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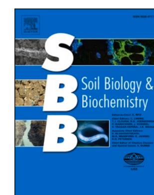

A DNA-barcode biodiversity standard analysis method (DNA-BSAM) reveals a large variance in the effect of a range of biological, chemical and physical soil management interventions at different sites, but location is one of the most important aspects determining the nature of agricultural soil microbiology

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ABSTRACT

There are significant gaps in our understanding of how to sustainably manage agricultural soils to preserve soil biodiversity. Here we evaluate and quantify the effects of agricultural management and location on soil microbiology using nine field trials that have consistently applied different soil management practices in the United Kingdom using DNA barcode sequence data. We tested the basic hypothesis that various agricultural management interventions have a significant and greater effect on soil bacterial and fungal diversity than geographic location. The analyses of soil microbial DNA sequence data to date has lacked standardisation which prevents meaningful comparisons across sites and studies. Therefore, to analyse these data and crucially compare and quantify the size of any effects on soil bacterial and fungal biodiversity between sites, we developed and employed a post-sequencing DNA-barcode biodiversity standard analysis method (DNA-BSAM). The DNA-BSAM comprises a series of standardised bioinformatic steps for processing sequences but more importantly defines a standardised set of ecological indices and statistical tests. Use of the DNA-BSAM reveals the hypothesis was not strongly supported, and this was primarily because: 1) there was a large variance in the effects of various management interventions at different sites, and 2) location had an equivalent or greater effect size than most management interventions for most metrics. Some dispersed sites imposed the same organic amendments interventions but showed different responses, and this combined with observations of strong differences in soil microbiomes by location tentatively suggests that any effect of management may be contingent on location. This means it could be unreliable to more widely extrapolate the findings from trials performed only at one location. The widespread use of a standard approach will allow meaningful cross-comparisons between soil microbiome studies and thus a substantial evidence-base of the effects of land-use on soil microbiology to accumulate and inform soil management decisions.

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1. Introduction

The increasing global demand for food has historically been met by increasing agricultural intensification (Tilman et al., 2011) but this has correspondingly caused significant soil degradation (Lal et al., 1998; Gomiero, 2016). The implementation of more sustainable agricultural management practices that enhance soil integrity in the long term, while allowing acceptable crop productivity in the short term, is essential to ensure future global food security (Zilberman et al., 2018). Any agricultural or land use management decisions that attempt to enhance soil integrity must be evidence-based (Bünemann et al., 2018). The role of soil physics and chemistry in agricultural productivity is reasonably well established and allows robust recommendations for soil management best practice to optimise crop productivity. Soil bacterial and fungal communities form part of one of the most complex and diverse ecosystems on the planet and underpin agricultural productivity as they drive nutrient availability and turnover (including carbon cycling and sequestration) (Bardgett and van der Putten, 2014; Paustian et al., 2016; Tao et al., 2023). Soil organisms also play a role in soil structural stability (Cock et al., 2012; Odelade and Babalola, 2019) and provide a range of other key ecosystem services, *e.g.*, they are sources of novel antibiotics (Ling et al., 2015). However, there is a paucity of understanding of how to manage agricultural soils generally to conserve biodiversity (Ortiz et al., 2021). Robust information on how soil management interventions can affect relative changes in soil microbiology is needed to inform future strategies attempting to maintain and enhance soil health and carbon-sequestration in the light of climate change and biodiversity loss (Bardgett et al., 2008; Turbé et al., 2010).

Previous studies evaluating indicators of soil biological health have focussed on key individual organisms (van Bruggen and Semenov, 2000) or physicochemical metrics like soil respiration (Logsdon and Karlen, 2004); other studies have taken more holistic approaches by evaluating microbial capabilities for restoring soil conditions in response to imposed stresses or to supress pathogen activity (van Bruggen and Semenov, 2000). Soil harbours a substantial fraction of the world's biodiversity; however, most soil bacteria and fungi do not grow on laboratory media making them difficult to analyse meaning soil microbial biodiversity studies have historically been constrained by their ability to measure the enormous variety of taxa (Yang et al., 2014; Guerra et al., 2020). The use of molecular technologies, such as the high-throughput sequencing of DNA extracted from soil, now offers an unprecedented opportunity to holistically investigate soil biodiversity (Orgiazzi et al., 2015) and may start to unravel how soil biology contributes to soil health (George et al., 2019; Harkes et al., 2019; Tedersoo et al., 2020). Metabarcoding is currently the most widely used soil biology molecular approach where 'barcode' genomic regions, whose sequences differ between taxa, are amplified from DNA extracted from soil, and their sequences are then determined and analysed. The most common barcodes include variable regions of the 16S rRNA gene for bacteria and the Internal Transcribed Spacer (ITS) regions for fungi, but other barcode areas are also commonly employed (Ruppert et al., 2019). Once raw sequence data are derived, there are two main analytical challenges: firstly, analysis must attempt to determine the biological signal in the data from errors that can derive from PCR and sequencing molecular processes; secondly, analysis must attempt to test whether very complex biological communities, represented by multivariate data, differ between treatments. There are large number of procedures available to analyse both these phases (Edgar, 2013; Callahan et al., 2016a; Semenov, 2021), and all have merits; for example, originally barcode sequences were clustered into operational taxonomic units (OTUs) at a prescribed genetic homology level thought to align with differentiation between genera or sometimes species. More recently amplicon sequence variant (ASV) algorithm approaches have been shown to control for sequence errors better than OTU approaches (Callahan et al., 2017). However, since there is no standardisation of the large number of options available at each of the many analytical steps, this makes individual studies analytically bespoke and prevents inter-study comparisons. There is an increasing appreciation that the lack of a standardized approach in the analyses of barcode sequences makes it extremely difficult to compare the findings from different studies which prevents an understanding of broader patterns of soil biology (Thompson et al., 2017). We address this here with the use of a standard set of ecological metrics for the molecular analyses of soil microbial biodiversity.

Fundamental ecological theory predicts that communities will differ in space due to the actions of both natural selection and neutral ecological processes and this naturally applies to soil microbial communities (Martiny et al., 2006). Both selective and neutral forces have been shown to play significant roles in defining agricultural soil fungal communities across space in New Zealand (Morrison-Whittle and Goddard, 2015). It is valuable to understand the relative magnitude of the effects of location and management interventions on soil biology. However, while individually robust, the range of soil studies to date have lacked analytical standardisation making it difficult to compare across different studies conducted in different places. As a result, the relationships between soil biodiversity, location, soil management, and crop health and productivity remain very poorly described. There is an urgent need for a standardised approach for measuring soil biology to allow meaningful inter-study comparisons to quantify the effects of soil management practices across agricultural systems, climates, and soil types on soil biodiversity.

Key agricultural soil management interventions revolve around physically manipulating soil structure or nutrient availabilities either by altering pH or adding organic and inorganic fertilisers. Soil pH, which defines nutrient availability (Binkley and Vitousek, 1989), is known to be a strong abiotic factor driving bacterial and fungal community assemblages (Borneman and Hartin, 2000; Fierer et al., 2005; Fierer and Jackson, 2006; Rousk et al., 2010), including in agricultural soils (Wang et al., 2019). Inorganic fertiliser application has been shown to affect the composition of bacterial and certain groups of fungal communities depending on the type of phosphate fertilisation (Silva et al., 2017), and there is evidence for an inhibitory effect of triple superphosphate fertiliser on mycorrhizal formation (Peine et al., 2019). Fertilisation by organic amendment (OA), such as green/food compost, food-based digestate, livestock manures and slurries, as a complement or even alternative to inorganic fertilisers have been shown to improve physical, chemical and biological features of soils, including soil organic carbon (Diacono and Montemurro, 2011). Organic amendments can potentially shape the structure of soil microbial communities by introducing additional diverse microbial pools plus a supply of metabolites into the soil (Lemanceau et al., 2015). However, DNA sequencing studies have not achieved an agreement on the nature or magnitude of the effect of agricultural management interventions on soil bacterial and fungal communities. For example, some studies report fertiliser application increases richness and diversity (Wang et al., 2017; Pan et al., 2020), while others found no significant influence of fertilisers (Yao et al., 2018). Recent work has also shown fungal and bacterial communities (Hartmann et al., 2015; Hannula et al., 2021) are affected by agricultural practices such as tillage, cover cropping and organic amendments. Recent soil DNA sequencing studies have focused on differences between conventional high-agrochemical input versus conservation low-agrochemical input agronomic management approaches. These studies, which include the simultaneous analyses of bacteria and fungi (Hartmann et al., 2015) and total soil biology across time and space (Giraldo-Perez et al., 2021), indicate significant but small and inconsistent differences between soil fungal (Morrison-Whittle et al., 2017; Hannula et al., 2021) and bacterial (Hendgen et al., 2018) diversity between the management approaches investigated.

Here we evaluate and quantify the effects of location and management approaches on agricultural soil microbial biodiversity using nine field trials that have consistently applied different soil management practices at different locations in the United Kingdom. The lack of

available molecular data for UK agricultural soil microbiology means we tested the basic hypothesis that various types of soil management interventions have a significant and greater effect on soil bacterial and fungal diversity than geographic location. To allow us to analyse and compare these data sets and quantify the size of any effects on soil bacterial and fungal biodiversity between sites, we developed and employed a soil post-sequencing DNA-barcode biodiversity standard analysis method (DNA-BSAM). The DNA-BSAM is based on a range of widely used bioinformatic tools and crucially suggests a standard set of ecological metrics. Our aim is to provide the DNA-BSAM resource to the community to allow future insightful meta-analyses to compare and quantify the effects of various factors on soil biology across diverse studies.

2. Materials and methods

2.1. Data generation

2.1.1. Trial sites and sample collection

Soil samples were collected between 2018 and 2020 from nine field trials in the United Kingdom (listed in Table 1 and Supplementary Fig. S1), some established as far back as 1922, that were each designed to compare one of three main types of management intervention, either: organic amendment, inorganic fertiliser addition, or mechanical and chemical soil disturbance (Table 1). The cropping rotations for each site as well as sampling times are detailed in Table S1. At sites 1–3, all treatments (including the controls) had manufactured fertiliser N based

Table 1
Trial sites, soil types, approximate altitude (https://en-us.topographic-map.com), climate (average temperature and precipitation during the month of sampling from the closest station; https://metoffice.gov.uk) and management details sampled.

Site	Soil texture (% clay)	Altitude (approx.)	Climate at sampling (max-min°C/mm)	Previous crop at sampling	Treatments & design
1.Terrington Bentinck Farm, Kings Lynn, Norfolk, England **Established in 1993 (pFYM) and 2005 (GC)** (Bhogal et al., 2009, 2011)	Silty clay loam (28% cl)	5 m	14.1–8.2/116.4	Sugar beet	Organic amendment 1. Control; manufactured fertiliser 2. Green compost (@250 kg N/ha; *c*.25 t/ha) 3. Pig FYM (@250 kg N/ha; *c*.35 t/ha) **3 replicates per treatment**
2.Harper Adams University Newport, Shropshire, England **Established in 1990 (cFYM) and 2005 (GC)** (Bhogal et al., 2009, 2011)	Sandy loam (12% cl)	65 m	13.4–7/93	Spring barley	Organic amendment 1. Control; manufactured fertiliser 2. Green compost (@250 kg N/ha; *c*.25 t/ha) 3. Cattle FYM (@250 kg N/ha; *c*.35 t/ha) **3 replicates per treatment**
3.Gleadthorpe Meden Vale, Mansfield, Notts, England **Established in 1993 (BL) and 2005 (cFYM, cSlurry and GC)** (Bhogal et al., 2009, 2011)	Loamy sand (6% cl)	50 m	12.6–6.7/135.6	Spring barley	Organic amendment 1. Control; manufactured fertiliser 2.10 t/ha Broiler litter 3. Green compost (@250 kg N/ha; *c*.25 t/ha) 4. Cattle FYM (@250 kg N/ha; *c*.35 t/ha) 5. Cattle slurry (@250 kg N/ha; *c*.80 m3/ha) **3 replicates per treatment**
4.Boxworth Cambridgeshire, England **Established in 2017**	Clay (35% cl)	53 m	14.1–8.2/116.4	Oilseed rape	Physical disturbance: Drainage 1. Improved drainage 2. Control; Poor drainage **3x2[a] replicates per treatment**
5.Loddington Leicester, Leicestershire, England **Established in 2010 (Zero till) and 2017 (Tillage)**	Clay (35% cl)	141 m	**2018:** 15–7.2/35.8 **2020:** 13.6–7.8/81.4	Wheat	Physical disturbance: Tillage 1. Zero till 2. Control; Conventional plough **3 replicates per treatment (2018)** **3x2[a] replicates per treatment (2020)**
6. Craibstone-F Scotland's Rural College, Craibstone Estate, Aberdeen, Scotland **Established in 1922**	Sandy loam (12% cl)	100 m	9.8–2/64.2	Pasture, wheat, spring barley and potatoes	Chemical additions: Fertiliser 1. Control; No fertiliser 2. Complete fertiliser + superphosphate **12[b] replicates per treatment**
7. Craibstone-pH Scotland's Rural College, Craibstone Estate, Aberdeen, Scotland **Established in 1961** (Walker et al., 2015)	Sandy loam (12% cl)	100 m	11.4–3/84	Pasture, wheat, spring barley and potatoes	Chemical additions: pH 1. pH 4.5 2. pH 6 3. Control; pH 6.5 4. pH 7.5 **12[b] replicates per treatment**
8.Gatsford Farm Ross on Wye, Herefordshire, England **Established in 2016** (Maskova et al., 2022)	Sandy loam (12% cl)	72 m	20.8–11.5/86	Asparagus	Organic amendments 1. Control; Bare soil 2. Mulch (Straw mulch or PAS 100 compost applied annually at 25 t ha^{-1} and 6 t ha^{-1} for PAS 100 and straw mulch, respectively) in combination with shallow soil disturbance. 3. Cover crop: Rye (*Sereale cecale* L.) or Mustard (*Sinapis alba* L.) **8 replicates per treatment**
9. Sustainability Trial in Arable Rotations (STAR) Stanaway Farm, Suffolk, England **Established in 2005** (Stobart and Morris, 2011)	Clay loam (19% cl)	58 m	13.5–6.9/59	Wheat	Physical disturbance: Cultivation 1. Control; Annual plough 2.Managed approach 3.Shallow tillage 4.Deep tillage **3 replicates per treatment**

[a] Two samples were taken from three replicate plots at Boxworth and Loddington (2020).
[b] Each group of 12 replicates were superimposed to 4 different stages of a rotation (pH trial: wheat, pasture, oats and potatoes; fertiliser trial: barley, pasture, oats and potatoes).

on MANNER-NPK predictions of the N supplied by the organic material (Nicholson et al., 2013) and manufactured fertiliser P, K, Mg and S to ensure that no major nutrient limited plant growth and that crop yields and residue returns were comparable across all treatments. At sites 4, 5 and 7, recommended rates of fertiliser based on the ADHB "The Nutrient Management Guide - RB209" (AHDB, 2017) were applied according to crop need. Physicochemical measures can be found in File S3.

Representative soil samples were taken in autumn, post-harvest and prior to any cultivations/treatment applications at all sites, except for the first sampling of the Sustainability Trial in Arable Rotations (STAR) at Stanway Farm in spring of 2018 and the Gatsford Farm site where samples were taken in the summer of 2020 post asparagus harvesting. For all trials sites, at least 25 soil cores were randomly taken from each plot to a depth of 15 cm (crops) or 7.5 cm (grass) and bulked together to achieve composite samples of soil comprising approximately 6 kg. Corers were cleaned to remove residual soil followed by alcohol flaming between soil cores. After the removal of root and plant material, samples were homogenized and 2 kg subsamples were transferred to Fera Science Ltd. (York, UK) at 4 °C within 48 h. In the laboratory, samples were further homogenized by thoroughly mixing and 10g of fresh soil subsampled into 50 ml tubes and stored at 4 °C. Because soil sieving steps are intended to isolate different sizes of soil aggregates and this has an effect on overall microbial community structures (Morita and Akao, 2021), samples were processed in their natural state to avoid methodological bias.

2.1.2. DNA extraction, amplification, and sequencing

DNA extractions were performed within two weeks of soil sample collection using the DNeasy PowerMax Soil Kit (Qiagen, Carlsbad, CA, United States) following the manufacturer's instructions. DNA quality and quantity were measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, USA). Bacterial V4-16S rRNA and fungal ITS1 barcodes were amplified via PCR using 515F (Parada et al., 2016) and 806R 16S primers (Apprill et al., 2015), and ITS1-F_KY02 ITS forward primer (Toju et al., 2012) with a modified ITS2 (White et al., 1990) including a wobble addition (Supplementary Table S2). Amplicons were quantified with Qubit fluorometric quantification and indexed amplicon libraries were prepared using Nextera (Illumina) adapters following the manufacturer's protocol. A PhiX internal control was added to the library pool before sequencing with 600 cycles using reagent kit v3 (2 × 300 PE) with a MiSeq Instrument (Illumina, San Diego, CA, USA) at Fera Science Ltd across 3 runs. Reads were demultiplexed with BaseSpace (Illumina's cloud-based resource) and imported and trimmed using CUTADAPT (Martin, 2011) with reads <50 bp discarded. PCR chimeras were removed, and the resulting reads were quality filtered, denoised, paired-end merged using DADA2.

2.2. The post-sequencing DNA-barcode biodiversity standard analysis method (DNA-BSAM) pipeline

The main steps of the DNA-BSAM pipeline are shown in Fig. 1 (code available from GitHub at https://github.com/matubiol/DNA-BSAM). Bioinformatic analyses were performed using the freely available QIIME2 v. 2021.2 (Bolyen et al., 2019) and R v. 4.0.4 (R Core Team, 2021).

2.2.1. mASV generation

Amplicon sequence variants (ASVs) were determined with DADA2 (Callahan et al., 2016b). 16S reads were truncated to 253 bp but ITS reads were not trimmed due to variation in ITS region length. All ASVs represented by a single read only (singletons) were removed and chimeras rechecked and removed with VSEARCH (Rognes et al., 2016). Genetic variation within the 16S and ITS regions means different isolates from the same bacterial and fungal species can typically vary by around 2% in genetic identity (Nilsson et al., 2008; Větrovský and Baldrian, 2013). All ASVs were therefore subsequently clustered into groups of greater than 98% genetic identities to merge highly similar ASVs at approximately species level: we term these merged ASV (mASVs). Taxonomic assignment for each mASV representative sequence was attempted using a naïve Bayes classifier trained using a primer-trimmed (515F and 806R) 16S database (release 138) from SILVA (Quast et al., 2012) and a modified ITS database (v. 8.2) from Unite (Nilsson et al., 2018) (Supplementary File S1, Table S3). Following Bokulich et al. (2018), the confidence parameter was set at 70 for 16S ASVs and any mASVs not assigned at phylum level or not assigned to the Bacterial kingdom was removed. The taxonomic assignment of ITS ASVs was performed by setting the confidence parameter at 95 to filter out all mASVs other than those assigned within the kingdoms Fungi or Stramenopila as these contain Oomycota, formerly classified as fungi, which include crop pathogens such as *Pythium* spp. (root rots) and *Phytophthora infestans* (potato blight); however, for simplicity both groups will be referred as 'Fungi' from here on.

2.2.2. Microbiome diversity analyses

From here on we use 'taxa' interchangeably with mASVs. mASV datasets were normalised by scaling with ranked subsampling (SRS) (Beule and Karlovsky, 2020) prior to analyses with sampling depths shown in Supplementary Fig. S2. The number of counts to which all samples were normalised was selected with the aim of preserving as many samples as possible while retaining as much diversity (suggested by a flattening of the diversity curve) as possible. Alternative commonly used conventional rarefaction and proportions normalisation methods were also applied to evaluate the effect of data normalisation on inferences of soil biology using this method. There are many biodiversity metrics that may be used (Roswell et al., 2021), but following Morrison-Whittle et al. (2017), Morrison-Whittle and Goddard (2018) and

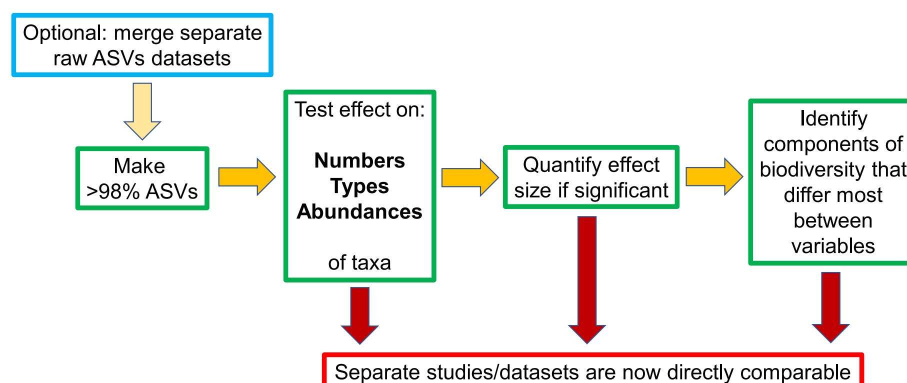

Fig. 1. The DNA-BSAM workflow and three standard measures of biodiversity used following Morrison-Whittle et al. (2017), Morrison-Whittle and Goddard (2018).

Giraldo-Perez et al. (2021), three intuitive but meaningful main classes of biodiversity metric were evaluated and quantified that comprise the core of the DNA-BSAM (Fig. 1 and S3), and these were: Numbers - differences in the total number (richness) of taxa between treatments; Types - differences in the presences/absences of taxa between treatments; and Abundances - differences in the relative abundances of taxa between treatments inferred by sequence read counts. Differences in taxa numbers were evaluated with Kruskal-Wallis tests and Eta^2 effect sizes calculated using the 'rstatix'(Kassambara, 2020) package. Differences in types and abundances of taxa were evaluated with PERMANOVA using binary and abundance Jaccard distance matrices, respectively (Anderson, 2017). PERMANOVA was conducted using 'vegan' (Oksanen et al., 2019), and 'pairwiseAdonis' (Martinez-Arbizu, 2017) with 999 permutations to determine pseudo-F ratios to calculate P values and R^2 estimates of effect sizes. Principal coordinate decomposition of distance matrices were computed using the 'ape' (Paradis and Schliep, 2019) package and visualised with 'phyloseq' (McMurdie and Holmes, 2013).

2.2.3. Merging data from different studies

While the outcomes from separate studies may be meaningfully compared if the same standard analyses methods in 2.2.1 and 2.2.2 are employed, there is also an option to merge raw ASVs from separate studies prior to analyses. There are three advantages to first merging raw ASV tables from different studies that use the same barcode regions and then analysing these together: 1) identical normalisation of all data controls for the effects of normalisation across studies; 2) taxa that are common across studies can be identified; and 3) the direction of effects of different treatments on taxa across studies can be directly estimated. When merged, the identification of matching ASVs between studies is possible because identical DNA sequences underlying ASVs produce the same MD5 identification tag between sequence runs with DADA2 (Callahan et al., 2016b). This correspondingly makes ASVs derived from independent sequencing runs directly comparable but only if sequences are in the same orientation and trimmed/truncated to exactly the same length and sites: if they are not highly similar ASVs will remain discrete. However, the clustering of ASVs into 98% mASVs circumvents this issue and allows the identification of mASVs that are common across datasets. ASV tables were merged using the 'qiime feature-table merge' tool (Bolyen et al., 2019) and subsequently clustered into groups of greater than 98% mASVs.

3. Results

A total of 17,842,718 16S and 14,891,875 ITS sequence reads were obtained from all samples across all trials, and sequence reads from each trial were independently processed to ASVs. When ASVs from each of the trials were independently merged into >98% sequence similarity ASVs (mASVs), the cumulative number of mASVs across all trials was 13,003 and 9016 for 16S and ITS, respectively; however, it is likely some of these mASVs represent duplicates of the same taxa in different trials. When raw ASVs from all trials were combined and collectively merged into >98% mASVs, 9090 16S and 11,083 ITS mASVs were obtained indicating that approximately 50–60% of these taxa are present across all trials. After taxonomic filters were applied 8786 bacterial and 7359 fungal mASVs were taxonomically assigned. 16S data from one replicate of each of the Craibstone-pH 6.5 and Gleadthorpe-cFYM samples were removed due to low read numbers (523 and 424 sequences, respectively).

3.1. Evaluating the effects of management and location on soil microbial diversity

The differential nature of experimental designs across the various trials, with different replicate numbers and levels of factors, meant that a full factorial two-way analysis was not possible. The DNA-BSAM was developed to circumvent such issues as it allows the comparison of the relative effects and magnitudes of effects of different factors on soil microbiology irrespective of differential experimental designs. We compared: 1) the outcomes of independent analyses of studies using the DNA-BSAM; and 2) where raw ASVs from separate studies were merged and then all analysed together with the DNA-BSAM, which emulates re-analysis of data from different studies.

3.1.1. Numbers of taxa

3.1.1.1. Effects of management interventions. Just one and three of the nine trials respectively reported a significant difference in numbers of bacterial and fungal taxa between management interventions within trials (Table 2). Fungal richness was significantly affected by organic amendments at the Gleadthorpe site with 1.2-fold more taxa in the cFYM treatment's than all other treatments, and by drainage at the Boxworth site with 1.1-fold more taxa in the undrained treatments compared to all other treatments. Both numbers of bacterial and fungal taxa differed by pH treatment at the Craibstone site with 1.4 and 1.1-fold more bacterial and fungal taxa in the pH 6 treatment than all other treatments; pH also had the largest effect size on taxa richness (Table 2). Different numbers of mASVs were found in each trial (on average, 5.5%, with a median of 1.93%) when ASVs across all trials were merged prior to the calculation of mASVs (Table S4). However, these differences in mASVs numbers between individual and merged analyses did not translate into any differences in statistical inferences than those reported in Table 2, except for the effect of fertiliser application on fungal taxa in Craibstone and the slight disparity observed with the effect of OA in Gleadthorpe (Table S5).

3.1.1.2. Effects of location. Irrespective of management intervention, a highly significant difference in both bacterial and fungal taxa richness between locations was revealed overall ($P < 5.5 \times 10^{-7}$, Fig. 2). Post-hoc Wilcoxon pairwise comparisons revealed no distinct group of sites in terms of bacterial richness, but fungal richness formed four distinct groups with greatest fungal richness at the Craibstone fertiliser site and least fungal richness at Boxworth, Gatsford Farm, Harper Adams and Terrington sites (Fig. 2). No correlation was apparent between the patterns of bacterial and fungal richness across the trial sites.

3.1.2. Types of taxa

3.1.2.1. Effects of management intervention. Comparisons of outcomes from the independent analyses of trials revealed a significant effect of management intervention on the types of bacterial and fungal taxa ($P < 0.049$, Table 2) due to organic amendments at Gleadthorpe and Gatsford, and fertiliser and pH at Craibstone. There was a significant effect on only the types of fungal taxa due to organic amendments at the Harper Adams trial, cultivation at the STAR trial and a weak but significant effect of tillage at Loddington (Table 2). There was no effect on the types of taxa due to organic amendments at the Terrington trial or drainage at the Boxworth trial (Table 2). Where significant, organic amendments at Gleadthorpe and Harper Adams, and pH treatments at Craibstone (mean 36% effect size across bacterial and fungal types) showed approximately 3-fold the effect size than observed for organic amendments at Gatsford and cultivation at Stanaway farm (mean 11%) and 5-fold the effect of fertiliser application at Craibstone and tillage practice at Loddington (7%). Supplementary File S2 shows the taxa that were unique to treatments in each of the trials, and when all raw ASVs were merged prior to analyses, taxa unique to treatments in any one trial were also unique across all trials.

3.1.2.2. Effects of location. Merging raw ASVs from separate data sets from each trial prior to calculating mASVs revealed that, irrespective of management intervention, there was a significant difference in the types

Table 2
The effect of management practices and location on soil microbial diversity estimated by >98% merged ASVs (taxa) and a comparison of the effects. P values from Kruskal-Wallis tests for mASVs richness and PERMANOVA test on binary (types) and abundance based Jaccard distances assessing the effect of management practices on bacterial (16S) and fungal (ITS) communities. Effect sizes (E2 and R2) for significant differences at P < 0.05 (in bold) are shown. OA = Organic amendments.

		OA (Terrington)		OA (Harper Adams)		OA (Gleadthorpe)		Drainage (Boxworth)		Tillage (Loddington)		Fertiliser (Craibstone)		pH (Craibstone)		OA (Gatsford Farm)		Cultivation (STAR; Stanaway Farm)		Location	
		All include Green compost @250 kg N/ha; c.25 t/ha treatment																			
Barcode		*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*	*16S*	*ITS*
Taxa richness	P	0.561	0.430	0.113	0.561	0.710	**0.035**	1	**0.016**	0.452	0.825	0.479	0.488	**0.001**	**$3x10^{-4}$**	0.528	0.998	0.670	0.407	**5.5^{-7}**	**2.7^{-25}**
	E^2	–	–	–	–	–	**0.036**	–	**0.026**	–	–	–	–	**0.073**	**0.089**	–	–	–	–	**0.211**	**0.730**
Types of taxa	P	0.167	0.105	0.214	**0.016**	**0.005**	**0.001**	0.659	0.149	0.203	**0.049**	**0.041**	0.077	**0.001**	**0.001**	**0.006**	**0.004**	0.297	**0.04**	**0.001**	**0.001**
	R^2	–	–	–	**0.309**	**0.386**	**0.379**	–	–	–	**0.079**	**0.056**	–	**0.411**	**0.325**	**0.105**	**0.113**	–	**0.150**	**0.294**	**0.368**
Abundances of taxa	P	0.335	**0.007**	0.224	**0.037**	**0.003**	**0.001**	0.649	**0.004**	**0.037**	**0.034**	**0.033**	**0.002**	**0.001**	**0.001**	**0.022**	**0.038**	0.322	0.066	**0.001**	**0.001**
	R^2	–	**0.330**	–	**0.375**	**0.416**	**0.477**	–	**0.174**	**0.088**	**0.090**	**0.063**	**0.112**	**0.51**	**0.364**	**0.112**	**0.112**	–	–	**0.362**	**0.347**

of bacterial and fungal taxa between all trials (P = 0.001, Fig. 3a). Principal Coordinate Analysis (PCoA) plots show clear clustering according to location, especially for fungi. The exception to this was the Craibstone pH trial where extreme soil pH values were maintained and three distinct sub-groups of taxa types were observed, with the pH 4.5 treatment forming the most distinct taxa types. One of these Craibstone pH taxa groups clustered with the spatially adjacent Craibstone fertiliser trial. Merging raw ASVs from separate data sets from each trial prior to calculating mASVs also revealed the absolute numbers of taxa that are shared with at least one other site, and the number of taxa that are unique to each site (Fig. 3b). The Craibstone pH trial had the greatest number of distinct bacterial and fungal taxa types across all sites. There were no mASVs that were found in all samples from all trials, and 257 mASVs were present in at least one sample from every location.

3.1.3. *Relative abundances of taxa*

3.1.3.1. Effects of management intervention. Comparisons of outcomes from the independent analyses of trials revealed a significant effect of management intervention (P < 0.038, Table 2) on the abundances of fungal taxa in all trials apart from the cultivation trial at Stanaway Farm. There was a significant effect of management intervention on the abundances of bacterial taxa (P < 0.037, Table 2) in five trials: organic amendments at Gleadthorpe and Gatsford, tillage at Loddington, and fertiliser and pH at Craibstone. From effect sizes, the greatest differential abundances of taxa were due to pH manipulation at Craibstone and organic amendments at Gleadthorpe, where an average of 44% of the variance in taxa abundances were explained by pH and organic amendment treatments, respectively, which was 25% greater than the effect of organic amendment treatments on fungi at Harper Adams and Terrington (mean 35%), 2.5-fold greater than the effect of drainage at Boxworth, and 4-fold greater than the effect of organic amendment and tillage at Gatsford Farm and Loddington respectively (mean 10%).

3.1.3.2. Effects of location. Merging raw ASVs from all trials prior to calculating mASVs allowed differences and similarities in taxa abundances between trials to be analysed. There was a significant difference in the abundances of bacterial and fungal taxa between sites overall, irrespective of management intervention (P < 0.001, Fig. 4a). PCoA ordination plots show clustering according to location again, but perhaps less tightly than for types of taxa (Fig. 3a). Again, the Craibstone pH trial was the exception to this rule as here taxa abundances, especially bacteria, were variable when compared with other locations, with taxa from the extreme pH 4.5 treatment forming the most distinct group. Again, one of these Craibstone pH groups clustered with the spatially adjacent Craibstone fertiliser trial. Merging raw ASVs from all trials prior to calculating mASVs also allowed a comparison of the taxonomic similarity in bacterial and fungal abundances between sites, which appeared remarkably similar overall at the phylum level (Fig. 4b). The bacterial mASVs spanned 39 phyla (of which 9 were candidate phyla), Acidobacteriota and Proteobacteria being the most abundant at all eight sites. Fungal mASVs represented 14 phyla where Ascomycota and Mortierellomycota were largely the most abundant at all sites.

3.2. *Testing the hypothesis that management has a greater effect than location on soil microbiology*

The DNA-BSAM allows an interrogation of the nature and magnitude of the effects of different soil management interventions and location on soil microbiology. Overall, the hypothesis that management has a greater effect than location on soil microbiology was not strongly supported. This is primarily because: 1) there was a large variance in the effects of various management interventions at different sites, and 2) that location irrespective of management had an equivalent or greater effect size than most management interventions for most metrics

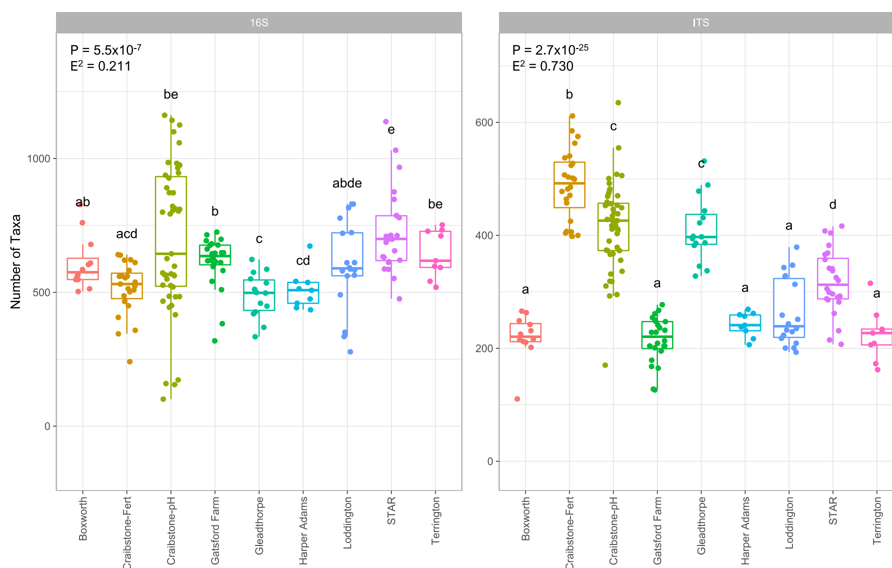

Fig. 2. Box plots showing significant general and pairwise differences in richness of 16S and ITS barcodes from the trial soils. Significant differences in numbers of taxa between trial sites as revealed by Wilcoxon pairwise tests are shown as compact letter displays (p-values have been adjusted using the Benjamini-Hochberg method).

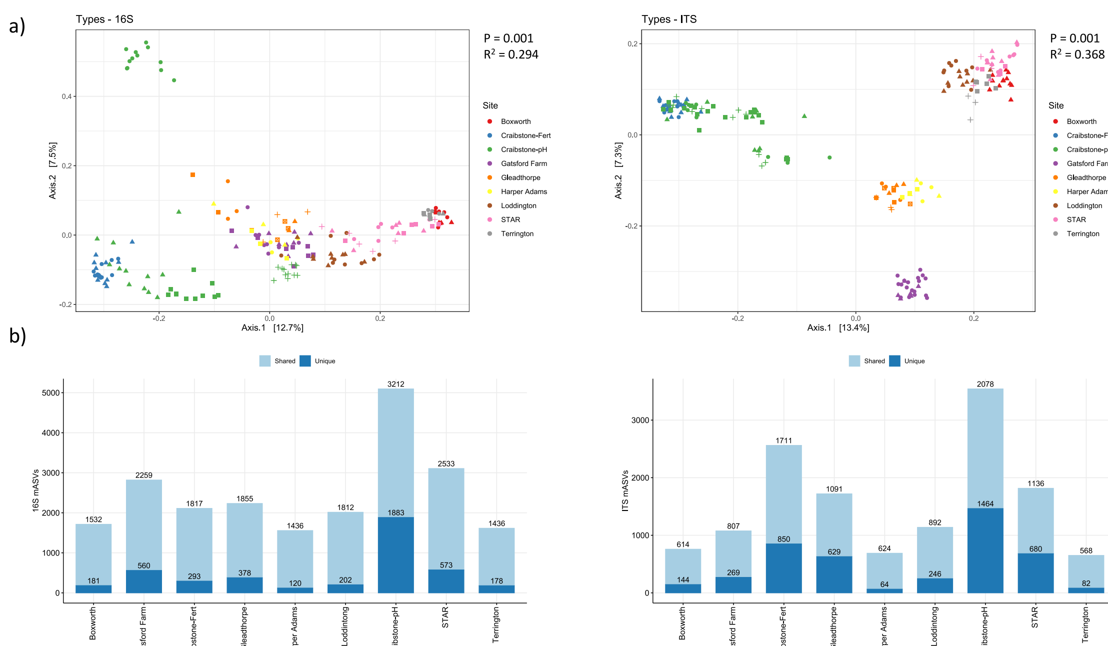

Fig. 3. (a) Principal Coordinate Analysis (PCoA) of the types of 16S and ITS mASVs in soils based on a binary Jaccard distance matrix derived from the merged dataset. The percent variance explained by each PCoA axis is indicated. Samples are coloured by Trial. (b) Bar-plots are stacked indicating the number of mASVs found in common (light blue) across all trials and the number of ASVs found exclusively in each trial (dark blue).

(Table 2 and Fig. 5). The pH trial at Craibstone was the only trial that consistently affected both bacterial and fungal taxa by all three biodiversity metrics (numbers, types and abundances of taxa; Table 2, Fig. 5), and pH manipulation had effect sizes that were greater than that of location (Fig. 5) on soil microbes. The organic amendments trial at the Gleadthorpe site consistently affected both bacterial and fungal taxa by all biodiversity metrics except the numbers of bacterial taxa (Table 2, Fig. 5), and had effect sizes that were greater than that of location (Fig. 5) on soil microbes. The effect sizes of pH at Craibstone and organic amendments at Gleadthorpe were approximately similar and on average accounted for 41% of variance in bacterial and fungal taxa types and abundances which compares to an average effect size of 34% for location. This is a comparatively large effect size for soil microbiology. Organic amendments at Gatsford caused the next largest significant effects for both the types and abundances of fungi and bacteria, but the effect size was only one-quarter that of pH at Craibstone and organic amendments at Gleadthorpe (mean 11%) and one-third the effect of location. Organic amendments at Harper Adam's site only affected fungal types and abundances modestly but with reasonable effect sizes (mean 34%). The effects of tillage at Loddington, inorganic fertiliser at Craibstone, and drainage at Boxworth were weaker and variously influenced only selected aspects of fungal or bacterial types or

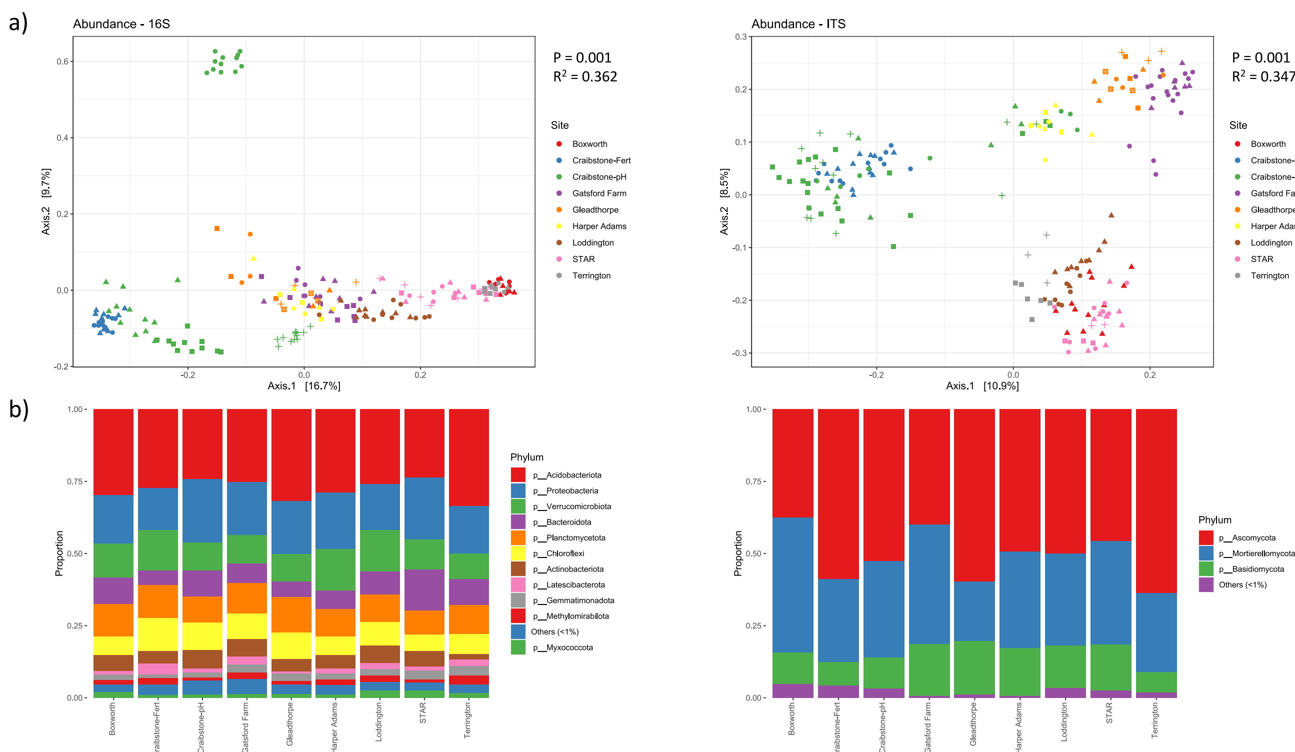

Fig. 4. (a) Principal Coordinate Analysis (PCoA) of the abundance of 16S and ITS mASVs in soils based on a Jaccard distance matrix derived from the merged dataset. The percent variance explained by each PCoA axis is indicated. Samples are coloured by Trial. (b) Comparison of trial sites showing bacterial (left) and fungal (right) taxonomic composition. Bars represent the relative abundance of the indicated phyla in all trials.

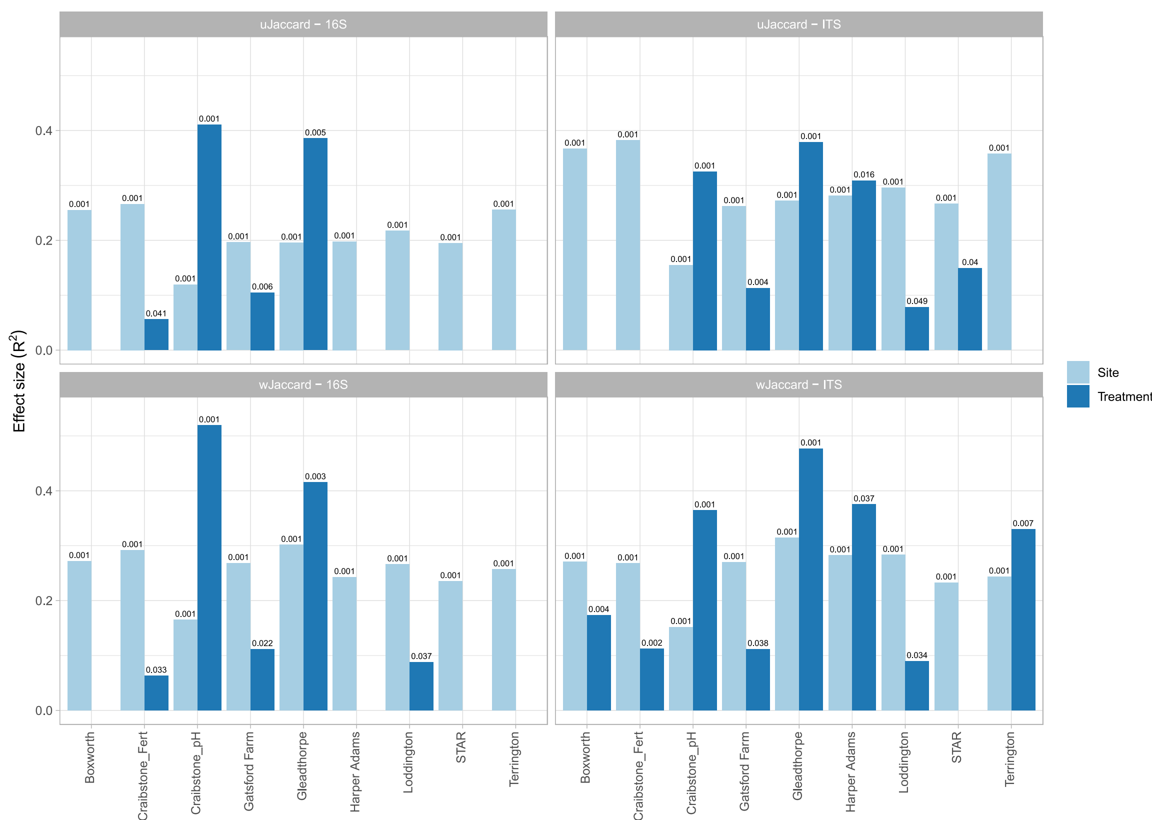

Fig. 5. Bar plots comparing average PERMANOVA effect size for the types (top) and abundances (bottom) of 16S and ITS mASVs by geographical location and total effect size by management practices, where p values are indicated at the top. Effect sizes were p value > 0.05 are not included.

abundances (Table 2, Fig. 5). On average, where significant, the effect sizes for these physical or chemical interventions was 9%. Two of the trials showed a very weak or no effect on soil microbiology. There was no effect of the four rotations and cultivation methods (annual ploughing, deep non-inversion, shallow non-inversion and a managed approach) on soil microbiology at the STAR trial in Suffolk other than a very weak signal for changes in the types of fungal taxa ($P = 0.04$; Fig. 4, Table 2). There was no effect of the organic amendment methods (green compost and pig farmyard manure) on soil biology at the Terrington trial in Norfolk other than for changes in the abundances of fungal taxa ($P = 0.007$; Table 2).

4. Discussion

While many studies have reported the effect of various agricultural management practices on soil microbiology, the analytical approaches and types of diversity indices used usually varies between studies making them difficult to compare. To directly address this, we have presented and tested the utility of a post-sequencing soil DNA biodiversity standard analysis method (DNA-BSAM) to precisely allow soil microbial diversity to be both assessed and to compare the effects of different treatments from independent site-specific studies. The utility of this standardized analysis allows comparison of both the relative significances and effect sizes of a range of soil management interventions from separate studies post-analyses even if different barcode regions are used (e.g. V3, V4 or V3-V4 region of the 16S for bacteria; ITS, 18S or 28S for fungi). If the same barcode regions are used, it also provides the detail of how to merge ASV tables from separate studies and analyse these together more powerfully. The advantage of merging ASV tables lies in being able to control for the effects of normalisation (i.e., all data are normalised together in the same way), and that more holistic analyses can be conducted, including being able to identify any differences in the direction of effects and to identify clusters of highly similar ASVs between studies. The difference in the mASV richness when mASVs were calculated for each trial separately and when ASVs were merged across all trials prior to mASV calculation (Table S4) is worth comment. In principle, this may be due to two reasons: 1) differences in sequencing depth cut-off during the normalisation step between individual trial datasets and a dataset where all trials were merged (lower read number cut offs in one or other will result in lower richness estimates); and 2) differences in mASV clustering between individual and merged datasets since the input pool of raw ASVs used to create mASVs differs in size and types between the two. To see if both these effects play a role, we compared the numbers of mASV derived from individual trials and where all trials are merged prior to mASV calculation but where all samples had the identical sequencing depth cut-off value of 6,270 16S and 7,842 ITS reads (Table S6). This shows that even with identical read-depths that there still may be slight differences in mASV richness when mASVs were calculated for each trial separately and when ASVs were merged across all trials prior to mASV calculation, and this is presumably since the difference in the size and types of ASVs in the input pool has an effect mASVs clustering. However, the aspect to focus on is not the absolute difference in the number of mASVs between the individual and merged approaches, which will be slightly different in each study, but if the trends in magnitudes and effect sizes are similar, which they are here (see Table S5), and we predict will also be in other studies that employ the DNA-BSAM for cross-study comparisons. Considering normalisation methods more generally, then there are several different data normalisation methods that may be employed, and one concern is that these hinder comparisons across studies (McMurdie and Holmes, 2014; Gloor et al., 2017; Weiss et al., 2017). We went on to analyse the effect of different normalisation methods on the analyses conducted here. The entire data set was also normalised with both rarefaction and by proportions and re-analysed, and this recovered the same pattern of significances and effect sizes (Tables S7 and S8) indicating the DNA-BSAM and main outcomes revealed in this study are insensitive to the method of data normalisation. Further, we feel the choice of taxonomic databases will not significantly influence the utility of the DNA-BSAM analyses per se: an insignificant proportion on the data were filtered out due to not being assigned to bacteria or fungi/Stramenopila (4% of and 2.9% of bacterial and 'fungal' reads, respectively). The use of alternative taxonomic databases would mainly only affect the lower taxonomic level naming of mASVs, and as we move forward with more complete databases more mASVs will be named but this will not affect the analyses of how these mASVs differ between treatments/trials. Overall, the DNA-BSAM allows comprehensive comparisons of the nature of effects, and the types of taxa that are common or different between different treatments in different studies.

We evaluated the effect of nine agricultural trials that have consistently applied key agricultural soil management interventions to develop and demonstrate the efficacy of the DNA-BSAM. The data and analysis presented here do not support the hypothesis that soil management interventions have a greater effect than location on soil microbiology due to the large variance in the effects of different management interventions at the different sites, and that location had an equivalent or greater effect size than most management interventions (Table 2 and Fig. 5).

The variance in the effects of the different interventions at different locations is large with no clear trend of effect strength between organic amendment, inorganic fertiliser addition, or mechanical and chemical soil disturbance categories, but we note this may not be formally analysed as there is no replication for many management categories. Some trials showed no significant effect on soil bacteria and fugal communities, but other interventions had a large effect (Table 2). The pH and organic amendment interventions at Craibstone and Gleadthorpe respectively had the greatest and most consistent effect on soil microbes (41% effect size). These data are in line with the well reported observations that pH has a large and significant effect on soil microbiology (Borneman and Hartin, 2000; Fierer et al., 2005; Fierer and Jackson, 2006; Rousk et al., 2010) and we show that even small changes in soil pH result in significant changes in bacterial and fungal community compositions. While the effects of organic amendments at Gleadthorpe were large, the effects of organic amendments at other sites were smaller and variable, which is in line with previous studies reporting inconsistent effects of organic amendments on soil microbes (e.g. (Li et al., 2019). Fernandez-Gnecco et al. (2022) have recently shown different disturbance signals by management practice such as tillage for bacterial and fungal communities. Here tillage and other cultivation methods at the Loddington and STAR sites only had a very weak effect.

The data revealed strong and significant differences between all trial locations irrespective of management interventions for both bacterial and fungal communities (Figs. 3 and 4), and on average 36% of the total variation in bacterial and fungal types and abundances is explained by location alone. However, soil microbiomes were not completely discrete between sites, as Fig. 3b illustrates. The signal for differentiated soil microbiology by location was relatively stronger and more consistent than the collective effects of soil management intervention (Fig. 5), with only the pH and organic amendment interventions at Craibstone and Gleadthorpe having effects that were larger than location. It is important to note that the effects of location include all variables at sites such as crops planted, climate and soil type which will each contribute to locational differences in soil microbes. These findings of differences by location are in-line with data from horticultural soils in New Zealand which show location is a strong driver of soil microbiology (Giraldo-Perez et al., 2021). The conclusion of differences by location revealed here are also in line with a recent study evaluating 12 European long-term experiments by Hannula et al. (2021) which reported over two thirds of the fungal species described were unique to each of the countries involved. Soil biodiversity differences between sites will be due to a combination of selective and stochastic ecological forces (Hendgen et al., 2018) but the precise balance of each would need to be empirically determined.

There are signals for both the differential effects of different management interventions at the same site, and different effects of the same management interventions at different sites. For example, the pH and fertiliser trials were immediately adjacent to one another at the Craibstone location, but the microbes from all pHs other than the 6–6.5 levels were different, and the adjacent inorganic fertiliser trial which was also maintained at pH 6 had the same microbial communities as the pH 6 trial ones. In contrast, the same 'Green compost (@250 kg N/ha; c.25 t/ha)' management interventions were applied at the Gleadthorpe, Harper Adams and Terrington sites, but these reported a range of very strong to very weak differences compared to the same manufactured fertiliser control treatments at each site (Table 2). A significant part of these observations may be explained by the finding of significant differences in soil microbiology between locations irrespective of management interventions: the differential response to the same management interventions at different locations could be due to different underling soil microbial communities. This suggests a revised hypothesis that can be tested going forward: it is the interaction between site and soil management intervention that better defines soil microbiology. To test this hypothesis an experimental design where identical management interventions are applied across multiple locations would be necessary, and the DNA-BSAM would be ideal to analyse the data from such a study. Indeed, the DNA-BSAM was precisely developed to test comparisons of the magnitude of effects between studies more generally going forward to increase understanding of the relative strength and direction of the effects of specific land, agronomic and soil managements at specific sites on biological communities over space and time.

The standardised analytical approach presented here does not extend to suggestions how soils or habitats should be sampled or processed once in the laboratory, including the methods of DNA extraction. The choice of these techniques may differ according to the nature of the study, and differences in these will affect inferences of changes in biodiversity, and so future work to standardise this aspect would be valuable. Here identical sampling and laboratory methodologies were applied to all samples, allowing valid comparisons between samples from all locations.

This DNA-BSAM can be used to compare any studies that use DNA barcodes (including extension to animal barcodes such as cytochrome *c* oxidase I (Giraldo-Perez et al., 2021)) and be extended to analyse soil biomes beyond agricultural soils (Morrison-Whittle and Goddard, 2018) to compare the effects of different management approaches or land-uses on biodiversity. This study significantly redresses the lack of available molecular data for UK agricultural soil microbiology and starts to address the urgent need for a standardized approach for measuring soil biodiversity to allow meaningful comparisons across studies to quantify the effects of soil management practices across agricultural systems, climates, and soil types. The suggestion revealed by the analyses here, that it is the interaction between site and soil management intervention that better defines soil microbiology, means it may be unreliable to extrapolate the soil microbiology findings of trials that manipulate soil management interventions at one site to other sites across the UK and more widely. This possibility is important to appreciate given the increased need for implementation of more sustainable agricultural management practices that strive to improve soil integrity in the long term. The widespread use of a standard approach going forward will allow meaningful cross-comparisons between soil microbiome studies and thus a substantial evidence-base on the effects of land-management on soil biology to accumulate and inform decisions to help enhance soil health for the global agriculture and conservation sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All high-throughput sequencing files were deposited in the Sequence Read Archive (SRA) under BioProject PRJNA835416.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109104.

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