Supplementary Information

Materials and Methods

Field Experiment and Soil Sampling – Soil used to generate biological (metagenomic) and physical (X-ray computed tomography) data was sampled in 2015 from four winter wheat plots of the Broadbalk Winter Wheat field experiment (51˚48'35" N, 00˚22'30" W, http://www.era.rothamsted.ac.uk/experiment/rbk1). The site was established in 1843 to investigate the long-term consequences of different fertiliser and manure applications on the yield of winter wheat, as well as the effect of cessation of all cultivation from a part of the site in 1882[1]. The plots were subject to contrasting fertility management: receiving 35 Mg ha^{-1} yr^{-1} composted farmyard manure from cattle (FYM); 144 kg-N ha^{-1} yr^{-1} as ammonium nitrate together with 90 kg-K ha^{-1} yr^{-1} as K_2SO_4, 35 kg-P ha^{-1} yr^{-1} as triple superphosphate [$Ca(H_2PO_4)_2 \cdot H_2O$], and 12 kg-Mg ha^{-1} yr^{-1} as $MgSO_4$ (^{144}NPK); 192 kg-N ha^{-1} yr^{-1} as ammonium nitrate together with 90 kg-K ha^{-1} yr^{-1} as K_2SO_4 and 12 kg-Mg ha^{-1} yr^{-1} as $MgSO_4$ (*i.e.* lacking phosphorus fertilisation, ^{192}NK); 90 kg-K ha^{-1} yr^{-1} as K_2SO_4, 35 kg-P ha^{-1} yr^{-1} as triple superphosphate, and 12 kg-Mg ha^{-1} yr^{-1} as $MgSO_4$ (*i.e.* lacking nitrogen fertilisation, PK); and soil which had never received any fertilisation for the whole duration of the experiment (nil). The managements are summarized in Table I of the main text. The experimental soil is a clay loam to silty clay loam over clay with flints (FAO Classification: Chromic Luvisol) and slightly calcareous. The experiment is under-drained and free draining. The arable treatment plots are on Section 1 of the experiment[2], sown continuously with winter wheat (*Triticum aestivum* L.) since 1843, except for occasional fallow years to control weeds. Crusoe (2013-2018) and Zyatt (2019) seed was coated with Redigo Deter (Bayer CropScience) combination insecticide/fungicide treatment. All arable soils are tilled conventionally each year to a depth of 23 cm. In addition to these regularly tilled arable soils, we also studied two soils which had not been subject to management for over a century: part of the original Broadbalk experiment which since 1882 has been allowed to revert naturally to a mixed species woodland of ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.) and hawthorn (*Crataegus monogyna* Jacq.)[3] which was sampled at the same time as the plots described above; soil

was also collected in 2015 from plots of the Highfield Ley-Arable experiment (http://www.era.rothamsted.ac.uk/experiment/rrn1) managed as a mown sward of mixed fescue (*Festuca pratensis* L.), Timothy grass (*Phleum pratense* L.) and white clover (*Trifolium repens* L.) since 1838 to generate soil structure information from X-ray computed tomography. Metagenomic data from this treatment was generated from soils collected in 2011 which have been described elsewhere[4-7] and deposited with the European Nucleotide Archive with accession number PRJEB43407. Since treatments are not replicated on the Broadbalk experiment, three *pseudo*-replicates were collected from each treatment plot for nucleic acid extraction and four replicates for X-ray computed tomography (CT) imaging. These pseudo-replicates were spaced equidistant along the 18-m long plot. Replicate plots are available on the Highfield Ley-Arable experiment and soil was sampled from each of three replicates. The top 10-cm of soil was sampled with a 3-cm diameter auger for nucleic acid extraction. For each *pseudo*-replicate, ten cores were pooled and mixed thoroughly whilst sieving through a 2-mm mesh. Samples were then frozen and stored at -80 °C. For X-ray computed tomography, cylindrical soil cores (6.8-cm diameter, 12.0-cm height) were collected and stored undisturbed in unplasticized polyvinyl chloride pipes at 4 °C until they were scanned.

Chemical properties of Broadbalk soils and crop performance have been measured routinely since the experiment inception. Historical data for soil organic carbon (SOC) and total nitrogen (N_{tot}) for the soils described above as well as soil receiving 240 kg-N ha^{-1} yr^{-1} as ammonium nitrate, 90 kg-K ha^{-1} yr^{-1} as K_2SO_4, 35 kg-P ha^{-1} yr^{-1} as triple superphosphate and 12 kg-Mg ha^{-1} yr^{-1} as $MgSO_4$ was taken from the *e*-RA database[8] maintained by Rothamsted Research. SOC and N_{tot} were measured by combustion using a Leco TruMac analyser (LECO, Stockport, UK). SOC was determined by subtraction of $CaCO_3$-C, measured by a calcimeter from total carbon estimates. SOC and N_{tot} stocks, as Mg ha^{-1}, were calculated from measured %SOC and %N and measured soil weight. Soil weight declines over time in the FYM-treated plots, as the addition of FYM reduces bulk density. We have adjusted for this decline by including an equal weight of subsoil[9]. Soil pH was measured in water (1:2.5 soil: solution). We also employed 2019-2020 data relating to precipitation (http://www.era.rothamsted.ac.uk/station/rms#measurements), measured on a 254 mm diameter

ARG100 rain gauge (Environmental Measurements Ltd., North Shields, UK) located 500 m east of Broadbalk and the temperature 20 cm below the surface of bare soil, measured by electronic temperature probe (type 107, Campbell Scientific, Loughborough, UK) from the *e*-RA database (http://www.era.rothamsted.ac.uk). Using precipitation data and estimates of water evaporation over grass ($EVAP_{grass}$), we calculated daily soil moisture deficits (*SMD*) as

$$SMD = (SMD_{acc} + EVAP_{grass}) - \text{precipitation}$$

where SMD_{acc} is an accumulated value, starting at the value for SMD at December 14th, 2018.

Soil Structure and Hydrodynamic Behaviour

X-ray Computed Tomography and Image Analysis – We generated X-ray CT images from all soils described above at 1.5 µm resolution and scales relevant to microbes (10^0-10^2 µm), requiring imaging of 0.7 – 2.0 mm diameter soil aggregates. The connectivity of pores within networks was assessed from binary images derived from X-ray CT. Aggregates were selected at random from soil collected from each plot of the Highfield experiment. Each was scanned using a Phoenix Nanotom system (GE Measurement and Control solution, Wunstorf, Germany) operated at 90 kV, a current of 65 µA and at a voxel resolution of 1.5 µm. Initial image analysis was performed using Image-J. Images were threshold-adjusted using the bin bi-level threshold approach[10] using QuantIm version 4.01.

Calculation of Diffusion in Soil Pore Networks - The hierarchical soil structures revealed in X-ray CT images indicate that gaseous O_2 in the atmosphere moves into soil primarily through its inter-aggregate pores and is then dissolved in water prior to moving into the aggregates largely by molecular diffusion. Since gaseous O_2 diffuses up to 10^3-fold more quickly than O_2 dissolved in water, microbial community activity is thus constrained mainly by O_2 diffusion within aggregates. The ability of aggregates to conduct dissolved O_2 and other soluble substrates depends on the intra-aggregate pore geometry, and we quantified it with effective diffusion coefficients calculated directly by mimicking solute movement through the pore geometry using numerical simulations. The movement of solutes, including O_2 and substrates, within the pore geometry is assumed to be

diffusion dominated. The temporal change in solute concentration inside any pore voxel can be calculated using the finite volume approach, as follows:

$$\frac{c_o^{t+\delta t}-c_o^t}{\delta t}=q_w+q_e+q_s+q_n+q_u+q_d,$$

$$q_w=\begin{cases}D\left(c_w^{t+\delta t}-c_o^{t+\delta t}\right) & if\ voxel\ w\ is\ pore\\ 0, & if\ voxel\ w\ is\ solid\end{cases},\ q_e=\begin{cases}D\left(c_e^{t+\delta t}-c_o^{t+\delta t}\right) & if\ voxel\ e\ is\ pore\\ 0, & if\ voxel\ e\ is\ solid\end{cases}$$

$$q_s=\begin{cases}D\left(c_n^{t+\delta t}-c_o^{t+\delta t}\right) & if\ voxel\ s\ is\ pore\\ 0, & if\ voxel\ s\ is\ solid\end{cases},\ q_n=\begin{cases}D\left(c_s^{t+\delta t}-c_o^{t+\delta t}\right) & if\ voxel\ n\ is\ pore\\ 0, & if\ voxel\ n\ is\ solid\end{cases}$$

$$q_d=\begin{cases}D\left(c_d^{t+\delta t}-c_o^{t+\delta t}\right) & if\ voxel\ d\ is\ pore\\ 0, & if\ voxel\ d\ is\ solid\end{cases},\ q_u=\begin{cases}D\left(c_u^{t+\delta t}-c_o^{t+\delta t}\right) & if\ voxel\ u\ is\ pore\\ 0, & if\ voxel\ u\ is\ solid.\end{cases} \quad (1)$$

where c is concentration, q is diffusive flux, D is molecular diffusion of the solute in liquid water, superscripts t and $t+\delta t$ represent time, δt is a time increment, subscript o represents the pore voxel being calculated, and subscripts w, e, s, n, u and d represents the face-to-face neighbours of voxel o on the west, east, south, north, top and bottom sides respectively. Applying Eq. (1) to all pore voxels leads to linear systems which was solved by the bi-conjugate gradient stabilized method[11].

Calculation of Diffusion Coefficients – To calculate the effective diffusion coefficient of each aggregate, we applied a constant concentration C_1 on the top and a constant concentration C_0 on the bottom of the image, and then simulated solute diffusion to steady state. The diffusive flux in the three directions in each pore voxel was calculated by Eq. 1. Taking the vertical direction as the z direction for the image, the effective diffusion coefficient of the image was calculated as follows:

$$D_{eff}=\frac{L_z\sum_{i=1}^{N}q_z(x_i)}{N\left(C_1-C_0\right)} \quad (2)$$

where D_{eff} is the effective diffusion coefficient, N is the total number of pore voxels in the simulated images, $q_z(x_i)$ is the vertical diffusive flux in pore voxel centred at location x_i, L_z is the height of the image. To address the impact of change in pore geometry due to management on the ability of the aggregate to diffuse solute, in result analysis we normalized the effective diffusion coefficient D_{eff} of all solutes by their associated molecular diffusion coefficient in non-constrained water, D.

Modelling of Oxygen Diffusion and Anoxia Within Soils - The influence of soil structure on O_2 diffusion and its subsequent consumption by microbes under various saturations was studied using pore-scale simulations. We first calculated the spatial distribution and connectedness of different pores and then determined water distributions in pores under different matric potentials (ψ_m). We assumed the soil was initially saturated and then applied a negative pressure p at the bottom to drain water. We assumed the soil was essentially hydrophilic in that only pores whose associated capillary pressure p_c, calculated by $p_c = \sigma / r$ with σ being water-air surface tension, is less than p and that they form clusters which stretch from the top to the bottom of the structure can be drained.

Once the water distribution was determined for a given ψ_m, we treated the water-air interfaces inside the structure as a boundary at which gaseous O_2 dissolves and then moves toward the solid-water interface to be reduced by microbial reactions. The partial pressure of gaseous O_2 in the simulated structure was assumed to be constant. Movement of dissolved O_2 in the liquid water was simulated using the following diffusion-reaction equation:

$$\frac{\partial c}{\partial t} = \nabla D \nabla c - s, \qquad c\big|_{\Gamma_{aw}} = c_s \tag{3}$$

where c is concentration of the dissolved O_2, D is molecular diffusion coefficient of O_2 in water, Γ_{aw} is the air-water interface, s is microbial consumption, c_s is the saturated dissolved O_2 concentration at the water-air interface calculated from Henry's law, in which H is the Henry constant and p_o is the partial pressure of the gaseous O_2 inside the structure. Microbial consumption was assumed to occur in water-filled voxels adjacent to the water-solid wall and described by the following Monod kinetic equation:

$$s = m_c k_0 \frac{[C]}{k_C + [C]} \frac{c}{k_O + c}, \tag{4}$$

where m_c is microbial biomass, k_0 is a kinetic parameter, [C] is the concentration of dissolved carbon. Since we are interested in impact of soil structure on development of anaerobic sites, we simulated O_2 diffusion and reduction to steady state. In all simulations, we normalized Eqs. (3) and (4) as follows

$$\frac{\partial c'}{\partial t'} = \nabla D' \nabla c' - s',$$
$$c'|_{\Gamma_{aw}} = 1,$$
$$s' = k' \frac{c}{k_o' + c}$$

where $t' = t/T_0$, $D' = DT_0/L^2$, and $k' = m_c k_0 T_0 [C]/(k_c + [C])$ in which L is the side length of the voxels and T_0 is a characteristic chosen to make $D' = 1$ in our simulations.

The above equation was solved by a finite volume method with each water-filled voxel being the element used to calculate the mass balance. In all simulations, water was assumed be initially free of O_2 and we simulated the system to steady state. As the development of anaerobic areas was a balance between the ability of soil to diffuse dissolved O_2 and the microbial consumption rate, to elucidate that the relative anaerobicity of soils under the same ψ_m is the consequence of their structures and does not change with microbial reactive rate, we simulated anoxic pore space under conditions of both fast (k□ = 1x10^{-2}) and slow (k□ = 1x10^{-4}) microbial decomposition. For each scenario, once the system was deemed to have reached a steady state, we sampled sites where concentration of dimension-less dissolved O_2 was less than 20%, assuming them be anaerobic[12]. We repeated the procedure to achieve different water distributions calculated by varying ψ_m and then calculated the proportional change in the volumetric anaerobic sites with the ψ_m for both the fast and slow microbial reactions.

Bioinformatic Analysis of Metagenome Sequences - To assess general abundance of genes in metagenomes, we mapped individual metagenomic sequences to the RefSeq non-redundant (NR) protein database held at NCBI (downloaded August 22nd, 2018) using DIAMOND version 0.8.27[13] in BLASTX mode using a bitscore cut-off of 55. For each sequence, only the match with the highest bitscore was considered. Sequences not matching the NR database were considered currently

unclassified. MEGAN Ultimate version 6.10.2[14] was used to associate metagenome sequences with Kyoto Encyclopaedia of Genes and Genomes[15] (KEGG) functional orthologs (ko) and modules (M).

From all of the reads associated with a ko, we selected those associated with nitrogen metabolism (ko00910), including nitrogen fixation (M00175); assimilatory nitrate reduction (M00531); dissimilatory nitrate reduction (M00530); denitrification (M00529); nitrification (M00528) and complete nitrification, comammox (M00804): amino acid metabolism (ko09105), including alanine, aspartate and glutamate metabolism (ko00250); glycine, serine and threonine metabolism (ko00260); cysteine and methionine metabolism (ko00270); valine, leucine and isoleucine degradation (ko00280); valine, leucine and isoleucine biosynthesis (ko00290); lysine biosynthesis (ko00300); lysine degradation (ko00310); arginine biosynthesis (ko00220); arginine and proline metabolism (ko00330); histidine metabolism (ko00340); tyrosine metabolism (ko00350); phenylalanine metabolism (ko00360); tryptophan metabolism (ko00380); phenylalanine, tyrosine and tryptophan biosynthesis (ko00400): and metabolism of other amino acids (ko09106), including beta-alanine metabolism (ko00410); taurine and hypotaurine metabolism (ko00430); cyanoamino acid metabolism (ko00460); *D*-arginine and *D*-ornithine metabolism (ko00472); and glutathione metabolism (ko00480) for detailed study of distribution differences between soils.

Emission of Nitrous Oxide from Soil – All N fertilisers were applied on April 12th, 2019, FYM was applied 17th September 2018 and 23rd September 2019. Measurement of gaseous emissions from ^{240}NPK, FYM and PK soils were taken on fourteen dates between April 11th and October 7th 2019, and measurements from ^{144}NPK and ^{192}NK soils were taken on eleven dates between April 23rd and October 7th 2019. Gas sampling was performed using in-field static chambers[16]. Three chambers (dimensions 40 cm x 40 cm x 25 cm height) were inserted to a depth of 5 cm in each treatment soil. Gas samples were taken with a syringe from the headspace at the time of chamber closure and again after 40 minutes. Samples were transferred in the field to pre-evacuated 20-mL vials. Once wheat plants had become too tall for a single chamber, additional chambers were stacked on top and a correction to the headspace volume was applied to calculate gas fluxes. On each sampling day, two chambers were selected to collect a third sample at 60 minutes to check the validity of the linearity

assumption of gas accumulation in the chamber headspace. Sampling was performed between 10:00 to 14:00 hours as this was considered representative of the daily mean. Sampling frequency was established following Cárdenas *et al*.[17] with higher frequency following fertiliser application (up to five samplings on the first two weeks), then twice per week for three weeks, followed by one sample every two weeks for five months followed by once per month until the end of the experiment. N_2O concentrations in the vials were analysed on a PerkinElmer Clarus 500 Gas Chromatograph (GC) equipped with an electron capture detector operating at 300 °C for N_2O analysis and a TurboMatrix 110 automated headspace sampler. The limit of detection of N_2O using this approach was estimated for each daily measurement: the range of detection limits across the experiment was 0.75 – 14.4 g N_2O ha^{-1} day^{-1}.

Statistical Analysis – To test our hypotheses, we generated several soil physical and biological metrics. Covariance (ρ) between soil organic carbon and total nitrogen stocks was tested using a geometric mean functional relationship. The effects of different land use and arable fertiliser treatments upon edaphic factors were analysed using analysis of variance (ANOVA) after testing for homogeneity of variances using Levene's test and normality using the Shapiro-Wilk test. Where significant heterogeneity of variances was detected we employed Welch's variant of ANOVA. In cases where significant treatment effects were identified by ANOVA, *post-hoc* pair-wise comparisons were performed using Tukey-Kramer Studentized *Q* following the step-down, recursive reject Copenhaver-Holland multiple comparison procedure, or Games-Howell Studentized *Q* in the case of Welch's ANOVA. An α of 0.05 was considered significant. Correlation, ANOVA, and associated tests were performed using PAST version 4.06b.

Analysis of covariance (ANCOVA) was initially used to compare N_2O emissions from the different soils. A Box-Cox transformation was necessary to meet model requirements. Soil temperature and potential soil moisture deficit (PSMD) were used as covariates. ANCOVA proceeded by first establishing homogeneity of slopes for each soil treatment relative to each covariate. Having established homogeneity, an equal slopes model was used to establish the influence of soil treatment and the covariates upon gas emission. Where a significant treatment effect was

identified, step-down, recursive reject Holm-Šidák *a priori* contrasts were used to evaluate the differences in treatment means compared to the ^{240}NPK treatment. ANCOVA and associated tests were performed using SigmaPlot for Windows, build 14.0.0.124.

For metagenome-associated multivariate data, we tested treatment effects upon the assemblages of nitrogen-associated genes. Hellinger distance-based analyses were performed, testing for heteroscedasticity of multivariate dispersion using the PERMDISP test. Hypothesis testing was based upon permutational multivariate analysis of variance (PERMANOVA) and *post hoc* pair-wise tests: probabilities were based upon 99,999 permutations (denoted p_{perm}). For *post hoc* pair-wise comparisons, since the number of observations was insufficient to allow a reasonable number of permutations, Monte Carlo probabilities were calculated based upon an asymptotic permutation distribution. All multivariate tests were performed in PRIMER PERMANOVA+ version 7.0.17 (PRIMER-e, Auckland, New Zealand).

Bi-hierarchical clustering was used to visualize gene distributions in the soils. Prior to clustering, gene abundance data were centred log-ratio transformed, generating the log of the ratio between each observed gene abundance and the gene's geometric mean abundance across all treatments. Minkowski distance was employed for heatmap visualization: gene and treatment clusters were identified using Ward's agglomerative hierarchical clustering procedure. To identify genes for which a significant difference in abundance between treatments was observed we used DESeq2[18] which applies a negative binomial generalized linear model to generate maximum-likelihood estimates of $\log_2$-fold change between treatments associated with each gene. Bayesian shrinkage - based upon a zero-centred normal distribution as a prior - reduces $\log_2$-fold changes towards zero for genes with low mean counts or a high dispersion in their count distribution. The resulting reduced fold-changes are used in tests of significance using Wald's test. DESeq2 has been shown to be particularly sensitive to differences in gene abundance on small datasets[19] such as those in this study. Before analysis, three genes with low abundance (mean count of less than 10) were removed as well as five genes associated with the lowest coefficients of variation. Differential abundance of the remaining genes was tested for significance employing $\alpha = 0.05$ and a Benjamini-Hochberg false

discovery rate (q) of 0.01 to control type I error rate arising from the multiple comparisons. DESeq2 was employed as implemented in MicrobiomeAnalyst[20].

Results

Co-metabolism of carbon and nitrogen – Since the field experiment was established, soils subject to different management or fertiliser treatment have developed distinct SOC and N_{tot} contents. A group of soils comprising unmanaged woodland and grassland soils together with arable soil amended with FYM have all consistently accumulated SOC since inception and by 2015 contained approximately 80 Mg ha^{-1} organic carbon (Fig. 1A). Significant linear increases in SOC were observed for all three management treatments over time (woodland r = 0.980, t = 11.1, p = 0.0001; grassland r = 0.781, t = 4.2, p = 0.0016; FYM arable r = 0.924, t = 8.7, p = 8.1x10^{-7}). A second group of treatments displayed consistent loss of SOC over time. These were soil which had received no fertiliser inputs (r = -0.527, t = 2.2, p = 0.043) and PK soil (r = -0.656, t = 3.1, p = 0.008). The remaining arable soils – ^{144}NPK, ^{192}NK and ^{240}NPK - showed no significant temporal change in SOC stocks. The effect of these changes was that by 2015 there was a measurable significant effect of management upon SOC, calculated for all treatments (except woodland for which there were insufficient annual measurements) for the years 1966 – 2015 (Welch ANOVA, $F_{6,23.1}$ = 313.1, p = 5.9x10^{-21}; ω^2 = 0.981: Supplementary Fig. 1A). Games-Howell *post hoc* pairwise tests indicated no significant difference between the treatments with highest SOC (77.4 ± 1.5 Mg ha^{-1} in FYM and 74.0 ± 1.4 Mg ha^{-1} grassland soils) or between inorganically fertilised arable soils (30.8 ± 0.7 Mg ha^{-1} in ^{240}NPK, 31.1 ± 0.6 Mg ha^{-1} in ^{192}NK and 31.7 ± 0.8 Mg ha^{-1} in ^{144}NPK soils). There was a significant difference in SOC between these two groups (smallest Q = 40.8, p = 0.0001). SOC stocks in arable soil which had never received either any fertiliser (nil, 25.1 ± 0.8 Mg ha^{-1}) or nitrogen fertiliser (PK, 25.9 ± 0.4 Mg ha^{-1}) were similar and significantly less than all other soils. The trends observed for total soil nitrogen (Fig 1B) were similar to those seen for total soil organic matter. N_{tot} was lost from the PK (r = -0.763, t = 2.6, p = 0.046) and ^{192}NK (r = -0.824, t = 3.6, p = 0.0481) management treatments, while FYM was the only management treatment associated with significant accumulation of N_{tot} (r = 0.928, t = 9.0, p = 6x10^{-7}). Although woodland and grassland soils also showed

accumulation of N_{tot}, regressions were not performed because of the limited number of temporal measurements available. As with SOC, there was a measurable significant effect of management upon N_{tot}, calculated for all treatments (except woodland) for the years 1966 – 2015 (Welch ANOVA, $F_{6,15.0} = 172.3$, $p = 5.5x10^{-13}$; $\omega^2 = 0.975$: Supplementary Fig. 1B). Games-Howell *post hoc* pairwise tests indicated no significant difference between the soils with highest N_{tot} (7.56 ± 0.13 Mg ha^{-1} in FYM and 6.92 ± 0.40 Mg ha^{-1} in grassland soils) or between inorganically fertilised arable soils (3.39 ± 0.07 Mg ha^{-1} in ^{240}NPK, 3.43 ± 0.08 Mg ha^{-1} in ^{192}NK and 3.44 ± 0.07 Mg ha^{-1} in ^{144}NPK soils). N_{tot} in arable soil which had never received either any fertiliser at all (nil, 2.83 ± 0.07 Mg ha^{-1}) or nitrogen fertiliser (PK, 2.78 ± 0.08 Mg ha^{-1}) were similar and significantly less than all other soils.

The link between nitrogen storage and physical processes – Parameters relating to soil structure were determined in grassland, woodland, FYM, ^{144}NPK, ^{192}NK and PK soils and are shown in Table II. Significant treatment-dependant differences were observed for all parameters.

For both total and connected porosity, soils separated into two general groups dependent upon their management and consistent with the groupings described for SOC and N_{tot}: woodland, grassland and FYM amended arable soils with greater than 30% porosity and inorganically fertilised arable soils with less than 30% porosity. Greatest total and connected porosity were associated with FYM amended arable soil, the least with PK inorganically fertilised arable soil. Games-Howell *post hoc* tests indicated that total porosity in FYM amended arable soil was not significantly different from that in woodland soil, however it was significantly greater than in grassland. In turn, grassland soil contained significantly greater total porosity than inorganically fertilised arable soils apart from ^{192}NK soil which was associated with a wide range of total porosity. There was no significant difference in total porosity between inorganically fertilised arable soils. The same trends were observed for connected porosity. These trends in the relative magnitude of porosity between the different soils and fertility management were characteristic of the differences observed in the other soil parameters used as descriptors of the structure-function relationship.

Greatest permeability was associated with grassland soil: Games-Howell *post hoc* tests indicated grassland permeability was significantly greater than in any other soil. There was no significant difference in woodland and FYM amended arable soil permeability and no significant difference in soil permeability between inorganically fertilised arable soils which were collectively associated with the least porosity. Soil porosity was associated with the largest effect size (ω^2) of all the topology-related parameters considered here. Tukey-Kramer *post hoc* tests indicated diffusion (D_e') was greatest in FYM amended arable soil and this was significantly greater than every other soil except woodland. There was no significant difference in D_e' between woodland or grassland soils, but D_e' in these three soils was significantly greater than in any inorganically fertilised arable soils, between which there was no significant difference in D_e'.

We used estimates of pore network topology to model the hydrodynamic behaviour of the pore networks under saturated conditions, estimating hydraulic conductivity (K) as a function of connected porosity. This measures the dynamical state of the pore space and the maximum potential rate at which resources can move through the networks—effectively the capacity for flux within the soil pore space. K was predicted to be greatest in grassland soil: Games-Howell *post hoc* tests indicated that this was not significantly different from K of woodland or FYM amended arable soils. K of grassland and FYM soils was significantly greater than any inorganically fertilised soils which shared statistically similar K.

Combined direct measurement and modelling indicate a power law relationship between connected porosity and K, and that SOC is associated with these changes (Fig. 2a). Greater SOC is associated with higher proportions of connected porosity and greater K. Regions of this relationship correspond to the different process-form states of fertilised and FYM amended arable soils and grassland and woodland soils. The relationship also indicates that the hydraulic conductivity and connected porosity in FYM amended soils are similar to grassland and woodland soils, despite regular physical disturbance by tillage. The combined influence of connected porosity and K upon the predicted anoxic proportion of each soil shows that addition of FYM to arable soils results in a matric potential – anoxic space profile distinct from inorganically fertilised arable soils (Fig 2b). Conventionally fertilised ^{192}NK and ^{144}NPK soils, as well as soil that had received no fertilization (nil)

all present large proportions of anoxic space under relatively dry conditions (50 – 65 kPa ψ_m) and are predicted to be completely anoxic at ψ_m between 38.4 and 32.0 kPa. In contrast, the process-form state of FYM amended soil resembles aspects of both woodland and grassland soils. Under relatively dry conditions FYM soil has low proportions of anoxic space most typical of grassland soil. At increased moisture content, both soils are completely anoxic at 22.6 kPa. Similarly, woodland soil is completely anoxic between 20.2 kPa.

Consequences of physical processes for biological function – Gene assemblages were determined in grassland, woodland, FYM, ^{144}NPK, ^{192}NK and PK soils. Forty-eight nitrogen associated KEGG functional orthologs were identified in the metagenomes. There was significant heterogeneity of multivariate dispersion between the assemblages (PERMDISP; *pseudo-*$F_{5,12}$ = 14.2, p_{perm} = 0.0095). Greatest multivariate dispersion was observed for woodland soil which was significantly more disperse than any other soil (smallest difference, woodland *vs.* grassland, *pseudo-t* = 4.1, p = 0.0152). There was no significant difference in multivariate dispersion between gene assemblages in any other soil. PERMANOVA indicated a significant treatment effect upon nitrogen associated gene assemblages (*pseudo-*$F_{5,12}$ = 22.5, p_{perm} = 1x10^{-5}). *Post hoc* pair-wise comparisons indicated that there was no significant difference in gene assemblages between ^{192}NK and PK soils, but all other comparisons were significantly different.

Centred log-ratio transformed gene distributions are shown in Fig. 3. Hierarchical clustering of soils identified that the nitrogen metabolism-related gene assemblages of unmanaged woodland and grassland soils were distinct from those associated with arable soils (^{144}NPK, ^{192}NK, FYM and nil). Genes separated into two broad clusters based upon their distribution between unmanaged and arable soils. The first cluster was associated largely with prokaryotic and fungal genes associated with amino acid metabolism and other nitrogen assimilation pathways. Prominent in the former group were several glutamate synthase (*gltB, gltD* and *gltS*), dehydrogenase (*gudB, gdhA* and *GDH2*) and glutamine synthetase (*glnA*) genes which perform central roles in regulating nitrogen assimilation. In addition, genes coding for formamidase (EC 3.5.1.49), associated with conversion of formamide ($HCONH_2$) to ammonia, and nitrilase (EC 3.5.5.1) associated with conversion of either alpha-

aminopropionitrile ($C_3H_6N_2$) or gamma-amino-gamma-cyanobutanoate ($C_5H_8N_2O_2$) to alanine and glutamate respectively are associated with this group. The second group of genes associated with this cluster were *nrtABC* coding for the ATP-binding cassette (ABC) nitrate/nitrite transport system, and *narK* coding for a nitrate/nitrite transporter of the major facilitator superfamily (all associated with nitrate assimilation, KEGG module M00615) and genes associated with assimilatory nitrate reduction (M00531) including the assimilatory nitrate reductase catalytic subunit (*nasA*), the ferredoxin-dependant nitrite reductase (*nirA*) and the fungal nitrite reductase gene *nit-6*. In addition to these assimilatory genes, several genes associated with dissimilatory nitrate reduction (M00530) including *napAB* of the periplasmic nitrate reductase, nitrate reductase A (*narI*) and the nitrite reductase (NADH) large subunit (*nirB*) and denitrification (M00529) including the nitric oxide reductase *norB* and the nitrate reductase *nirK* were associated with this first cluster. All these genes were significantly more abundant in woodland and grassland soils—which hierarchical clustering of soils by ortholog abundance indicated were most similar to each other—than arable soils. More subtle than this general pattern, there was evidence that a suite of genes was more abundant in FYM amended soil than inorganically fertilised arable soils. These included genes associated with nitrate assimilation (*nrtABC* and *nasA*), *nirK*, *nirB* and *napA*.

A second gene cluster was generally significantly more abundant in arable soils. These represented several modules including nitrification (M00528), denitrification (M00529), dissimilatory nitrate reduction (M00530) and complete nitrification (comammox) (M00804). The genes *hao* (hydroxylamine dehydrogenase) and *amoABC* (ammonia monooxygenase) are associated with the nitrification and comammox pathways; genes *norC* (nitric oxide reductase), *nirS* (cytochrome cd_1 nitrite / hydroxylamine reductase), *nosZ* (nitrous-oxide reductase) and *CYP55* (cytochrome P450 fungal nitric oxide reductase) genes are associated with denitrification; genes *nrfA* (cytochrome c_{552} nitrite reductase), *nrfH* (cytochrome *c* nitrite reductase) and *nirD* (nitrite reductase) are associated with the dissimilatory nitrate reduction pathway; while genes *nxrAB* (nitrate reductase / nitrite oxidoreductase) are common to the denitrification, dissimilatory nitrate reduction and comammox pathways. Genes associated with a fourth module, M00175 (nitrogen fixation), namely *nifH* (nitrogenase iron protein), *nifDK* (nitrogenase molybdenum-iron protein) and *anfG* (nitrogenase delta

subunit) were all associated with this cluster, but their general abundance was low and abundance differences between soils were not significant.

This analysis presents clear evidence for a direct influence of process-form state upon nitrogen associated gene assemblages in soil. Clearest evidence of this was the association of nitrogen assimilatory pathways with soils having the greatest SOC and N_{tot}, connected porosity and hydrodynamic conductivity; unmanaged woodland and grassland (*cf.* Fig. 2 and 3). Conversely, soils having low SOC (and N_{tot}) and thus low connected porosity and hydrodynamic conductivity were characterized as associated generally with dissimilatory pathways, utilizing oxidized forms of nitrogen as alternative electron acceptors for respiration. Counter to this general trend in arable soils, the increased abundance of genes associated with nitrogen assimilation in FYM amended soil is suggestive of with this soil having a similar process-form state to woodland and grassland soils.

Nitrous oxide emissions from soil – Emissions were measured during the growing season between April and November 2019 from FYM, ^{240}NPK, ^{192}NK, ^{144}NPK soils and compared to emissions from soil which had never received nitrogen fertiliser (PK), considered for the purposes of these measurements as a control. Mean emissions of N_2O are shown in Supplementary Fig. 2 together with daily measures of precipitation and estimates of potential soil moisture deficit (PSMD) for the measurement period. In general, emission episodes coincided with periods of soil saturation- represented as negative PSMD estimates- however, emissions were also recorded in April, July, August and September when soil was not predicted to be saturated. For each measurement date, the largest emissions were typically measured from ^{240}NPK soils and the least from PK soils. Initially, we tested the influence of the covariates soil temperature and potential soil moisture deficit (PSMD) upon the overall treatment effect using Box-Cox transformed N_2O emissions (lambda = 0.222; log-Likelihood = -177.74). There was no significant influence of either soil temperature (ANCOVA; $F_{1,134} = 0.901$, $p = 0.344$) or PSMD (ANCOVA; $F_{1,134} = 0.272$, $p = 0.603$) upon any observed treatment effect. We therefore employed a one-factor ANOVA, testing the main effect of soil treatment upon N_2O emission. This indicated a significant influence of soil treatment (ANOVA; $F_{4,116} = 8.7$, $p = 3.3\text{x}10^{-6}$).

References

1. Macdonald A.J., Poulton P.R., Clark I.M., Scott T., Glendining M.J., Perryman S.A.M., Storkey J., Bell J.R., Shield I.F., Mcmillan V.E., Hawkins J.M.B. (2018). *Guide to the Classical and other Long-term Experiments, Datasets and Sample Archive*, Rothamsted Research, Lawes Agricultural Trust Ltd, Harpenden UK. 10.23637/ROTHAMSTED-LONG-TERM-EXPERIMENTS-GUIDE-2018.
2. Rothamsted Research (2021). *Broadbalk Wheat Experiment plan and cropping 1968-2017*. Electronic Rothamsted Archive, Rothamsted Research. 10.23637/rbk1-plan1968-2017-01.
3. Poulton P.R., Pye E., Hargreaves P.R., Jenkinson D.S. (2003). Accumulation of carbon and nitrogen by old arable land reverting to woodland. *Global Change Biol.*, 9, 942-955 10.1046/j.1365-2486.2003.00633.x
4. Neal, A.L., Rossmann, M., Brearley, C., Akkari, E., Guyomar, C., Clark, I.M., Allen, E. and Hirsch, P.R. (2017). Land-use influences phosphatase gene microdiversity in soils. *Environ. Microbiol.* 19: 2740-2753. https://doi.org/10.1111/1462-2920.13778
5. Neal, A.L., Blackwell, M., Akkari, E. *et al.* (2018). Phylogenetic distribution, biogeography and the effects of land management upon bacterial non-specific acid phosphatase gene diversity and abundance. *Plant Soil* **427,** 175–189 doi.org/10.1007/s11104-017-3301-2
6. Neal, A.L., Bacq-Labreuil, A., Zhang, X. *et al.* (2020). Soil as an extended composite phenotype of the microbial metagenome. *Sci. Rep.* **10,** 10649 doi.org/10.1038/s41598-020-67631-0
7. Neal A.L., Hughes D., Clark I.M., Jansson J.K., Hirsch P.R. (2021). Microbiome Aggregated Traits and Assembly Are More Sensitive to Soil Management than Diversity. *mSystems*. 29;6(3):e0105620. doi: 10.1128/mSystems.01056-20.
8. Perryman, S., Castells-Brooke, N., Glendining, M. *et al.* (2018). The electronic Rothamsted Archive (e-RA), an online resource for data from the Rothamsted long-term experiments. *Sci. Data* **5,** 180072 doi.org/10.1038/sdata.2018.72
9. Powlson D.S., Bhogal A., Chambers B.J., Coleman K., Macdonald A.J., Goulding K.W.T., Whitmore A.P. (2012). The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: A case study. *Agric. Ecosys. Environ.* 146, 23-33.
10. Vogel, H.-J., Weller, U. & Schlüter, S. (2010). Quantification of soil structure based upon Minkowski functions. *Comput. Geosci.* 36, 1236–1245.
11. van der Vorst, H.A. (1992). BI-CGSTAB—A fast and smoothly converging variant of BI-CG for the solution of nonsymmetric linear systems. *Siam J. Sci. Stat. Comp.* **13**, 631–644.
12. Harrison, D.E.F., Pirt, S.J. (1967). The influence of dissolved oxygen concentration on the respiration and glucose metabolism of *Klebsiella aerogenes* during growth. *J. Gen. Microbiol.* **46**, 193–211.
13. Buchfink, B., Xie, C., Huson, D. H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nat. Method.* **12**, 59–60.
14. Huson, D. H. *et al.* (2016). MEGAN Community Edition—interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Comput. Biol.* **12**(6), e1004957. https://doi.org/10.1371/journal.pcbi.1004957
15. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M. (2015). KEGG as a reference resource for gene and protein annotation. *Nucl. Acids Res.* **44**, D457–D462.
16. Chadwick D.R., Cardenas L., Misselbrook T.H., Smith K.A., Rees R.M., Watson C.J., McGeough K.L., Williams J.R., Cloy J.M., Thorman R.E., Dhanoa M.S. (2014). Optimizing chamber methods for measuring nitrous oxide emissions from plot□based agricultural experiments. *Eur. J. Soil Sci*. 65, 295-307.

17. Cárdenas, L.M., Bhogal, A., Chadwick, D.R., McGeough, K., Misselbrook, T., Rees, R., Thorman, R., Watson, C.J., Williams, J., Calvet, S. (2019). Nitrogen use efficiency and nitrous oxide emissions in five UK fertilised grasslands. *Sci. Total Environ.* 661, 696–710.
18. Love, M.I., Huber, W., Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550. https://doi.org/10.1186/s13059-014-0550-8
19. Weiss, S. *et al.* (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **5**, 27. https://doi.org/10.1186/s40168-017-0237-y
20. Dhariwal A., Chong J., Habib S., King I.L., Agellon L.B., Xia J. (2017). MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucl. Acids Res*. 3; 45(W1): W180-W188. doi: 10.1093/nar/gkx295.