS* (denitrification), **c** *gltA* (iron

243 reduction), **d** *dsrAB* (sulfate reduction), and **e** *mcrA* (methanogenesis) in clay (C) and loam (L)

244 soils under conventional (CT) and no-till (NT) management. DNA was extracted from soil sampled

245 directly from the field at the beginning of the experiment. Boxplot centre lines represent the median

246 ($n = 3$ independent field replicates per treatment), box limits indicate the first and third quartiles,

whiskers extend to the minimum and maximum values within 1.5x of the interquartile range and points represent individual observations. Statistical analyses were performed using two-way ANOVA with texture and tillage as fixed factors. *P* values for texture and tillage effects are shown within each panel.

Pore-scale anaerobic potential does not scale to bulk carbon turnover

Soil texture and matric potential were the dominant controls on cumulative SOC mineralization, whereas tillage had no detectable effect despite its strong influence on pore structure and anaerobic microbial functional potential (Fig. 4a). Loam soils showed 63–82% higher cumulative SOC mineralization per unit SOC than clay soils across all matric potentials ($p < 0.001$), consistent with their greater oxygen supply capacity and more connected macropore network (Figs. 1 and 2). Tillage had no significant effect on cumulative mineralization across any texture or matric potential combination ($p = 0.884$). Mineralization declined significantly with decreasing matric potential across all treatments ($p < 0.001$), more pronouncedly in loam soils (29–34% reduction) than clay soils (17–29%), reflecting a shift from oxygen to substrate transport limitation as water film connectivity restricts solute diffusion to microbial cells under drier conditions. This is consistent with prior evidence that CO₂ emissions from intact cores rise sharply once soils drain past saturation and then plateau across drier matric potentials⁴¹.

SOC concentrations measured at the end of the incubation reflected long-term field differences: clay soils contained significantly higher SOC than loam soils ($p < 0.001$), and no-till soils showed higher SOC than conventionally tilled soils across both textures ($p = 0.004$; Fig. 4b), with no texture × tillage interaction. Despite these differences, and the higher abundance of anaerobic microbial functional genes under no-till (previous section), cumulative SOC mineralization per unit SOC did not differ between tillage systems. This indicates that the pore-scale anaerobic potential associated with no-till management was insufficient to alter carbon turnover at the scale of the intact soil core^{17,29,42}. This decoupling between microsite-level anaerobic metabolism and whole-

soil respiration in turn challenges the assumption, common to current conceptual models, that greater anaerobic potential should suppress bulk mineralization. Differences in crop inputs are unlikely to drive this pattern: clay soils showed higher wheat yields than loam soils⁴³ yet consistently lower mineralization, and X-ray-derived particulate organic matter and root volumes showed no treatment effects (Fig. 1g), a result unlikely to reflect methodological limitations given the established agreement between X-ray-derived and physically fractionated organic matter pools in intact soils^{44,45}.

Instead, higher SOC concentrations under clay and no-till management likely reflect two complementary stabilization mechanisms operating at different structural scales: management-driven expansion of the 1–30 μm pore class promoting anoxic microsites that may protect SOC independently of texture, and organo-mineral association in clay-rich soils providing complementary chemical stabilization through mineral surface binding⁴⁶. These mechanisms likely interact, with anoxic microsites offering transient protection that complements longer-term mineral stabilization as soils dry and anaerobic conditions dissipate.

Fig. 4: Soil organic carbon concentration and cumulative mineralization across texture, tillage, and moisture conditions.

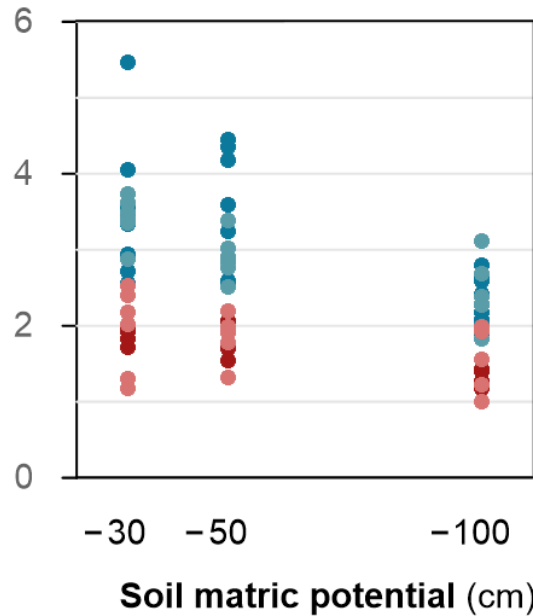
a. Cumulative SOC mineralization

(mg CO₂-C g SOC⁻¹)

Matric potential: $p < 0.001$

Texture: $p < 0.001$

Tillage: $p = 0.884$

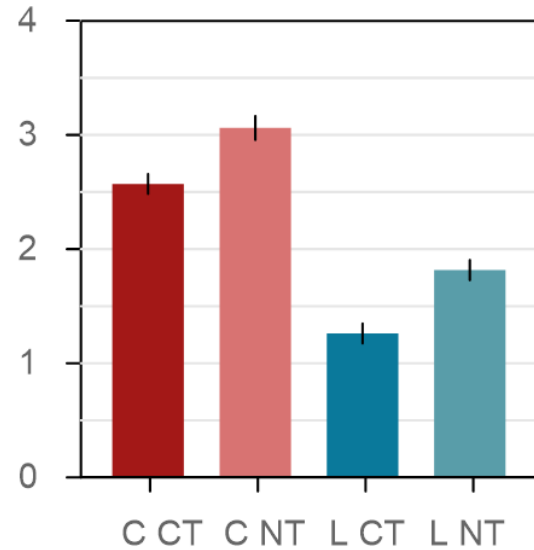


b. SOC content

(%)

Texture: $p < 0.001$

Tillage: $p = 0.004$



Clay Conv. ● Clay No-till ● Loam Conv. ● Loam No-till ●

288

289 **a.** Cumulative SOC mineralization normalized by SOC content (mg CO₂-C g SOC⁻¹) integrated
 290 over 5 days of incubation at -30, -50, and -100 cm matric potential in clay (C) and loam (L) soils
 291 under conventional tillage (CT) and no-till (NT) management. Cumulative mineralization was
 292 analyzed using a linear mixed-effects model with matric potential, texture, tillage, and their
 293 interactions as fixed effects and sampling plot as a random factor. Points represent individual
 294 observations ($n = 4$ for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT). **b.** SOC concentrations
 295 (%) measured at the end of the incubation on the same intact cores. SOC was analyzed using a
 296 linear mixed-effects model with texture and tillage as fixed factors and sampling plot as a random
 297 factor. Bars represent means $\pm$ standard errors. P values shown above each panel correspond to
 298 the main effects of texture and tillage.

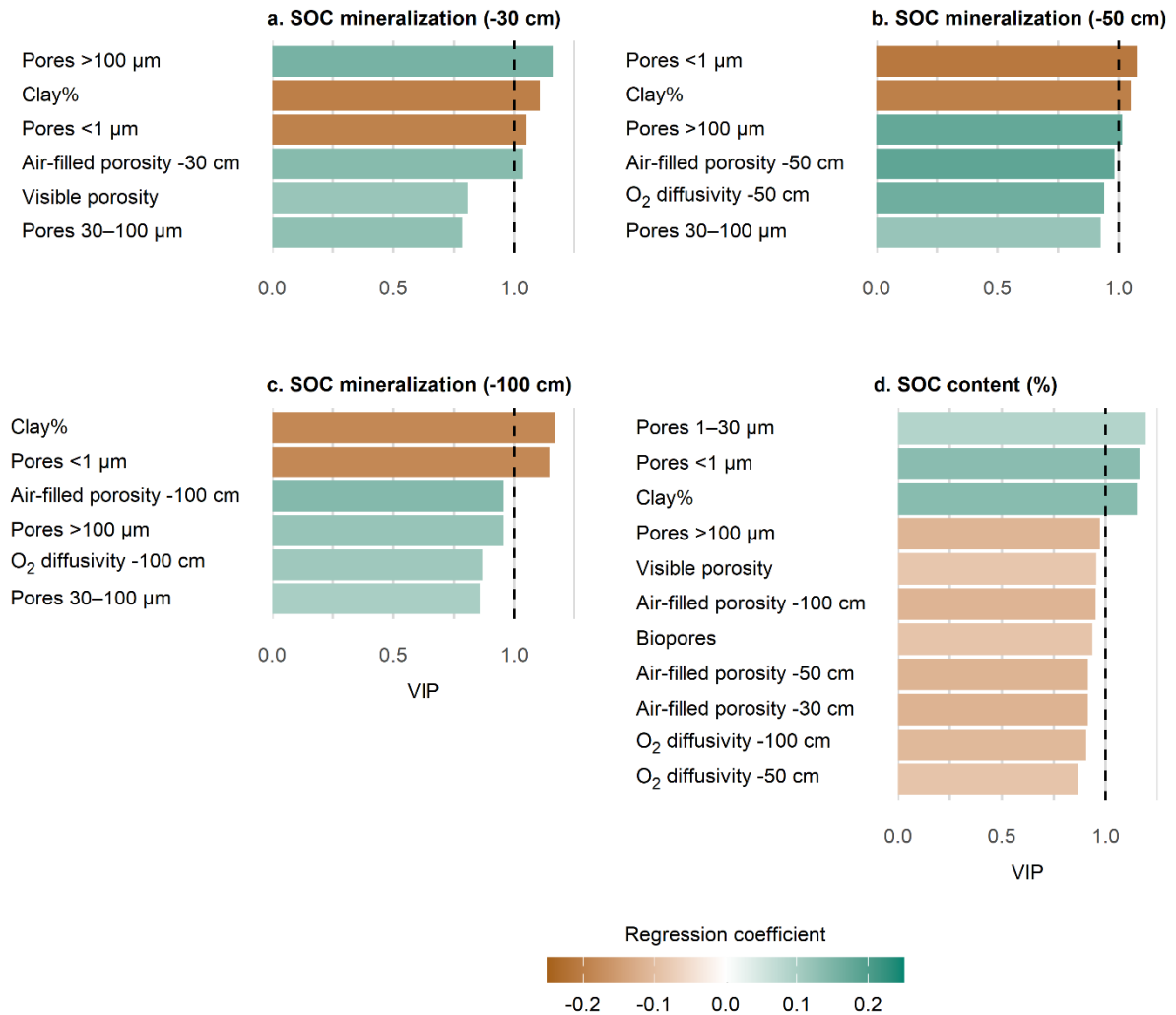
Pore architecture and soil texture jointly explain SOC mineralization and concentration

Pore architecture metrics and soil texture collectively explained 53–85% of the variance in cumulative SOC mineralization across moisture conditions and in SOC concentration, with distinct and partially opposing predictor sets for carbon turnover versus carbon storage (Fig. 5). Macropore abundance and air-filled porosity were positively associated with cumulative mineralization but not SOC concentration, while fine pore abundance and clay content showed the opposite pattern. At –30 cm matric potential, the wettest condition tested, >100 μm pores and air-filled porosity emerged as the strongest positive predictors of mineralization ($\text{VIP} \geq 1$), alongside clay content and <1 μm pores as negative predictors; as matric potential decreased, the importance of large pores and air-filled porosity diminished while clay content and <1 μm pores remained consistently negative predictors. This shift indicates that mineralization depends on bulk aeration near saturation but becomes increasingly constrained by fine-pore architecture and clay content as soils dry.

For SOC concentration, the 1–30 μm pore class, <1 μm pores, and clay content were the most influential predictors ($\text{VIP} \geq 1$), all positively associated with SOC. The positive association of the 1–30 μm class with SOC concentration is notable given that this pore domain tracks tillage rather than texture (Fig. 1a), suggesting a management-driven contribution to SOC stabilization distinct from clay-associated mineral protection. The collinearity between clay content and <1 μm pores reflects their shared structural origin, meaning the relative contributions of mineral surface interactions and pore-scale oxygen accessibility cannot be fully disentangled here.

Together, these results indicate that macropore abundance and air-filled porosity promote carbon turnover, particularly under wetter conditions, while fine-pore abundance and clay content favor SOC storage but constrain mineralization. This reinforces the partial decoupling, established in the previous section, between pore-scale conditions favoring anaerobic microbial potential and bulk SOC mineralization measured at the core scale.

Fig. 5: Partial least square regression (PLSR) variable importance and regression coefficients for SOC concentration and cumulative mineralization.



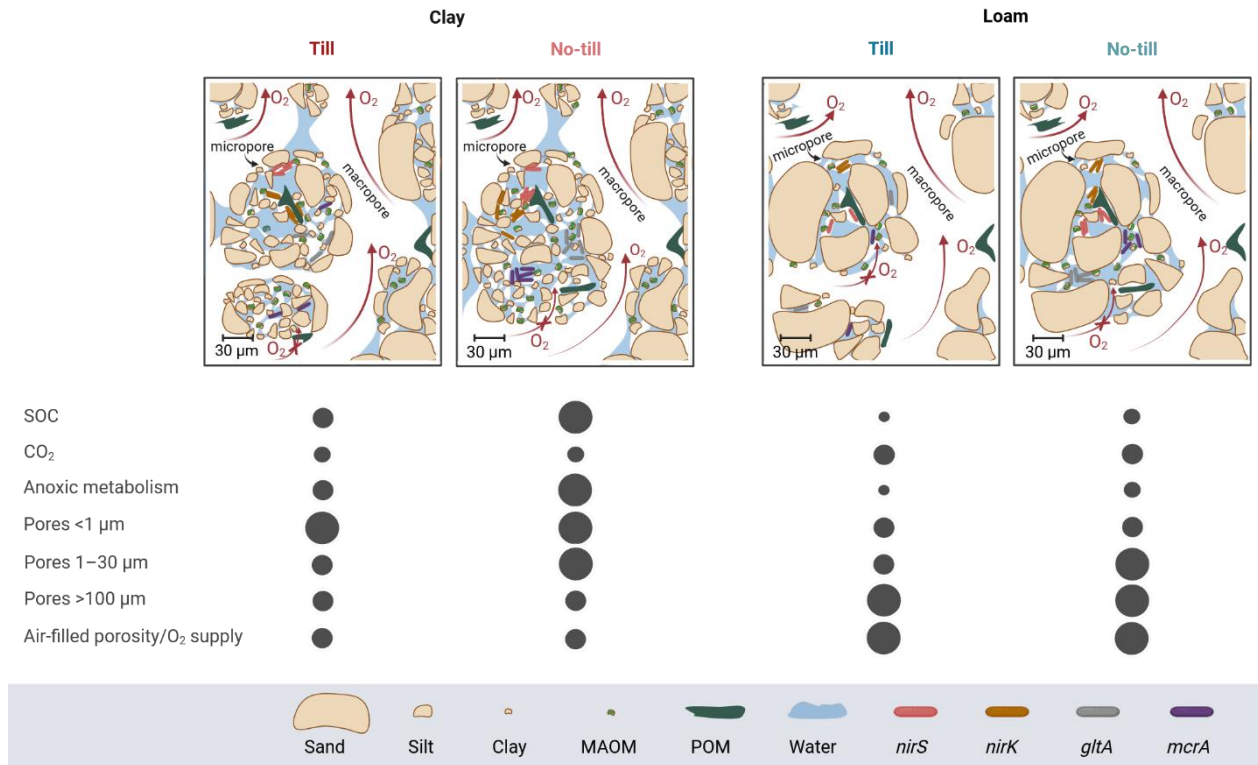
Regression coefficients and variable importance (VIP) scores from PLSR models relating soil structural and aeration variables to (a) cumulative SOC mineralization at -30 cm, (b) cumulative SOC mineralization at -50 cm, (c) cumulative SOC mineralization at -100 cm matric potential, and (d) SOC content. Predictors were standardized and pre-screened prior to model fitting. The dashed vertical line indicates a VIP threshold of 1, with predictors exhibiting VIP ≥ 1 considered the most influential contributors to model performance. Teal bars indicate positive regression

coefficients and orange-brown bars indicate negative regression coefficients. Final models retained one latent component and explained 61%, 76%, 53%, and 85% of the variance in cumulative mineralization at -30, -50, and -100 cm and SOC concentration, respectively.

Pore-scale oxygen accessibility as an underquantified component of physical protection

Physical protection has long been invoked to explain SOC accrual under conservation management, but the specific pore-scale processes underpinning it have remained poorly quantified^{11,47}. Prior studies report shifts toward finer pore classes under no-till relative to conventional tillage^{23,24,48}, but most pore-size classifications are too coarse to isolate the 1–30 μm range specifically. Our findings demonstrate that no-till selectively expands this pore class, a structural domain associated with anaerobic microsite development and SOC stabilization that operates independently of texture-driven mineral protection and without suppressing bulk respiration (Fig. 6). Together, these findings support pore-scale oxygen accessibility as an important, previously underquantified component of the physical protection of SOC, complementary to organo-mineral stabilization. Incorporation of these pore-scale controls on oxygen accessibility into SOC models may help us improve predictions of carbon persistence under changing management and climate conditions.

Fig. 6: Conceptual synthesis linking functional pore domains, oxygen accessibility, microbial metabolism, and SOC dynamics across texture and tillage treatments.



Schematic representation of clay and loam soils under conventional tillage and no-till management. Visual elements summarize the main structural and functional patterns observed across treatments. Clay-rich soils are depicted with greater <1 μm pore volume and lower air-filled porosity, whereas loam soils are depicted with greater >100 μm pore volume and higher oxygen supply. No-till management is represented as selectively increasing the 1–30 μm pore domain, which was strongly associated with anaerobic functional gene abundances. Below each treatment panel, relative levels of SOC concentration, cumulative SOC mineralization, anaerobic functional potential, <1 μm pores, 1–30 μm pores, >100 μm pores, and air-filled porosity are shown qualitatively. Anaerobic functional potential is illustrated using symbolic representations of microbial genes involved in denitrification (*nirK* and *nirS*), iron reduction (*gltA*), and methanogenesis (*mcrA*). The synthesis illustrates that pore-scale conditions associated with anaerobic microbial potential do not necessarily scale to lower bulk SOC mineralization,

highlighting a partial decoupling between microbial habitat structure and whole-core carbon turnover. Some elements were created in BioRender.

Methods

Study site and long-term field experiment

The soil was collected from a long-term tillage field experiment located at Agroscope Nyon, Switzerland (46°24' N, 06°14' E; 430 m.a.s.l.), established in 1969. The site experiences a mean annual temperature of 11.2 °C and a mean annual precipitation of 942 mm, based on 2000-2020 climate averages. The soil is classified as a Cambisol and comprises two spatially different soil textures within the same experimental area: a clay soil (48% clay, 37% silt, 15% sand) and a loam soil (25% clay, 45% silt, 30% sand)⁴³. The site contains a natural small-scale textural heterogeneity, resulting in contrasting clay and loam soils within the same experimental field.

The experiment follows a randomized block design with replicated tillage treatments on both soil textures. Here, we focused on two contrasting tillage systems: conventional tillage (CT), consisting of deep inversion ploughing to 20-25 cm, and no-till (NT), consisting of direct seeding without soil inversion. Conventional tillage has been applied continuously since the establishment of the experiment in 1969, whereas the no-till treatment was introduced in 2007 following the conversion from non-inversion tillage. Each tillage treatment is replicated three times on the clay soil and four times on the loam soil, with individual plots measuring ~304 m² (38 m × 8 m; Supplementary Fig. 10).

The cropping system follows a standardized rotation dominated by winter wheat (*Triticum aestivum* L.), interspersed with rapeseed (*Brassica napus* L.) and maize (*Zea mays* L.), with identical crop varieties, sowing dates, fertilization regimes, and plant protection measures applied across all treatments⁴³. This experimental design provides a unique opportunity to disentangle the

interactive effects of soil texture and long-term tillage management on soil structure, aeration, and carbon dynamics under otherwise identical climatic and agronomic conditions.

Soil sampling and experimental design

Soils were sampled from the topsoil (0–10 cm) on 30 April 2024, during the active growth phase of winter wheat and after sufficient post-tillage reconsolidation to ensure stable soil structure and suitable field conditions for sampling. Sampling focused on the central zone (~2.5–7.5 cm depth), where tillage effects on structure and SOC are typically most pronounced^{5,49}. Intact soil cores were collected using aluminum or steel sampling rings (5.0 cm inner diameter, 5.0 cm height; ~100 cm³ volume) with an Eijkelkamp sampler inserted carefully to preserve soil structure.

Initially, six intact soil cores were sampled per treatment (two soil texture and two tillage systems) by collecting two cores from each of three experimental plots. However, post hoc particle-size analysis showed that one plot classified as clay under conventional tillage had a loam texture. Consequently, this plot was reclassified, resulting in the following final number of intact cores per treatment and per set: clay conventional tillage (CCT, $n = 4$), clay no-till (CNT, $n = 6$), loam conventional tillage (LCT, $n = 8$), and loam no-till (LNT, $n = 6$). Detailed soil texture data for each plot are provided in the Supplementary Information (Supplementary Table 1).

Two independent sets of intact soil cores were established. The first set of cores ($n = 24$), hereafter set A, was used for determination of soil water retention curves, air permeability, and oxygen diffusivity. The second set of cores ($n = 24$), hereafter set B, was used for the SOC mineralization incubation experiment conducted under controlled matric potentials of –30, –50 and –100 cm (corresponding to pF values of 1.5, 1.7, and 2.0, respectively), and subsequently scanned by X-ray imaging, and finally destructively sampled for SOC content determination. In parallel, bulk soil samples were collected directly from the field plots at the time of intact core sampling to quantify microbial functional genes associated with anaerobic metabolism as described below.

Water retention curves and pore size distribution

Soil water retention curves were determined using 24 intact soil cores collected with steel cores (set A). A nylon mesh was attached to the bottom of each ring to retain soil during equilibration. Samples were saturated and placed on a pF laboratory station equipped with suction plates (ecoTech, Bonn, Germany) and sequentially equilibrated at matric potentials of -10, -30, -50, -100, and -300 hPa. At each pressure step, samples were weighed after equilibrium to determine volumetric water content. Additional water contents at -1000 and -3000 hPa were determined using a pressure plate apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Equivalent pore neck diameters corresponding to each matric potential were calculated using Jurin's law and perfect pore surface wettability, $d \approx -3000/\Psi$, where d is pore neck diameter (μm) and Ψ is matric potential (hPa). From these relationships, pore volume fractions ($\text{cm}^3 \text{ cm}^{-3}$) were derived for the following size classes: <1, 1–3, 3–10, 10–30, 30–60, 60–100, and 100–300 μm ^{27,50}.

For interpretation, pore size classes were grouped into four functionally defined domains based on their relevance for microbial activity and transport processes: <1 μm (mineral-associated and largely inaccessible to microorganisms), 1–30 μm (microbial-accessible but potentially oxygen-limited)^{11,26,28,39}, 30–100 μm (optimal balance of water and oxygen supply)^{11,25–27}, and >100 μm (macropores, well-aerated but water and nutrient limited). Pore size classes <100 μm were quantified from water retention data and represent equivalent pore neck diameters controlling water and gas transport.

Gas transport measurements

Gas transport properties were measured on the same 24 intact steel cores used for water retention analyses at matric potentials of -30, -50, and -100 cm (corresponding to approximately -3, -5, and -10 kPa, respectively; set A). These matric potentials represent relatively wet soil conditions ranging from near saturation (-30 cm) toward field capacity (-100 cm), where oxygen diffusion

constraints are expected to be most relevant in structured agricultural soils. Air permeability was measured first, immediately followed by oxygen diffusivity measurements, to ensure consistent soil physical conditions.

Air permeability (k_a) was determined using a steady-state airflow method similar to that described by Iversen et al. (2001)⁵¹ and Martínez et al. (2016)³⁶. Air was applied to the top of the soil core, while the base remained open and supported on a metal mesh to allow throughflow. A constant pressure difference of 2 hPa was applied across the soil core, and the stabilized volumetric airflow rate was recorded. Air permeability was calculated from the measured airflow rate, applied pressure gradient, sample geometry, and air viscosity, following Darcy's law³⁶. Instrument precision was assessed through calibration tests, and air permeability values below 2.3 μm^2 were considered below measurement precision. These values were assigned a value of 1.15 μm^2 (50 % of the detection threshold) for subsequent statistical analyses.

Oxygen diffusivity was measured using a one-chamber diffusion apparatus with O_2 as the diffusing gas, following the methodology described by Schjønning et al. (2013)⁵² and Martínez et al. (2016)³⁶. Soil cores were mounted in the diffusion chamber, which was flushed with O_2 -free N_2 prior to each measurement. The effective soil gas diffusion coefficient (D_p^*) was derived from the temporal change in the logarithm of the O_2 concentration difference between the chamber and ambient air, assuming steady-state diffusion conditions. Measured diffusion coefficients were corrected to standard temperature (293 K) and pressure (1000 mbar). Relative oxygen diffusivity (D_p/D_0) was calculated by normalizing D_p to the diffusion coefficient of O_2 in free air.

Instrument bias and precision were evaluated using airtight samples, yielding an apparent diffusivity of 0.00061 and an estimated limit of detection (LOD) of 0.001. Diffusivity values below the LOD were replaced with 0.0008, corresponding to the midpoint between the instrumental bias and detection limit. Subsequently, the instrumental bias (0.00061) was subtracted from all

diffusivity measurements prior to statistical analyses, resulting in corrected below-LOD values of approximately 0.0002.

Soil incubation experiment and SOC mineralization

The batch with 24 intact aluminum soil cores (set B) were used for the incubation experiment at ~20 °C under matric potentials –30, –50, and –100 cm. Prior to CO₂ flux measurements, all cores were pre-incubated for 10 days at –100 cm matric potential to re-establish microbial activity after sampling. Following pre-incubation, soils were saturated with demineralized water and subsequently equilibrated on a sandbox system (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) to a matric potential of –30 cm until hydraulic equilibrium was reached. Equilibrium was defined as a mass change of <0.2 g between two consecutive weighing days. After equilibration, soil CO₂ efflux was measured at days 1, 3, and 5. The same procedure was subsequently repeated after stepwise adjustment of soil matric potential to –50 cm and –100 cm, with equilibration to each matric potential followed by CO₂ measurements at days 1, 3, and 5. In total, CO₂ efflux was quantified nine times per core throughout the incubation, which lasted approximately 44 days from the first measurement at –30 cm.

Soil CO₂ efflux was measured using a closed-chamber approach^{34,53}. Each soil core was placed in an opaque airtight chamber with a fixed headspace volume of 0.2796 L. Chambers were equipped with a battery-powered fan to ensure air mixing and a pressure vent to avoid pressure artifacts during measurement. Changes in headspace CO₂ concentration were monitored in real time using a cavity ring-down spectroscopy analyzer (G2201-i isotopic CO₂/CH₄ analyzer, Picarro, United States) in a closed-loop (recirculating) set-up. Chambers were sequentially connected to the analyzer, and CO₂ concentrations were recorded every 1-2 s over a measurement period of ~10 min.

A linear model was fitted to the increase in headspace CO₂ concentration over time, and the slope was used to calculate soil CO₂ efflux rates. Fluxes were converted to mass-based units (mg CO₂-C kg⁻¹ dry soil d⁻¹) using the ideal gas law. To account for differences in SOC content among treatments, CO₂ efflux rates were normalized by SOC concentration, yielding units of mg CO₂-C g⁻¹ SOC d⁻¹ (Supplementary Fig. 9). Cumulative SOC mineralization was calculated by integrating CO₂ efflux over time. Fluxes measured at days 1, 3, and 5 were multiplied by the corresponding mid-interval durations since the start of each matric potential phase and summed sequentially to obtain cumulative emissions (mg CO₂-C g⁻¹ SOC).

X-ray imaging

X-ray imaging was performed on the 24 intact aluminum soil cores (set B) following completion of the SOC mineralization experiment at -100 cm. Scanning was conducted using a SkyScan 1273 system (Bruker, Billerica, MA, USA). Images were acquired with an X-ray source voltage of 130 kV and a current of 115 μ A, using a 0.5 mm copper filter to reduce beam-hardening artefacts. Each scan consisted of 2000 radiographs acquired with four-frame averaging, an exposure time of 1224 ms per frame, and an isometric voxel edge length of 33 μ m. The total scan duration per sample was approximately 2 h.

Raw projections were reconstructed into three-dimensional image stacks using GPUReconServer and NRecon software (Bruker). Subsequent image processing and analysis were performed in ImageJ/FIJI using the SoilJ plugin⁵⁴. To reduce noise and enhance structural features, reconstructed images were filtered using a 2 $\times$ 2 $\times$ 2 median filter followed by a 3-D unsharp mask (σ = 2, weighting factor = 0.6). The aluminum core walls were automatically detected in SoilJ and used to define the region of interest.

Greyscale calibration was applied by scaling each horizontal slice between the 0.1 percentile (representing air-filled pores) and the greyscale value of the aluminum wall, allowing normalization

across samples and construction of a joint greyscale histogram. Air-filled porosity was segmented from the soil matrix using the minimum method applied to the joint histogram.

From the segmented air phase, we quantified visible porosity, pore surface area, and pore diameter distributions using the method of maximally inscribable spheres. These metrics represent geometrically resolved macropores ($>100\text{ }\mu\text{m}$), given the effective resolution of the X-ray imaging, and therefore complement pore neck diameter information derived from water retention curves. Air-phase connectivity was characterized using multiple metrics, including the fraction of air-filled porosity connected to the top surface, the percolating air phase connecting top and bottom of the core, connection probability and Euler number^{55,56}. Biopores were identified using morphological criteria implemented in SoilJ, following the approach of Lucas et al. (2022)⁵⁷. Particulate organic matter (POM) was delineated using greyscale thresholding, with thresholds determined by visual inspection and refined using morphological operations to minimize partial volume effects³⁵.

Finally, distances from each soil matrix voxel to the nearest air-filled pore connected to the soil surface were calculated⁵⁸, providing an estimate of the spatial extent of potentially oxygen-limited domains. These distance maps were further combined with the binary POM phase to quantify the spatial distribution of organic matter relative to venting pores.

Soil organic carbon (SOC) concentration

After completion of the incubation experiment and X-ray scanning, all soil cores were destructively sampled. Soils were homogenized, oven-dried, sieved, and finely ground prior to SOC analysis. Total organic carbon content was determined using a Rock-Eval® 6 analyzer (Vinci Technologies, Rueil-Malmaison, France)⁵⁹, which quantifies organic carbon through programmed pyrolysis under inert atmosphere followed by oxidation, across a temperature range of 200–850 °C. To correct for potential misattribution of mineral carbon to organic carbon during Rock-Eval® analysis, the SOTHIS correction method was applied to all samples following Hazera et al. (2023).

Microbial functional gene quantification

At the time of intact core sampling, disturbed bulk soil was collected from 0-10 cm depth for microbial functional gene analysis. For each treatment, three composite soil samples were obtained by collecting soil from six randomly distributed points within each experimental plot. Soils were sampled under field-moist conditions, corresponding to mean WFPS values of approximately 84% in clay soils and 74% in loam soils. Subsamples of the homogenized soil were transferred into sterile Whirl-Pak® plastic bags and immediately placed in a cooler with ice packs. Samples were shipped with ice packs to the University of Bari (Italy) and received within 48 h of sampling. Upon arrival, soils were stored at -80 °C until DNA extraction.

Total soil DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals™, USA) following the manufacturer's protocol. DNA concentrations were quantified using a Qubit® 3.0 fluorometer with the dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific Inc., USA). Quantitative PCR (qPCR) was performed in triplicate to quantify gene copy numbers associated with anaerobic microbial metabolism, including denitrification (*nirK* and *nirS*), iron reduction (*gltA*), sulfate reduction (*dsrAB*), and methanogenesis (*mcrA*). Primer sequences, annealing temperatures, and template DNA dilutions are reported in Supplementary Table 2. Primer sets and assay conditions followed established protocols^{30,60}.

qPCR reactions were carried out in 20 µL volumes containing iTaq Universal SYBR Green Supermix (KAPA Biosystems, USA), ROX reference dye, gene-specific primers, bovine serum albumin, and template DNA (<10 ng per reaction). Amplifications were conducted on an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Specificity of amplification was verified by melting curve analysis following each qPCR run. Standard curves were generated using 10-fold serial dilutions of purified PCR products obtained from positive control samples. PCR products were excised from agarose gels, purified using the NucleoSpin®

Gel and PCR Clean-up kit (Macherey-Nagel, Germany), and quantified prior to dilution. Finally, functional gene abundances were expressed as gene copy numbers per gram of dry soil.

Statistical analysis

All statistical analyses were conducted in R (v4.5.1) and SAS (v9.4; SAS Institute Inc.), with significance set at $p < 0.05$. Linear mixed-effects models were implemented in SAS and in R using the lme4⁶¹ package. Model assumptions were evaluated by visual inspection of residual diagnostics (Q-Q plots, histograms, residuals vs. fitted values) and by the Shapiro-Wilk test; response variables were log-transformed when necessary. Soil structural properties, gas transport parameters, spatial oxygen saturation, SOC concentrations, and SOC mineralization were analyzed using linear mixed-effects models to account for hierarchical structure and repeated measurements. Soil texture, tillage, and moisture were included as fixed effects where appropriate, and sampling plot, soil core, incubation ring, or time were specified as random effects to account for spatial and temporal dependence. Functional gene abundances were analyzed using two-way ANOVA with texture and tillage as fixed factors.

Pearson or Spearman correlations were computed for normally and non-normally distributed variables, respectively, to assess collinearity among soil structural variables, and were visualized using the R package corrplot⁶².

Relationships between soil structural and aeration variables and SOC concentration and cumulative SOC mineralization at -30, -50, and -100 cm matric potential were evaluated using PLSR (R pls⁶³ package). Predictors were pre-screened by retaining variables with $|r| \geq 0.5$ (Pearson) with the response variable. All predictors were standardized (mean = 0, SD = 1), and models were fitted using leave-one-out cross-validation. The optimal number of latent components was determined by identifying the minimum adjusted root mean squared error of prediction (adjRMSEP). Final models retained one component for SOC concentration, and cumulative

mineralization at –30, –50 cm and –100 cm matric potential. Influential predictors were identified using VIP scores (>1), and standardized regression coefficients were used to determine relationship direction. Final PLSR models explained 85% of the variance in SOC concentration and 61%, 76%, and 53% of the variance in cumulative SOC mineralization at –30, –50, and –100 cm matric potentials, respectively.

Data availability

Source data are provided with this paper. The complete source dataset has been deposited in Zenodo (DOI: <https://doi.org/10.5281/zenodo.21362312>). The dataset will be made publicly available upon publication of the manuscript.

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776 **Author contributions**

777 O.M. conceived and designed the study, performed water retention curve measurements, gas
778 transport measurements, X-ray imaging, SOC mineralization experiments and SOC
779 determinations, analyzed all data, and wrote the first draft of the manuscript. J.K. assisted with
780 water retention curve and gas transport measurements, and X-ray data analysis. L.B. and T.G.
781 assisted with field sampling and provided access to the long-term field experiment. B.P. assisted
782 with X-ray scanning. M.Y. performed microbial functional gene quantification and analysis. H.D.
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785 research project within which this study was embedded and supervised the research. All authors
786 reviewed and edited the manuscript and approved the final version.

787 **Competing interests**

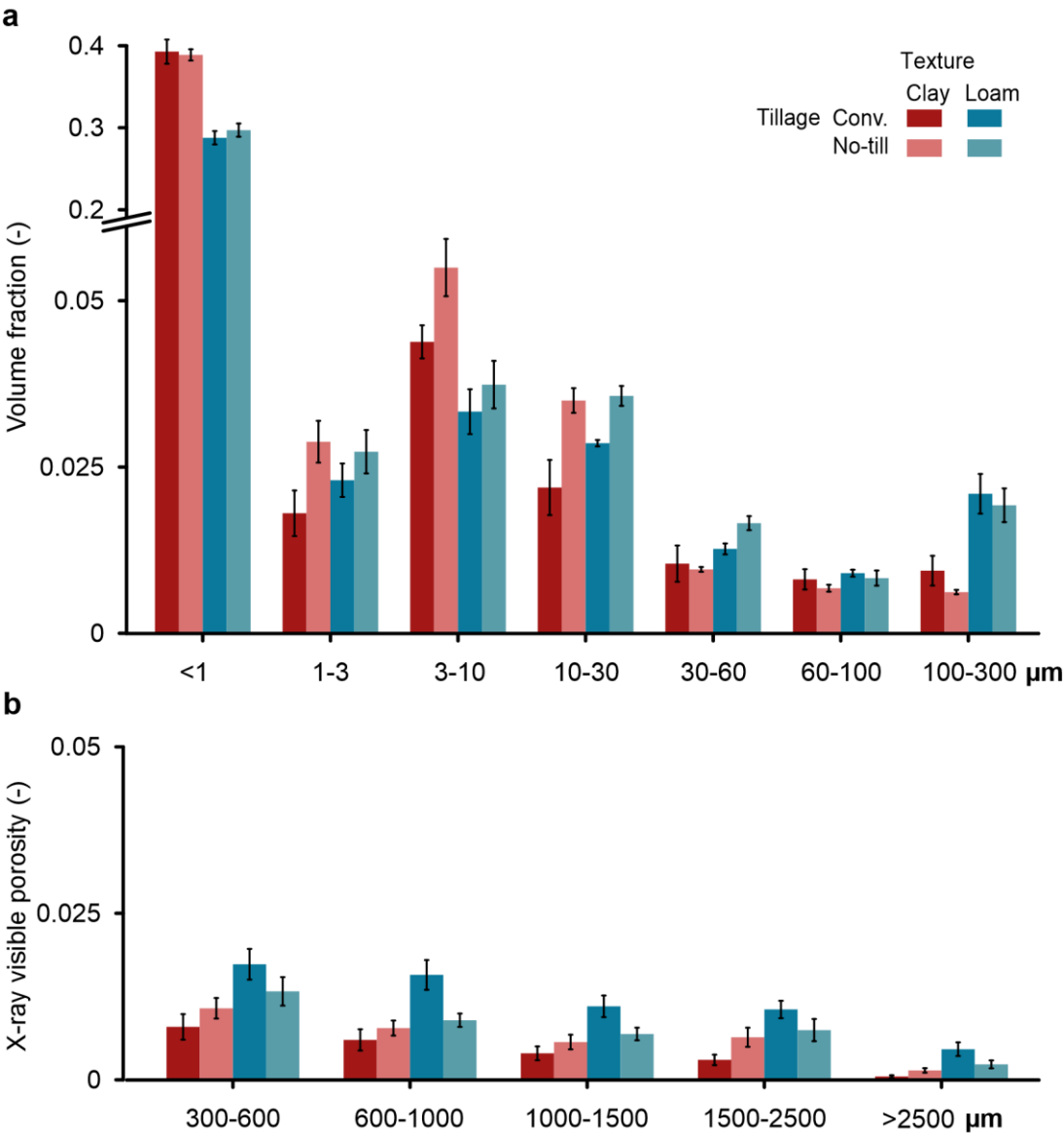
788 The authors declare no competing interests.

Supplementary material

Soil pore architecture regulates carbon persistence via anaerobic microsites

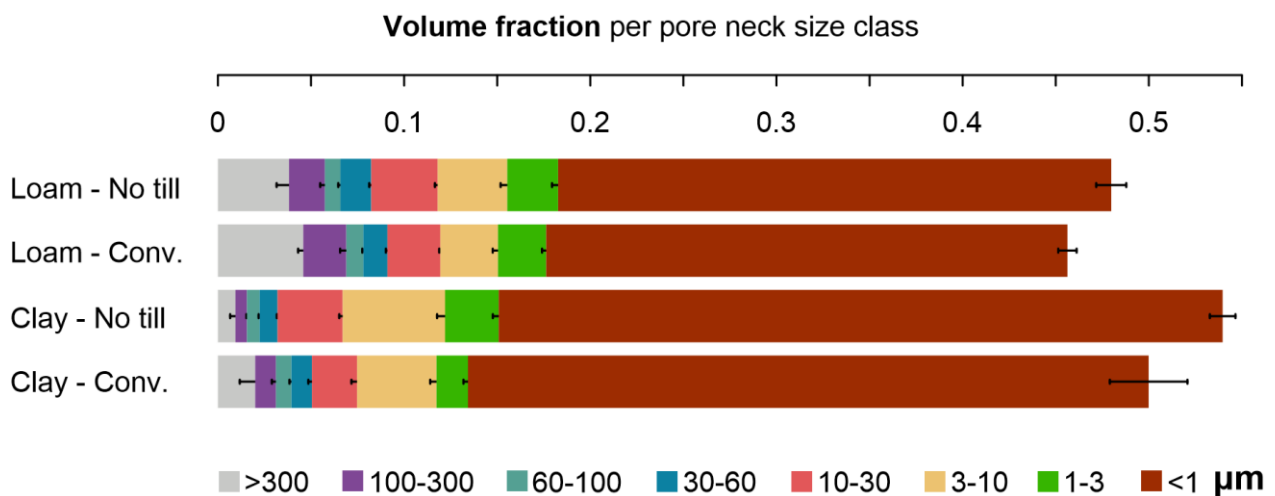
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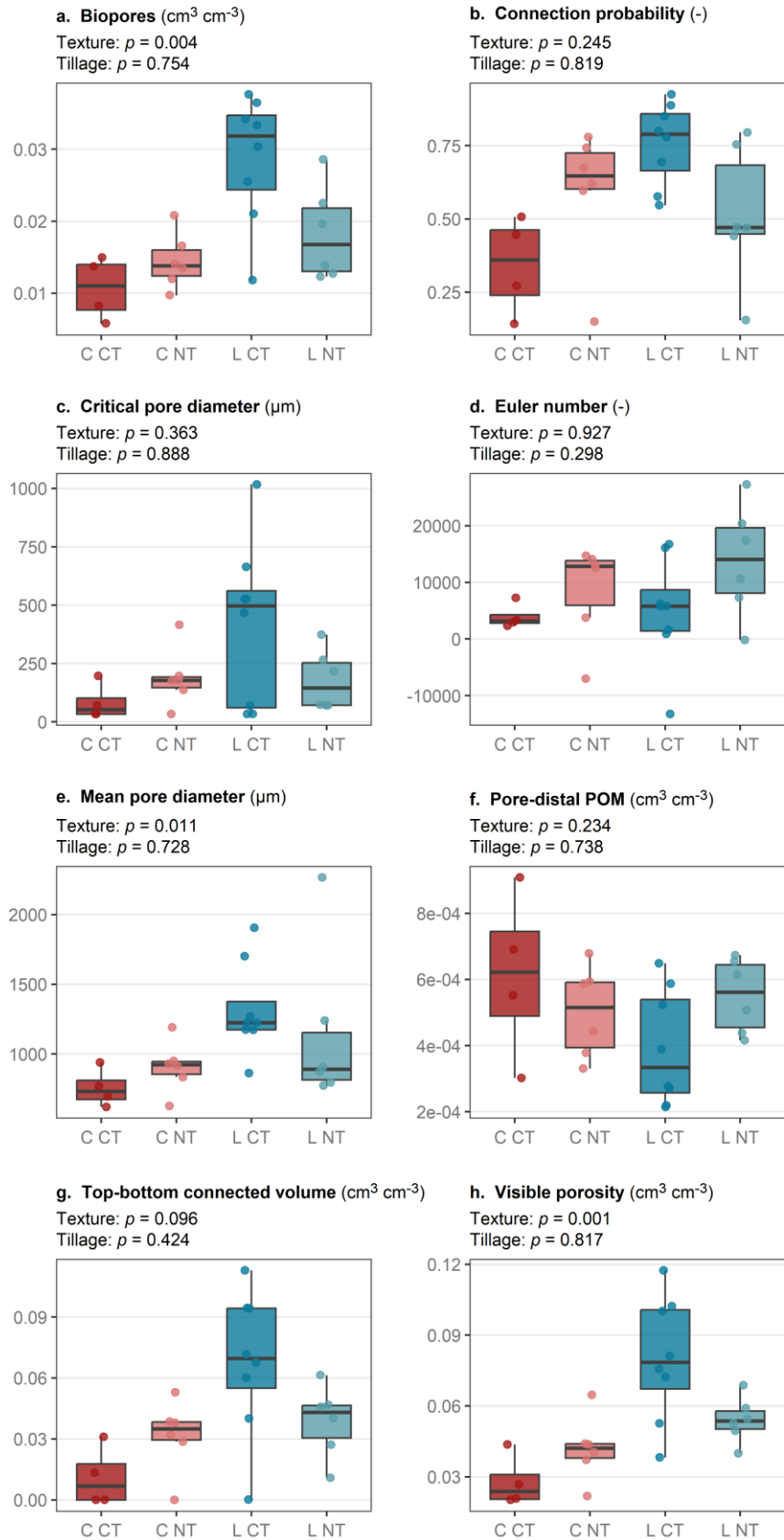


Supplementary Fig. 1. a Pore volume fractions (cm³ cm⁻³ soil) across pore neck size classes <1, 1–3, 3–10, 10–30, 30–60, 60–100, and 100–300 μm derived from water retention curves using an

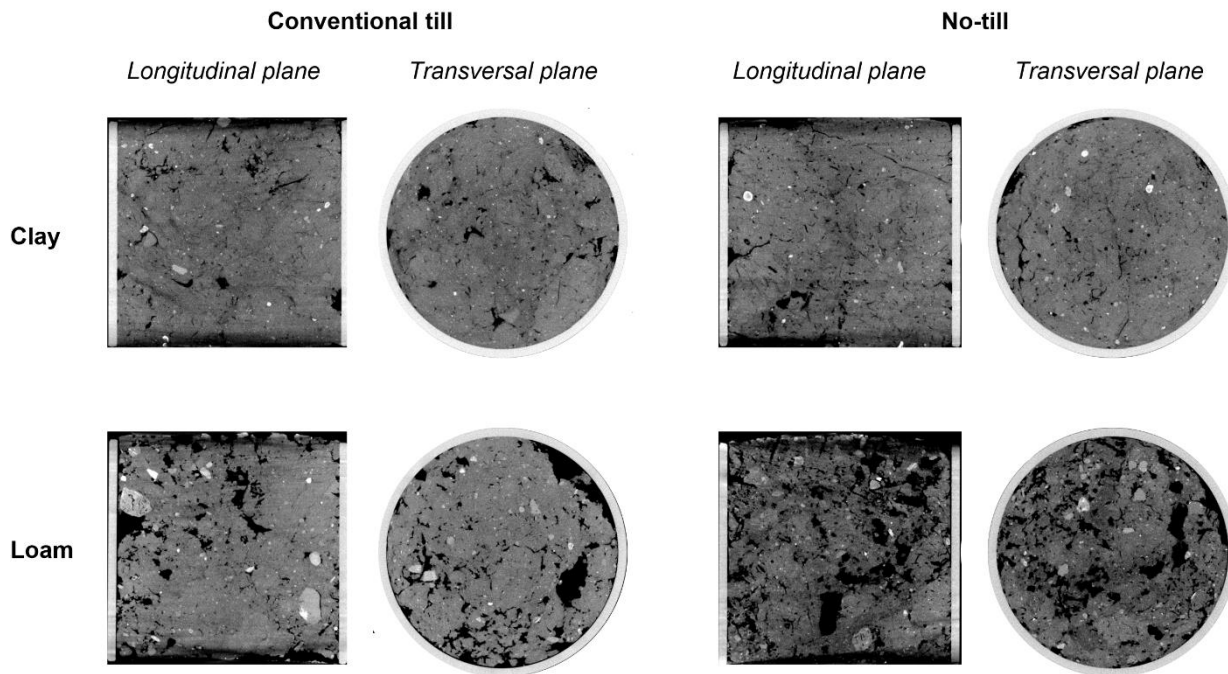
independent set of intact soil cores (set A). **b** Visible pore volume fractions across pore size classes 300–600, 600–1000, 1000–1500, 1500–2500, and >2500 μm derived from X-ray imaging of intact soil cores following the incubation experiment (set B). Data are shown for clay (C) and loam (L) soils under conventional tillage (CT) and no-till (NT) management. For both panels, means of $n = 4$ independent replicates for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT are shown with standard errors as errors bars.



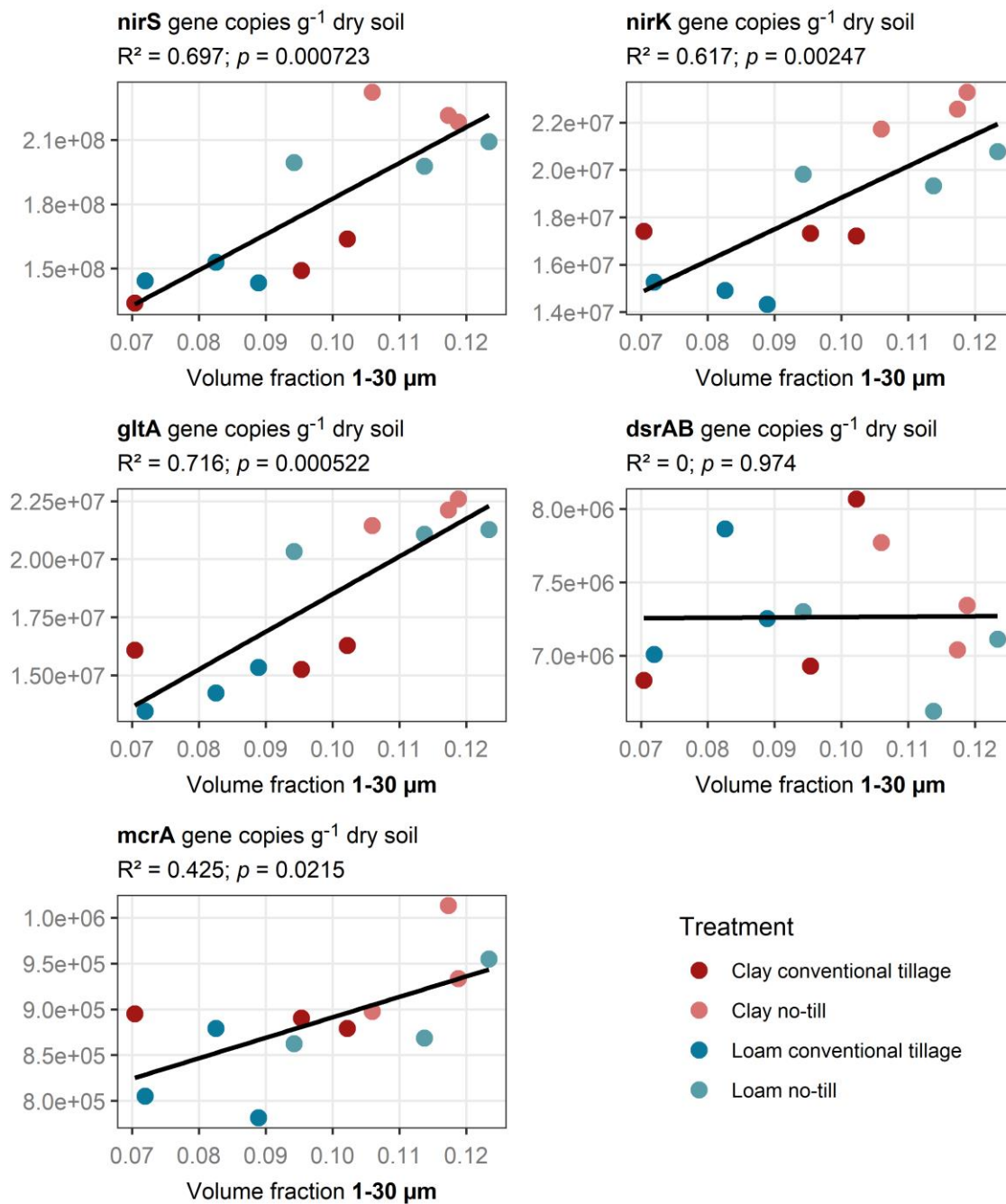
Supplementary Fig. 2. Total porosity and volume fractions across pore size classes in intact soil cores from clay and loam soils under conventional and no-till management, derived from water retention curves. Bars represent means of $n = 4$ independent replicates for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT, and standard errors as error bars.



Supplementary Fig. 3. Three-dimensional pore network metrics derived from X-ray images of intact soil cores collected after the incubation experiment, including (a) biopores ($\text{cm}^3 \text{cm}^{-3}$), (b) connection probability (-), (c) critical pore diameter (μm), (d) Euler number (-), (e) mean pore diameter (μm), (f) pore-distal particulate organic matter (POM; $\text{cm}^3 \text{cm}^{-3}$), (g) top-bottom connected pore volume ($\text{cm}^3 \text{cm}^{-3}$), and (h) visible porosity ($\text{cm}^3 \text{cm}^{-3}$). Data are shown for clay (C) and loam (L) soils under conventional tillage (CT) and no-till (NT) management. For all panels, individual data points and boxplots are shown for $n = 4$ independent replicates for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT. Statistical analyses were conducted using linear mixed-effects models with texture, tillage, and their interaction as fixed factors and sampling plot as a random factor. P values for texture and tillage effects are shown within each panel.

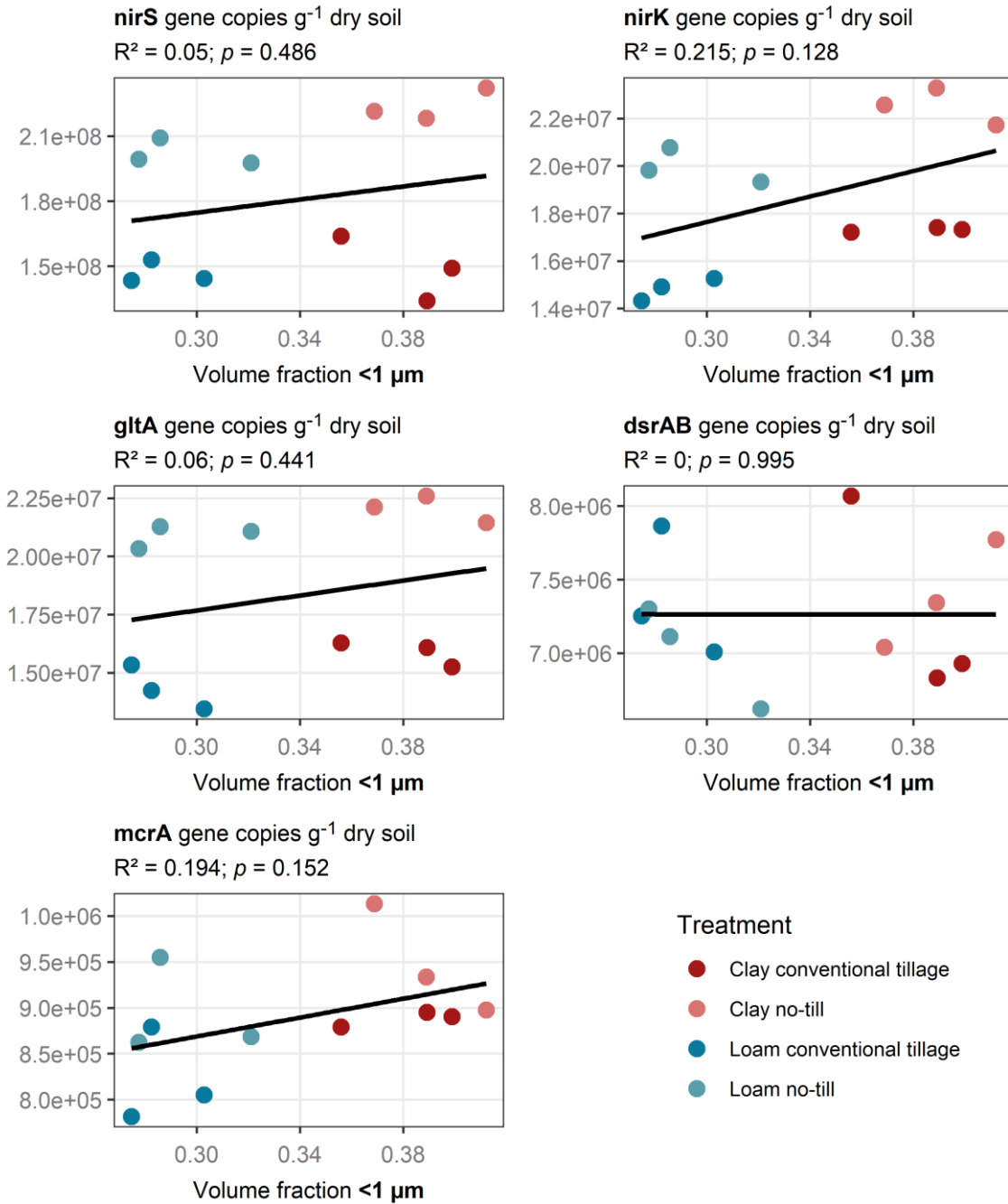


Supplementary Fig. 4. Longitudinal and transversal views of representative X-ray images of intact soil cores (5 cm diameter, 5 cm height) collected from clay and loam soils under conventional and no-till management.



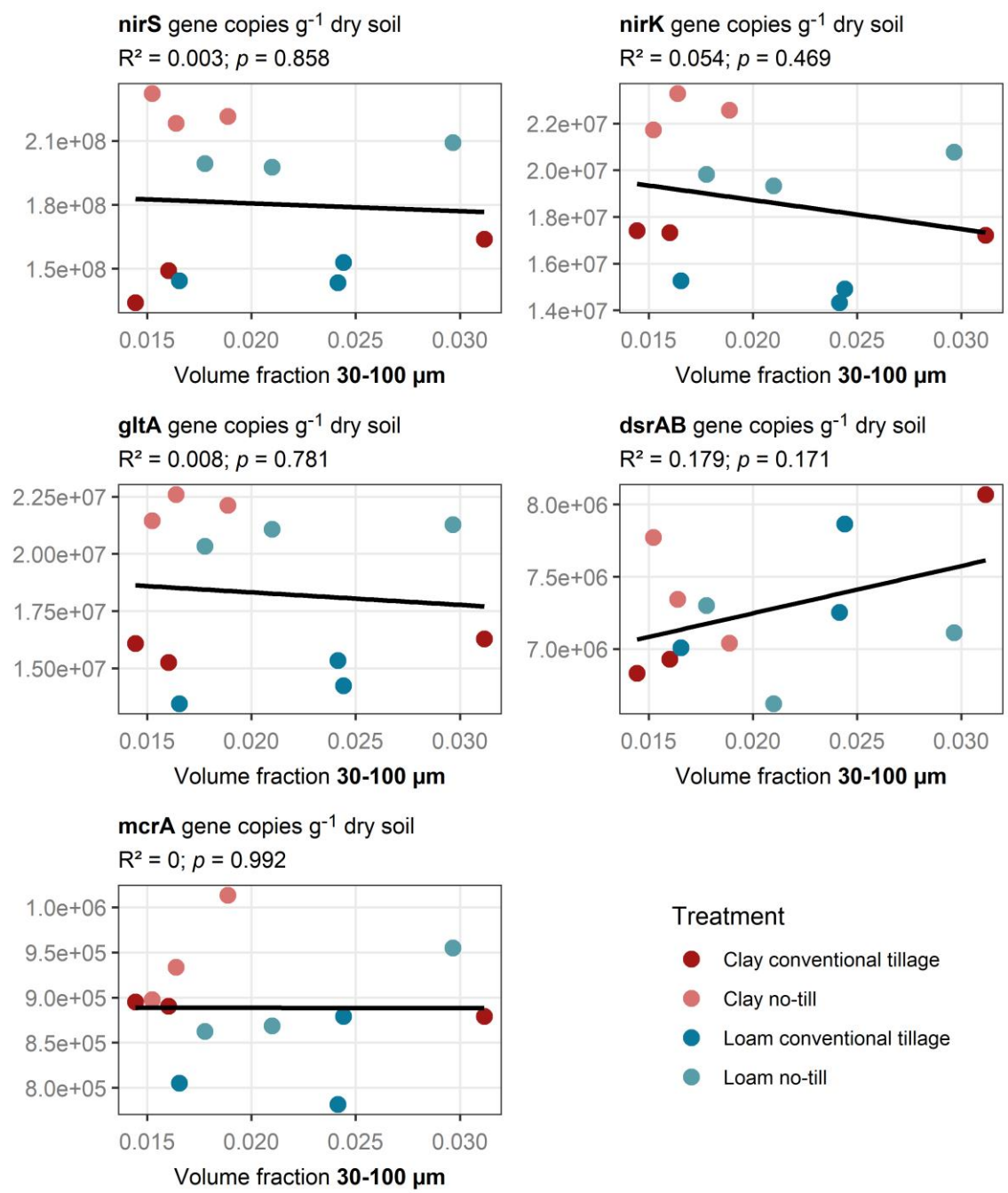
Supplementary Fig. 5. Scatter plots show linear regressions between the volume fraction of 1–30 μm pores (cm³ cm⁻³ soil) and gene copy numbers per gram dry soil for **a.** *nirS*, **b.** *nirK* (denitrification), **c.** *gltA* (iron reduction), **d.** *dsrAB* (sulfate reduction), and **e.** *mcrA*

(methanogenesis). For each regression, treatment means of pore volume fractions ($n = 12$ treatment-level observations, randomly subsampled to match the $n = 3$ field replicates per treatment available for gene quantification) were paired with corresponding gene copy numbers. Points are colored by treatment: clay conventional tillage (CCT), clay no-till (CNT), loam conventional tillage (LCT), and loam no-till (LNT). R^2 and p-values are shown for each regression.



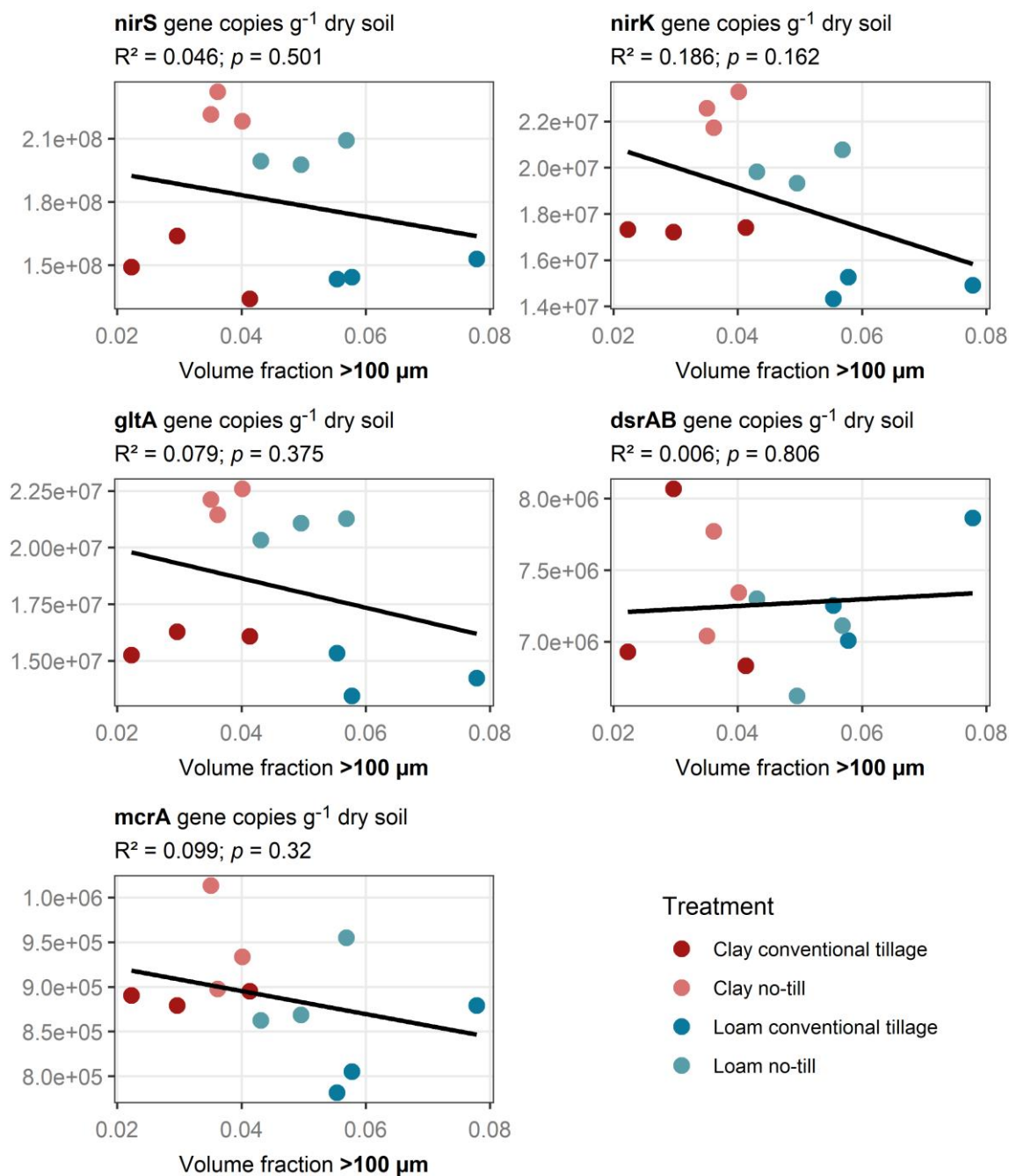
52
53
54 **Supplementary Fig. 6.** Scatter plots show linear regressions between the volume fraction of <1
55 μm pores (cm³ cm⁻³ soil) and gene copy numbers per gram dry soil for **a.** *nirS*, **b.** *nirK*
56 (denitrification), **c.** *gltA* (iron reduction), **d.** *dsrAB* (sulfate reduction), and **e.** *mcrA*
57 (methanogenesis). For each regression, treatment means of pore volume fractions (*n* = 12

58 treatment-level observations, randomly subsampled to match the $n = 3$ field replicates per
 59 treatment available for gene quantification) were paired with corresponding gene copy numbers.
 60 Points are colored by treatment: clay conventional tillage (CCT), clay no-till (CNT), loam
 61 conventional tillage (LCT), and loam no-till (LNT). R^2 and p -values are shown for each regression.



62

64 **Supplementary Fig. 7.** Scatter plots show linear regressions between the volume fraction of 30-
65 100 μm pores ($\text{cm}^3 \text{ cm}^{-3}$ soil) and gene copy numbers per gram dry soil for **a.** *nirS*, **b.** *nirK*
66 (denitrification), **c.** *gltA* (iron reduction), **d.** *dsrAB* (sulfate reduction), and **e.** *mcrA*
67 (methanogenesis). For each regression, treatment means of pore volume fractions ($n = 12$
68 treatment-level observations, randomly subsampled to match the $n = 3$ field replicates per
69 treatment available for gene quantification) were paired with corresponding gene copy numbers.
70 Points are colored by treatment: clay conventional tillage (CCT), clay no-till (CNT), loam
71 conventional tillage (LCT), and loam no-till (LNT). R^2 and p-values are shown for each regression.



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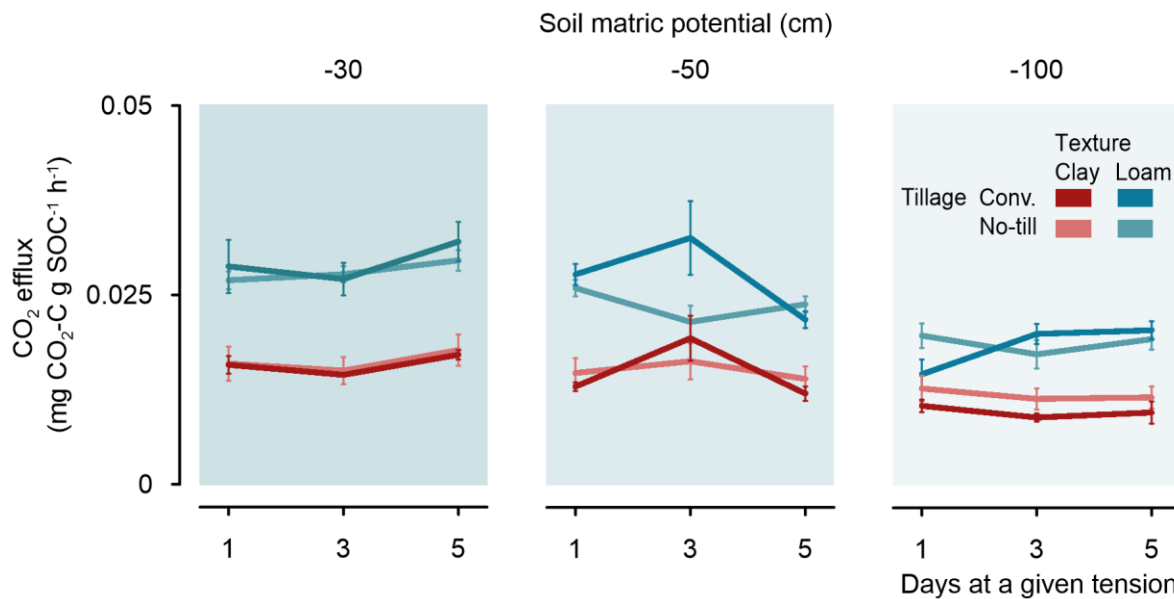
73

74 **Supplementary Fig. 8.** Scatter plots show linear regressions between the volume fraction of >100

75 μm pores ($\text{cm}^3 \text{ cm}^{-3}$ soil) and gene copy numbers per gram dry soil for **a.** *nirS*, **b.** *nirK*

76 (denitrification), **c.** *gltA* (iron reduction), **d.** *dsrAB* (sulfate reduction), and **e.** *mcrA*

(methanogenesis). For each regression, treatment means of pore volume fractions ($n = 12$ treatment-level observations, randomly subsampled to match the $n = 3$ field replicates per treatment available for gene quantification) were paired with corresponding gene copy numbers. Points are colored by treatment: clay conventional tillage (CCT), clay no-till (CNT), loam conventional tillage (LCT), and loam no-till (LNT). R^2 and p-values are shown for each regression.



Supplementary Fig. 9. CO₂ fluxes normalized by SOC content (mg CO₂-C g SOC⁻¹ h⁻¹) measured over a 5-day incubation at -30, -50, and -100 cm matric potential. Measurements were conducted on intact soil cores incubated under controlled moisture conditions, with repeated measurements at days 1, 3, and 5 in clay (C) and loam (L) soils under conventional (CT) and no-till (NT) management. Dots and bars show means of $n = 4$ independent replicates for CCT, $n = 6$ for CNT, $n = 8$ for LCT, and $n = 6$ for LNT, and standard errors as error bars. CO₂ efflux was analyzed using a mixed-effects model including matric potential, texture, tillage, day, and their interactions as fixed effects and incubation ring as a random factor.

collected. Two independent sets of intact cores were sampled from each plot (48 cores in total): set A for water retention and gas transport analyses, and set B for incubation and X-ray imaging analyses.

Supplementary Table 1. Particle size distribution of the 12 sampled plots in the long-term experiment comprising two soil textures (clay and loam) and two tillage treatments (conventional and no-till).

Field plot	Soil texture	Tillage treatment	Clay (%)	Silt (%)	Sand (%)
1	clay	conventional	50.3	35.6	14.1
2	clay	conventional	51.2	34.3	14.4
3	clay	no till	51.4	36.6	12.0
4	clay	no till	51.8	37.3	11.0
5	clay	no till	52.2	34.6	13.2
6	loam	conventional	28.6	45.2	26.3
7	loam	conventional	27.6	44.4	28.0
8	loam	conventional	25.9	46.9	27.2
9	loam	conventional	28.5	42.0	29.5
10	loam	no till	26.5	44.7	28.8
11	loam	no till	25.0	45.6	29.5
12	loam	no till	27.7	41.5	30.8

113 **Supplementary Table 2.** Primers and annealing temperatures used for qPCR quantification of
 114 microbial functional genes.

Target process	Gene	Primer name	Primer sequence (5'–3')	Annealing temperature (°C)
Denitrification	<i>nirK</i>	nirK876	ATYGGCGGVCA YGGCGA	54
		nirK1040	GCCTCGATCAGRTT RTGGTT	54
Denitrification	<i>nirS</i>	nirScd3aFm	GTSAACGTSAAGGARACSGG	57
		nirSR3cdm	GASTTCGGRTGSGTCTTGA	57
Iron reduction	<i>gltA</i>	CS375nf	AACAAGATGRCMGCCTGGG	52
		CS598nR	TCRTGGTCGGARTGGAGAAT	52
Sulfate reduction	<i>dsrAB</i>	DSR1F+	ACSCACTGGAAGCACGGCGG	58
		DSR-R	GTGGMRCCGTGCAKRTTGG	58
Methanogenesis	<i>mcrA</i>	mlas	GGTGGTGTMGGDTTCACMCARTA	56
		mcrA-rev	CGTTCATBGCGTAGTTVGGRTAGT	56

115