A meta-analysis of the effect of organic and mineral fertilizers on soil microbial diversity

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ABSTRACT

The Green Revolution of agriculture was in part driven by application of synthetic mineral fertilizers, largely supplanting organic manure as a source of the major nutrients nitrogen, phosphorous and potassium (NPK). Though enhancing crop production and global food security, fertilizers have contributed to soil acidification, eutrophication of water bodies, and greenhouse gas emissions. Organic agriculture, employing manures or composts, has been proposed as a way of mitigating these undesirable effects. Of particular interest is the effect of fertilizer regime on soil microbes, which are key to nutrient cycling, plant health and soil structure. Meta-analyses of experimental studies indicate that mineral fertilizer increases soil microbial biomass over unfertilized controls, and that organic fertilizers increase microbial biomass and activity over mineral fertilizers. However, the effect of fertilizers on soil microbial diversity remains poorly understood. Since biological diversity is an important determinant of ecosystem function and a fundamental metric in community ecology, the effects of fertilizer regimes on soil microbial diversity are of theoretical and applied interest. Here, we conduct a meta-analysis of 31 studies reporting microbial diversity metrics in mineral fertilized (NPK), organically fertilized (ORG) and unfertilized control (CON) soils. Of these studies, 26 reported taxonomic diversity derived from sequencing, gradient gel electrophoresis, RFLP, or dilution plate assay. Functional diversity, derived from Biolog Ecoplate™ measures of carbon substrate metabolism, was reported in 8 studies, with 3 studies reporting both diversity metrics. We found that functional diversity was on average 2.6 % greater in NPK compared with CON, 6.8 % greater in ORG vs CON and 3.6 % greater in ORG vs NPK. Prokaryote taxonomic diversity was not significantly different between NPK and CON, 4.2 % greater in ORG vs CON and 4.6 % greater in ORG vs. NPK. Fungal taxonomic diversity was not significantly different between NPK or ORG vs CON, but 5.4 % lower between ORG and NPK. There was very high residual heterogeneity in all meta-analyses of soil diversity, suggesting that a large amount of further research with detailed analysis of soil properties is required to fully understand the influence of fertilizer regimes on microbial diversity and ecosystem function.

INTRODUCTION

Diversity plays a key role in the resilience and adaptability of complex systems (Page, 2011), and biological diversity has been central to understanding of the structure and function of ecological communities (Ricklefs & Schluter, 1994). Human activities are rapidly eroding global biodiversity (Ceballos *et al*., 2015), hence understanding how human activities influence diversity and how negative impacts can be avoided is an important goal of applied ecology (Sutherland *et al*., 2006; Rudd *et al*., 2011). Recent advances in environmental DNA sequencing and metabarcoding have revealed enormous and unexpected microbial diversity in all habitats, particularly soils (Thompson *et al*., 2017; Lloyd *et al*., 2018). Given the fundamental importance of soils in terrestrial ecosystems, agriculture and food security, there is growing interest in the role of microbial diversity in processes such as nutrient cycling (Delgado-Baquerizo *et al*., 2016), and how soil diversity can be managed for maintenance of ecosystem services (Lemanceau *et al*., 2015). In agriculture, soil microbes are critical to desirable functions such as nutrient cycling, carbon storage, erosion control via soil aggregation, and disease suppression (Rillig *et al*., 2002; Mazzola, 2004; Sahu *et al*., 2017). A large fraction of the world's agriculture soils are in poor and deteriorating condition largely due to agricultural activities (Wuepper *et al*., 2020). Therefore, understanding how to manage soils for microbial diversity could help to prevent further deterioration.

Modern intensive, or conventional, agricultural methods, including application of mineral and chemical fertilisers, regular tillage, and use of synthetic pesticides and herbicides, aim to increase soil nutrition and suppress harmful species to produce higher crop yields, but these methods are environmentally damaging (Robertson, 2000). For example, growing pressure on agricultural land has resulted in soil nutrient depletion and soil erosion (Wopereis, Tamèlokpo, & Ezui, 2006; Lal, 2009). Mineral fertilizers supply nitrogen (N) as ammonium nitrate or urea, phosphorus (P) and potassium (K), with around 100 Tg N applied globally each year (FAO, 2020). The nitrogen sources are derived from the Haber-Bosch process which reduces dinitrogen to ammonia, accounting for around one third of the nitrogen fixed from the atmosphere (Fowler *et al*., 2013). Soil and plant-symbiotic microbes fix another third, marine cyanobacteria another third, with small contributions from lightning and fossil fuel combustion (Fowler *et al*., 2013). Typically less than half the N applied is taken up by crops, the remainder contributing to water pollution and the release of NOX greenhouse gases (Zhang *et al*., 2015). Organic agriculture, which applies manure- or compost-based fertilizers and soil conditioners and does not employ chemical pest controls (with certain exceptions), has been proposed as a means of both increasing soil nutrition and reducing environmental impacts (Reganold & Wachter, 2016), but evidence for organic benefits remains equivocal. For example, eutrophication potential appears greater in many organic than conventional crop systems (Clark & Tilman, 2017)

Soil health, the capacity of soils to function as a living system and sustain and promote plant and animal communities, is linked to microbial diversity (Kibblewhite *et al*., 2008; van Bruggen *et al*., 2019).

Microbial diversity is correlated with soil ecosystem multifunctionality including plant productivity, microbial biomass, availability of nitrate, ammonium and phosphorus, and nitrogen mineralisation rates (Delgado-Baquerizo *et al*., 2016). Hence, management to enhance soil microbial diversity could improve soil health. Soils are exceedingly complex and biologically-diverse ecosystems, varying in physico-chemical and biological composition across spatial scales (Howe *et al*., 2014; Thompson *et al*., 2017). Meta-analyses of multiple individual studies can reveal general trends and patterns in this complexity and determine the effects of management interventions. For example, observational data suggest that biological richness (measured by DNA sequences) is greatest at neutral pH and at a mean temperature of 10 °C (Thompson *et al*., 2017). Meta-analysis of experimental studies shows that pH has the greatest influence on soil microbial diversity among global change factors, alpha diversity rising with pH (Zhou *et al*., 2020). Nitrogen and NPK have negative or non-significant effects on alpha diversity depending on microbial group, with significant negative influences of N alone on diversity in agricultural soils. Soil functionality, defined as the range of biogeochemical processes carried out by soils, increases with N and NPK. Changes in diversity are negatively correlated with changes in functionality, perhaps due to functional redundancy (Zhou *et al*., 2020). Other meta-analyses have demonstrated that microbial diversity (Venter *et al*., 2016) and biomass (McDaniel *et al*., 2014) increase in crop rotations compared with monocultures. Organic agriculture greatly increases microbial biomass carbon, microbial biomass nitrogen, and enzymatic activity compared with conventional systems (Lori *et al*., 2017). Previously, a small meta-analysis found no significant effect of organic agriculture on soil organism diversity, but this only included five studies and occurred before widespread use of sequencing to soil microbial diversity (Bengtsson *et al*., 2005). However, despite growing application of sequencing technologies in microbial ecology, results on the effects of organic and mineral fertilizers on measures of soil microbial diversity remain unsynthesized.

Here, we conduct a meta-analysis quantifying the effects of organic fertilizers (manures and manure composts) and mineral fertilizers (NPK) on soil microbial diversity, in comparison with unfertilized controls. We compare results for taxonomic and functional diversity, and investigate the influence of factors such as soil chemistry and duration of organic treatment on these effects. We hypothesise that organic inputs will increase microbial diversity as compared to mineral fertilizer inputs, based on the observation that organic fertilizers increase microbial biomass and enzymatic activity compared with NPK (Lori *et al*., 2017).

MATERIALS AND METHODS

Criteria for meta-analysis

We used meta-analysis to test for differences in soil microbial functional (H_{fun}) and taxonomic (H_{tax}) diversity between soils treated with manure-based organic fertilizer (ORG), mineral fertilizer (NPK) and control (CON). Here, H_{tax} is defined as Shannon's diversity index calculated from the relative abundance of species, operational taxonomic units (OTUs) or amplicon sequence variants (ASVs; Callahan *et al*., 2017) in a sample. H_{fun} is defined as Shannon's diversity calculated from the relative conversion rates of various carbon sources in Biolog Ecoplates (e.g. Ros *et al*., 2006). We conducted a literature search using combinations of the terms 'soil', 'organic', 'agriculture', 'microbial', 'diversity', 'bacteria*', 'fung*', 'communit*', 'fertilizer', 'manure' and 'compost' (where * indicates a wildcard search, where appropriate) on Google Scholar, Scopus and Web of Science between August 2018 and June 2019. Additionally, the reference lists of the papers were browsed to find potentially appropriate studies which were not identified during the online literature search. All potentially appropriate papers identified for the meta-analysis came from peer-reviewed journals.

Papers were considered eligible for the analysis if they met the following criteria, adapted from Lori et al. (2017): Comparisons of the farming systems should be pairwise, meaning the organic- and mineral-fertilised treatments were subject to the same climatic conditions before and during sampling; The organic treatment must have been applied for a minimum of two consecutive years prior to soil sampling, and the treatments must be defined by the study; Results must report the mean Shannon diversity index (H) per treatment, uncertainty of the mean (either standard error of the mean or standard deviation of the sample distribution) and sample size (n); experiments were conducted in open fields or under cover, not in pots or containers; the study must have been published no earlier than the year 2000; the mineral and organic treatments must be clearly described (description of fertilizer, application rate). If there were several different organic or conventional treatments within the same study, all appropriate combinations were reported and treated as individual comparisons but with non-independent errors (see meta-analysis methods). Results of combination treatments (e.g. manure plus NPK fertilizer) were not included.

For all valid studies, we extracted mean, SE or SD, and sample size for H, for control, NPK and organic treatments. Where SE was reported, this was converted to SD by multiplying by the square root of sample size. When data were presented in graphical form, we extracted means and errors using the online tool WebPlotDigitalizer (https://automeris.io/WebPlotDigitizer/). Additionally we abstracted the following variables: study location; reported soil type, duration of organic treatment, organic treatment type (i.e. source of manure), organic treatment application rate (mass per hectare per year or equivalent nitrogen mass per hectare per year); mineral fertilizer application rate (nitrogen mass per hectare per year); crops grow; taxonomic group of microbes analysed (e.g. all, bacteria, fungi); median soil depth sampled (usually the mid-point of the range of depths sampled); brief summary of the methodology used (e.g. 16S sequencing, 16S DGGE, Biolog Ecoplate). Where reported we abstracted mean and SE of soil pH; and mean and SE of soil nitrogen content (mg kg^{-1}).

Meta-analysis

Meta-analysis was conducted using the *metafor* package v. 2.4–0 for R v. 4.0.2 (Viechtbauer, 2010). Effect size was calculated as the log–transformed ratio of means (Hedges *et al.*, 1999). There was at least one pairwise comparison of H in each study. However, several studies reported data from more than one pairwise comparison, with multiple organic and/or conventional fertilisation treatments investigated. We fitted multi-level random– and mixed–effects (i.e. testing for the effects of moderator variables) models by restricted maximum likelihood, with random intercepts per study. Other similar meta-analyses have ignored potential non-independence among results from the same study and treated each reported mean as an independent replicate (Lori *et al.*, 2017). Standard errors and confidence limits for parameters were estimated from the *t*–distribution. We report the I^2 statistic and Q-test for heterogeneity (Higgins & Thompson, 2002), and provide funnel plots rank correlation tests for funnel plot asymmetry (Viechtbauer, 2010).

RESULTS

We abstracted data from 31 studies which met our requirements (Table S1). Our search criteria provided a long-list of 259 studies, of which 195 were rejected on the basis of the abstract, leaving a shortlist of 64. A further 33 were rejected after detailed reading of the full text. Around half (16) of the included studies reported one set of comparisons, with either or both of unfertilized control (CON) and mineral fertilizer (NPK), 10 reported two comparisons, and five studies reported up to six comparisons, giving 57 comparisons in total. The majority of treatments were replicated three or four times. Bacteria were the most commonly analysed group (24 studies, including one of actinomycetes only, one of bacteria and archaea, and one of nitrogen-fixing bacteria), followed by fungi (6 studies, including one of arbuscular mycorrhizal fungi only). The functional diversity studies, and the single study reporting soil dilution plate assays (Mahanta *et al.*, 2017), were taxonomically non-specific. Most studies were conducted in China (19), followed by India (6), with single studies from Austria, Canada, Denmark, Kenya, the Netherlands, and the USA.

Soil types were not reported according to any standard taxonomy. Soils were sampled to a median depth of up to 15 cm (median 10.0 cm, IQR 7.5 - 10.0 cm). Soil nitrogen content in unfertilized plots was reported in 14 studies (mean mg N kg^{-1} median 77.9, IQR 28.5 - 99.6). Manure-based organic fertilizers were applied in all studies, derived variously from cattle, pigs, poultry, horses, sheep, or mixtures of these. Composted manures were employed in 8 studies, fresh manures in 24 studies (one study employed both). A small number of studies reported more than one manure treatment or level (Mahanta *et al.*, 2017; Hu *et al.*, 2018b; Liu *et al.*, 2019). Application rates were reported as either total mass per area per year (24 studies, median 10 t ha^{-1} y^{-1}, IQR 4.9 - 18.6 t ha^{-1} y^{-1}) and/or as nitrogen addition (15 studies, median 102.0 kg ha^{-1} y^{-1}, IQR 60.0 - 140.0 kg ha^{-1} y^{-1}). We did not attempt to estimate nitrogen content in manures where this was not reported, due to the large variability in the nitrogen fraction among different treatments. The reported nitrogen fraction varied from 0.4 % in horse manure to 2.7 % in pig manure, with overall median 1.0 % (IQR 0.7 - 2.0 %) among the treatments reported in the studies. The level of nitrogen in the manure treatment was similar to that in corresponding NPK fertilizer treatments, where reported (absolute percentage difference median 13.4 %, IQR 0.0 - 56.0 %). The duration of organic treatment was right-skewed, but many studies reported treatment periods of several decades (median 20 y, IQR 11 - 34.5 y). A variety of crops were grown, either as monocultures or in mixtures, the most common being maize (9 studies), wheat (7 studies), and rice (6 studies). Soil pH in unfertilized plots was reported in 16 studies (mean pH median 6.5, interquartile range 6.0 - 7.7). Meta-regression showed that soil pH declined under NPK treatment by -0.53 ± 0.20 compared to control soil (Fig. S1a). Manure-based fertilizers increased pH in acid soils and reduced pH in alkaline soils (Fig. S1b). Funnel plots showed no asymmetry (Fig. S2). There was a large degree of residual heterogeneity in the meta-analyses of soil pH (Table S2).

H_{fun} was reported by 8 studies, all employing Biolog Ecoplates (Table S1). H_{fun} varied between 1.12 and 4.61 with values in three clusters comprising a single study from Austria with $H_{fun} \sim 1.2$, four studies from China and one from India with $H_{fun} \sim 2.9$, and two studies from India with $H_{fun} \sim 4.5$ (Fig. 1a-c). H_{fun} increased by an average of 2.6 % in NPK vs. CON, 6.8 % in ORG vs. CON, and 3.6 % in ORG vs. NPK (Fig. 2, Table 1). We found no influence of the duration of organic amendment on the change in H_{fun} compared with control (LRM change per year of organic treatment -0.0008 ± 0.0013 y^{-1}, $t = -0.58$, $p = 0.56$) or NPK (0.0006 ± 0.0007 y^{-1}, $t = 0.84$, $p = 0.40$) treatments. We found no effect of the level of NPK addition relative to control (-0.0013 ± 0.0009, $t = -1.42$, $p = 0.15$), nor of level of organic fertilizer addition quantity (0.0000 ± 0.0000, $t = -0.35$, $p = 0.72$) or nitrogen equivalent relative to control (0.0001 ± 0.0005, $t = 0.18$, $p = 0.86$). We found a marginally-significant effect of control soil pH on the effect size of ORG vs. CON (-0.052 ± 0.025, $t = -2.10$, $p = 0.036$), but not of soil pH on NPK vs. CON (0.0045 ± 0.0527, $t = 0.085$, $p = 0.93$). Soil N did not influence the effect size of ORG vs. CON (0.000 ± 0.001, $t = -0.01$, $p = 0.99$). Funnel plots did not exhibit significant asymmetry (Fig. S3). There was a large degree of residual heterogeneity in all meta-analyses of H_{fun} ($I^2 > 75$ %, Table S2).

H_{tax} was reported in 31 studies, employing DNA amplicon sequencing (17 studies), various forms of gradient gel electrophoresis (11 studies), soil dilution plate assay (1 study) and T-RFLP (1 study). H_{tax} tended to be higher in prokaryotes than fungi, and in sequencing compared with DGGE methods (Fig. 1d-f). Median H_{tax} for prokaryotes was 6.36 using sequencing and 2.85 using DGGE. Median H_{tax} for fungi was 3.36 using sequencing and 1.40 using DGGE. H_{tax} of NPK-fertilized soils did not differ from control for prokaryotes or fungi, nor in the single study using dilution plate assays (Fig. 3, Table 2). H_{tax} of prokaryotes was greater in ORG soils than both NPK-fertilized soils and control. Fungi were less taxonomically diverse in ORG than NPK, but there was no significant difference in fungal H_{tax} between ORG and CON. In the soil dilution plate assay, organically fertilized soils were more diverse than NPK or CON. We found no evidence that duration of organic amendments (0.0006 ± 0.0009, $t = 0.66$, $p = 0.51$), pH (-0.0054 ± 0.0038, $t = -1.42$, $p = 0.15$) or soil N (-0.0008 ± 0.0004, $t = -2.1$, $p = 0.051$) influenced the effect size of ORG vs. CON. Funnel plots did not exhibit significant asymmetry (Fig. S4). There was a large degree of residual heterogeneity in meta-analyses of H_{tax} ($I^2 > 95$ %, Table S2).

DISCUSSION

We found significant, but varying, effects of both NPK and organic fertilizers on soil microbe taxonomic and functional diversity. Taxonomic diversity in organic treatments compared with NPK was greater for prokaryotes but lower for fungi, and was greater for organic treatments compared with control for prokaryotes with no significant difference in fungi. We found no significant difference in taxonomic diversity between NPK and control for either prokaryotes or microbes. Functional diversity was significantly greater in NPK compared with control, and in organic treatment compared with NPK. We did not detect any significant influence of factors such as duration or type of organic treatment, soil chemistry or experimental methodology on effect sizes. Importantly, the residual heterogeneity in all meta-analyses was very high.

Several factors must be considered when interpreting the effects of fertilizers on soil microbial functional and taxonomic diversity (Fig. 4). First, while in many studies the amount of nitrogen applied in organic and NPK treatments was similar, farmyard manure is far more physically, chemically and biologically complex. In addition to NPK, manures contain undigested plant matter (lignin, cellulose, hemicellulose), lipids, carbohydrates, proteins, and nutritive elements (e.g. magnesium, iron, manganese, zinc, copper) (Levi-Minzi *et al*., 1986). If structural complexity and heterogeneity are enhanced by the addition of organic matter across spatial scales (Lehmann *et al*., 2008), this could in itself increase microbial diversity through provision of ecospace that reduces interspecific competition (Vos *et al*., 2013). Provision of additional energy (organic carbon) and micronutrients in manures could sustain a greater diversity of microbes via the species-energy hypothesis which predicts that more species can be sustained in ecosystems supporting more individuals (Clarke & Gaston, 2006), since manure increases soil microbial biomass more than NPK (Lori *et al*., 2017). Second, microbial communities may be indirectly influenced by changes in crop plants. While organic agriculture tends to be less productive in terms of crop yields (de Ponti *et al*., 2012; Hijbeek *et al*., 2017), there is some evidence that root development is greater in organic farming (Hu *et al*., 2018a). Plant rhizospheres tend to harbour the greatest microbial diversity of all terrestrial ecosystems (Thompson *et al*., 2017), hence it is possible that enhanced root development and exudation of organic compounds influence the soil diversity measured in these studies. Third, there is the gut microbial community residing within the manure itself. Both fresh and composted manures have high fungal and prokaryote diversity, with community composition changing as composting proceeds (Meng *et al*., 2019). Bacterial diversity has been found to peak during the thermophilic phase of aerobic composting during which temperatures exceeded 55 °C, while fungal diversity was lowest during this stage (Meng *et al*., 2019). Composting can reduce the presence of undesirable microbes in manure, for example those carrying antimicrobial resistance genes (Gou *et al*., 2018), but gut microbes (including human pathogens) can survive for long periods in compost-amended soils (Sharma & Reynnells, 2016). Hence, increased soil microbial diversity in organic systems may be due to persistence of gut microbes.

Few studies reported both taxonomic and functional diversity, hence we were unable to determine a relationship between these two metrics. Functional diversity has been found to increase with species diversity in plant communities (Mensah *et al*., 2016; Zhang *et al*., 2017), but less clearly in bird communities (Morelli *et al*., 2018). The relationship between soil taxonomic diversity and ecosystem function (as opposed to functional diversity) tends to be positive (Philippot *et al*., 2013; Bardgett & Putten, 2014; Maron *et al*., 2018). Given that ecosystem functions like nitrogen cycling require multiple interacting processes, a positive relationship between taxonomic diversity and functional diversity might be expected. Soil ecosystem multifunctionality (the capacity of soils to sustain many functions simultaneously) increases with microbial diversity (Delgado-Baquerizo *et al*., 2016), while taxonomic and functional gene diversity are closely correlated (Zhang *et al*., 2019). However, taxonomic and functional diversity can be decoupled by the process of horizontal gene transfer, because different taxa can perform similar tasks through shared genes (Zhang *et al*., 2019). In addition, the presence of physiologically-distinct subgroups within OTUs commonly defined by > 97 % sequence similarity (so-called ‘microdiversity’) means that functional diversity can be large in groups of apparently identical microbial taxa (Larkin & Martiny, 2017). Microbial functional diversity tends to be considered from the perspective of potential functions inferred from gene sequences (Escalas *et al*., 2019). In contrast, we report results from studies of the diversity of actual functions (metabolization of carbon sources) carried out by the soil microbial community. These genotypic vs. phenotypic measures of functional diversity are analogous to the fundamental and realized niches of species, whereby the potential functions identified in gene sequences are likely to exceed the observed functions carried out by the community under particular environmental conditions (Escalas *et al*., 2019). Discovering the relationships between potential and observed functions of microbial communities is a key goal of microbial ecology.

Organic farming has been promoted as a more sustainable alternative to conventional agriculture, because of greater energy efficiency, potentially closed nutrient cycles and increased biodiversity

(Reganold & Wachter, 2016). Certainly, the rapid decline of insect populations around the world due to intensive agriculture and pesticides, is an urgent impetus for reconsidering the way we produce food (Sánchez-Bayo & Wyckhuys, 2019). These potential benefits come at a cost of reduced productivity compared with convential farming (de Ponti *et al*., 2012; Hijbeek *et al*., 2017), though there is some evidence that organic production can eventually catch up with conventional yields and provides greater spatial and temporal stability (Schrama *et al*., 2018). Environmental impacts of organic agriculture include greater land use and eutrophication potential per unit of food produced, contradicting the aim of closed nutrient cycles (Clark & Tilman, 2017). Our results suggest that, when considering organic crop fertilization alone and as a strict alternative to NPK (rather than a mixed system of manure-derived and NPK fertilizer), microbial functional diversity and bacterial taxonomic diversity are slightly, but significantly greater in organic systems while fungal taxonomic diversity is slightly but significantly lower. The enhanced eutrophication potential of organic farming, due to temporal mismatching between fertilizer addition and plant demand, is much larger than the increases in taxonomic or functional diversity we found (Clark & Tilman, 2017). Hence, it appears that increased soil microbial diversity under organic farming does not translate to improved nutrient cycling and retention. While we did not compare combined treatments (e.g. NPK with manure or compost) with single treatments, farmers commonly apply a diversity of fertilizers. For example, a comparison of low-input organic systems with conventional mixed (NPK and manure) and conventional NPK-fertilized systems in Switzerland found the greatest bacterial α-diversity in the organic systems, followed by the mixed and NPK-only systems (Hartmann *et al*., 2015).

Many studies addressing soil microbial diversity in response to fertilization could not be included in our meta-analysis because of incomplete or unclear reporting. Without giving specific examples, we found that many studies did not provide uncertainties (standard error or standard deviation) for parameter estimates, gave unclear descriptions of the fertilisation routines and inputs, or reported alternative measures of diversity. Of the studies we included, several did not report basic soil chemistry metrics such as pH and nitrogen content. Given the importance of pH in determining soil microbial diversity (Zhou *et al*., 2020), and the central relevance of nitrogen in these experiments, any future research should report these variables at minimum. We were only able to include a small number of studies in our meta-analysis, which revealed very large residual heterogeneity among effect sizes, i.e. most of the variation in effect sizes among studies remains unexplained. Further research will reveal whether the mean effects we detected are general, and what other factors help explain variation among soils, climates, locations and experimental treatments. To achieve this, it is critical that detailed and complete data on key variables are reported. In addition, better understanding of soil ecosystem functioning will be achieved via analysis of the taxa and functional genes identified within samples, in addition to summary metrics like diversity (Hartmann *et al*., 2015). In this way, the powerful new bioinformatics tools at our disposal can be harnessed to fully understand the relationships between agriculture and soil health.

DATA AVAILABILITY

The data used in the meta-analysis will be made available on publication of the manuscript.

AUTHORS CONTRIBUTIONS

DB developed the research. DB and VR collected data, analysed results and wrote the paper.

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TABLES

Table 1. Summary statistics for meta-analysis of functional diversity (H_{fun}) differences between fertilizer treatments and control. One study (Ros *et al*., 2006) reported three comparisons, hence a multi-level random model was employed to control for within-study effects. Studies (N) refers to the number of studies and number of comparisons in this estimate. LRM is the log-transformed ratio of means, giving mean ± SE and 95 % Confidence Interval. t and P are a t-test of the effect size vs. zero. Change (%) is the effect-size back-transformed to percentage change.

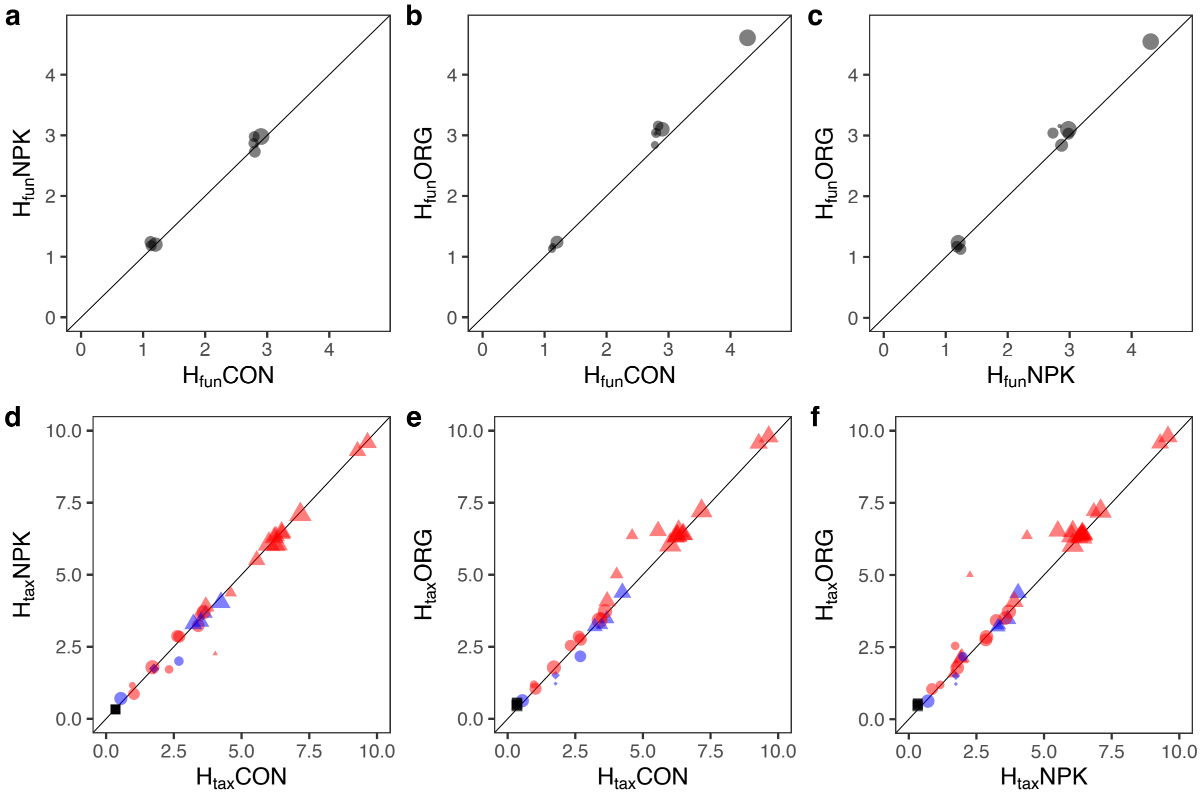
Comparison	Studies (N)	LRM	LRM (95% CI)	t	P	Change (%)	Change 95 % CI
NPK vs. CON	6 (8)	0.026 ± 0.004	0.016, 0.035	6.51	0.0003	2.6	1.7, 3.6
ORG vs. CON	7 (9)	0.066 ± 0.011	0.041, 0.090	6.21	0.0003	6.8	4.2, 9.4
ORG vs. NPK	7 (9)	0.035 ± 0.012	0.008, 0.063	3.00	0.0171	3.6	0.8, 6.5

Table 2. Summary statistics for meta-analysis of taxonomic diversity differences between fertilizer treatments and control. Statistics as in Table 1.

Comparison	Class	Studies (N)	LRM	LRM (95% CI)	t	P	Change (%)	Change % (95 % CI)
NPK vs. CON	All*	1 (1)	-0.042 ± 0.100	-0.239, 0.155	-0.42	0.67	-4.1	-21.1, 16.7
	Fungi	6 (7)	0.031 ± 0.020	-0.007, 0.069	1.58	0.11	3.1	-0.73, 7.5
	Prokaryotes	18 (23)	-0.006 ± 0.016	-0.037, 0.026	-0.35	0.73	-0.5	-3.6, 2.6
ORG vs. CON	All*	1(6)	0.372 ± 0.065	0.245, 0.499	5.76	10^{-8}	46.1	35.4, 57.7
	Fungi	6 (8)	0.016 ± 0.022	-0.028, 0.059	0.70	0.48	1.6	-2.7, 6.1
	Prokaryotes	18 (23)	0.041 ± 0.014	0.013, 0.069	2.84	0.004	4.2	1.3, 7.1
ORG vs. NPK	All*	1(6)	0.419 ± 0.072	0.017, 0.072	5.79	10^{-8}	52.9	40.1, 66.8
	Fungi	6 (8)	-0.055 ± 0.021	-0.097, -0.013	2.61	0.009	-5.4	-9.2, -1.4
	Prokaryotes	23 (33)	0.045 ± 0.014	0.017, 0.073	3.20	0.001	4.6	1.8, 7.5

*A single study (Mahanta *et al*., 2017) contributed to this estimate.

574 FIGURES



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576 Fig. 1. Comparison of H_{fun} (a-c) and H_{tax} (d-f) in (a,d) NPK vs. CON, (b,e) ORG vs. CON, (c,f) ORG
577 vs. NPK. Points show reported values with size proportional to log(1/variance). Results for Fungi are
578 in blue, Bacteria in red. Gel electrophoresis results are circles, sequencing results are triangles, TRFLP
579 are diamonds. The single soil dilution plate assay is shown as black squares.

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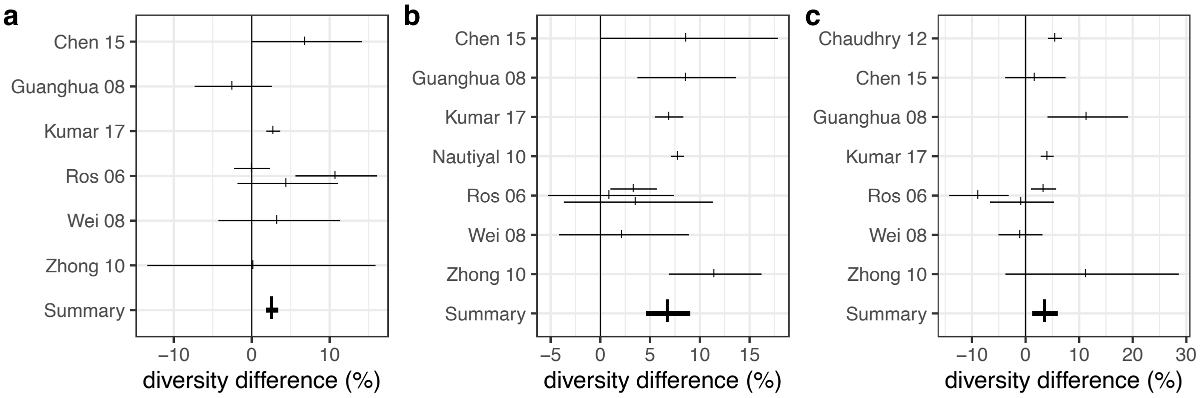

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582 Fig. 2. Functional soil diversity differences. a) NPK vs. CON, b) ORG vs. CON, c) ORG vs. NPK. Horizontal bars and ticks show 95% confidence intervals and means for effect sizes from the log-transformed ratio of means, back-transformed to give percentage differences. Summary estimates are given at the bottom of each plot. Random intercepts were fitted per Study.

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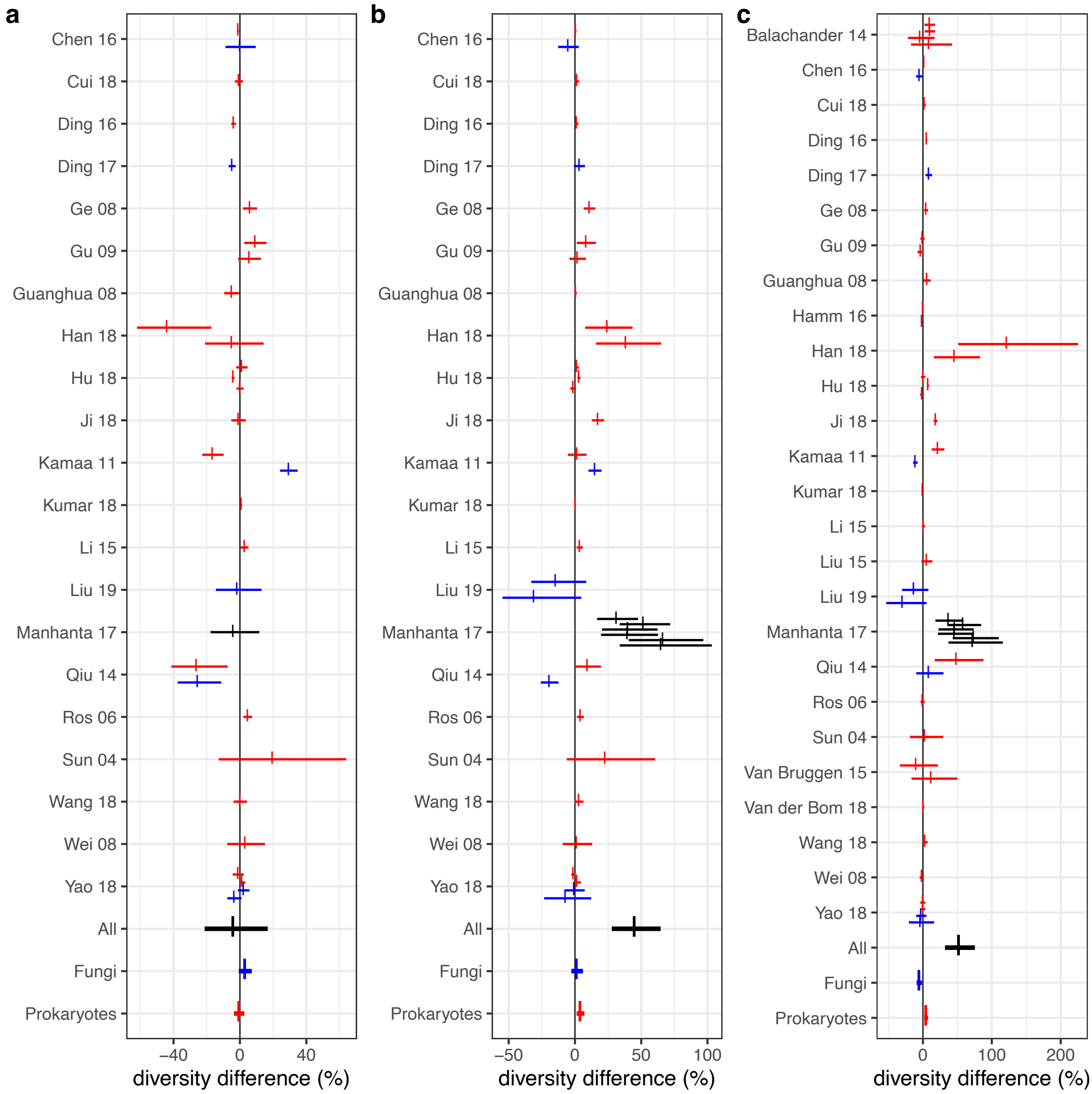


Fig. 3. Taxonomic diversity effect sizes. a) NPK vs. CON, b) ORG vs. CON, c) ORG vs. NPK. Horizontal bars and ticks show 95% confidence intervals and means for effect sizes from the log-transformed ratio of means, back-transformed to give percentage differences. Summary estimates are given at the bottom of each plot, for All microbes (black), Fungi (blue) and Bacteria (red). Random intercepts were fitted per Study. “All” refers to results of a single study (Mahanta *et al.*, 2017) which used soil dilution plate assays.

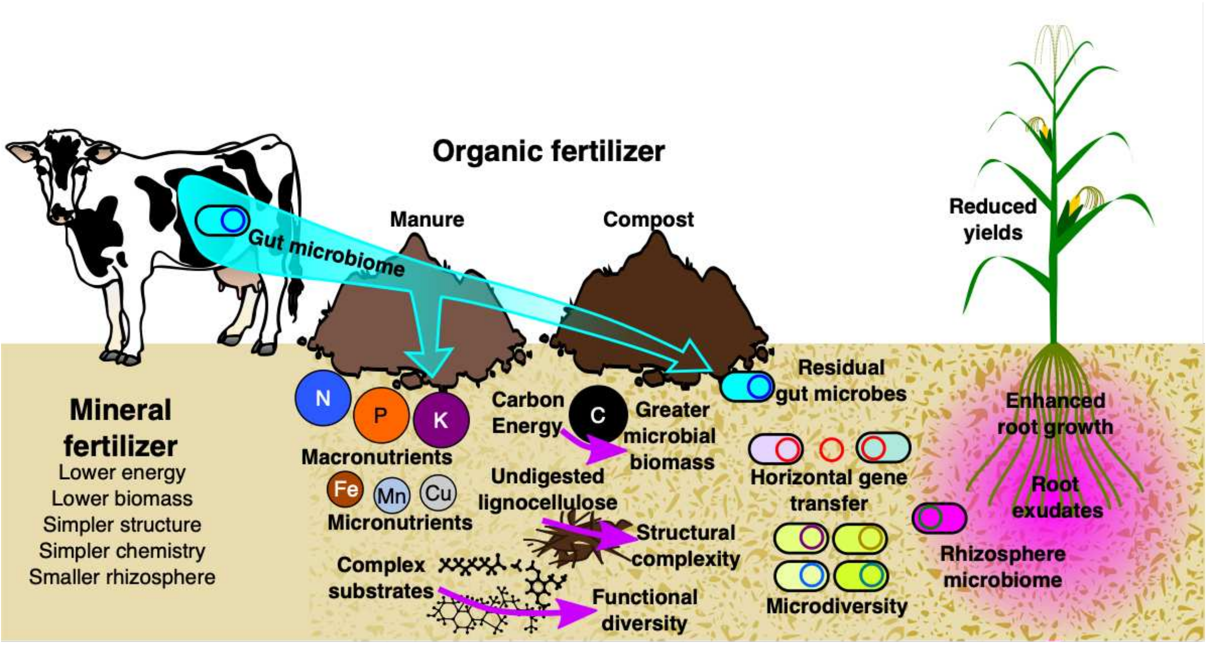


Fig. 4. Representation of processes influencing organic fertilizer affects on soil microbial diversity. See Discussion for details.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES

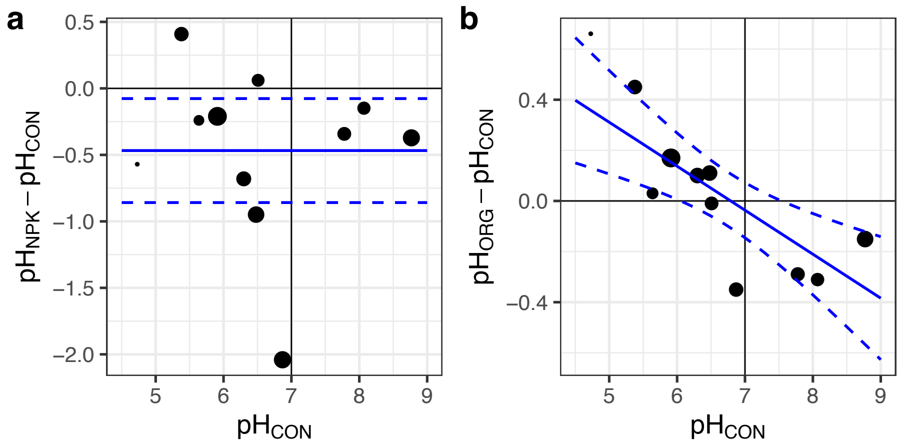


Fig. S1. Effect of fertilization on soil pH. a) Mean difference between NPK and CON vs. mean CON pH. Blue solid and dashed lines show mean difference (-0.46 ± 0.20 SE, Z = -2.34, p = 0.019) and 95 % CI (-0.86, -0.08) of meta-analysis fit. b) Mean difference between ORG and CON vs. mean CON pH. Blue solid and dashed lines show mean and 95% CI of meta-regression fit. Slope mean -0.174 ± 0.050, Z = -3.47, p = 0.0005, 95% CI = -0.272, -0.076. Points show means from individual studies with size proportional to log(1/variance).

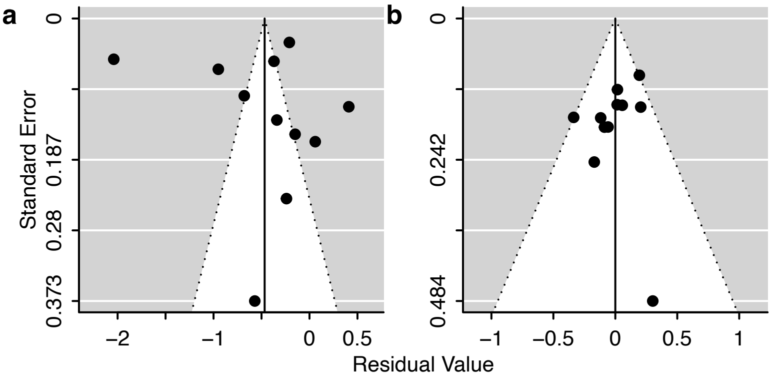
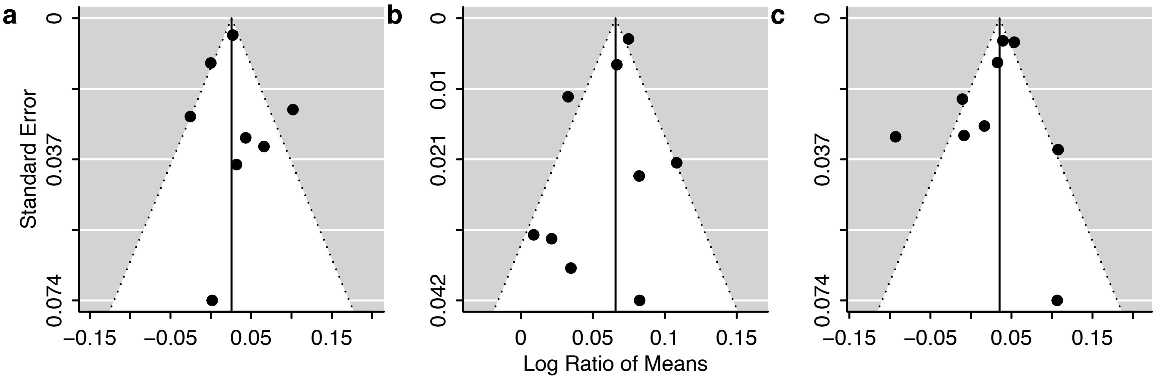


Fig. S2. Funnel plots for soil pH meta-analysis. Kendall's τ and p-values are given for rank correlation tests for funnel plot asymmetry. a) NPK vs. CON (τ = -0.055, p = 0.88), b) ORG vs. CON (τ = -0.018, p = 1.0).



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616 Fig. S3. Funnel plots for functional diversity meta-analysis. Kendall's τ and p-values are given for rank 617 correlation tests for funnel plot asymmetry. a) NPK vs. CON (τ = -0.071, p = 0.90), b) ORG vs. CON 618 (τ = -0.167, p = 0.61), c) ORG vs. NPK (τ = -0.167, p = 0.61).

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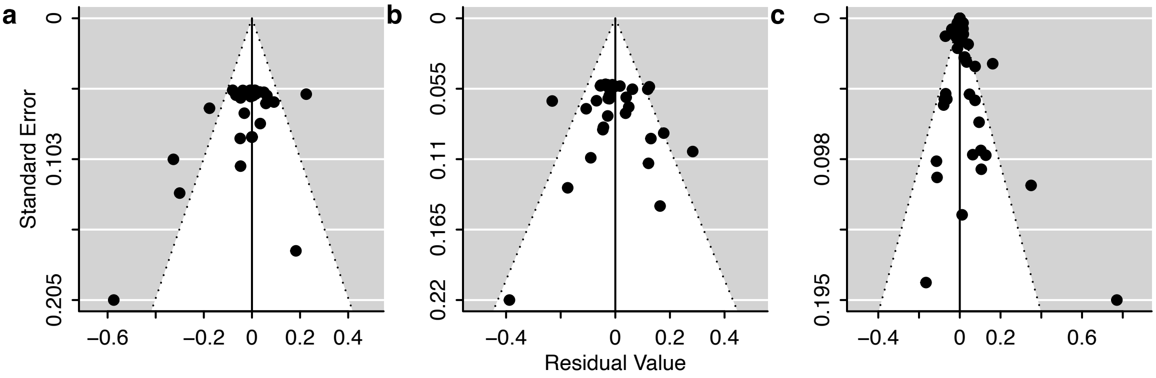

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621 Fig. S4. Funnel plots for taxonomic diversity meta-analysis. Kendall's τ and p-values are given for rank 622 correlation tests for funnel plot asymmetry. a) NPK vs. CON (τ = -0.123, p = 0.34), b) ORG vs. CON 623 (τ = 0.156, p = 0.18), c) ORG vs. NPK (τ = 0.149, p = 0.14).

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SUPPLEMENTARY TABLES

Table S1. Summary of studies included in the meta-analysis (alphabetical order).

Study	Location	Measure	Methodology
(Balachandar *et al*., 2014)	Tamil Nadu, India	Taxonomic	16S seq
(Chaudhry *et al*., 2012)	Maharashtra, India	Functional	Biolog Ecoplate
(Chen *et al*., 2015)	Jiangxi, China	Functional	Biolog Ecoplate
(Chen *et al*., 2016)	Jiangsu, China	Taxonomic	18S seq
(Cui *et al*., 2018)	Hunan, China	Taxonomic	16S seq
(Ding *et al*., 2016)	Heilongjiang, China	Taxonomic	16S seq
(Ding *et al*., 2017)	Heilongjiang, China	Taxonomic	ITS seq
(Ge *et al*., 2008)	Fengqiu, China	Taxonomic	16S seq
(Gu *et al*., 2009)	Sichuan, China	Taxonomic	16S DGGE
(Guanghua *et al*., 2008)	Jilin, China	Taxonomic	16S DGGE
		Functional	Biolog Ecoplate
(Hamm *et al*., 2016)	Manitoba, Canada	Taxonomic	16S seq
(Han *et al*., 2018)	Hunan, China	Taxonomic	nxr seq
(Hu *et al*., 2018b)	Northeast China	Taxonomic	16S seq
(Ji *et al*., 2018)	Fujian, China	Taxonomic	16S seq
(Kamaa *et al*., 2011)	Kabete, Kenya	Taxonomic	16S and 28S DGGE
(Kumar *et al*., 2017)	Odisha, India	Functional	Biolog Ecoplate
(Kumar *et al*., 2018)	Odisha, India	Taxonomic	16S seq
(Li *et al*., 2015)	Shandong, China	Taxonomic	16S DGGE
(Liu *et al*., 2015)	Jiangxi, China	Taxonomic	16S seq
(Liu *et al*., 2019)	Hebei, China	Taxonomic	TRFLP
(Mahanta *et al*., 2017)	Uttarakhand, India	Taxonomic	Soil dilution plate
(Nautiyal *et al*., 2010)	Maharashtra, India	Functional	Biolog Ecoplate
(Qiu *et al*., 2014)	Fujian, China	Taxonomic	16S and 18S TGGE
(Ros *et al*., 2006)	Linz, Austria	Taxonomic	16S PGGE
		Functional	Biolog Ecoplate
(Sun *et al*., 2004)	Oklahoma, USA	Taxonomic	16S DGGE
(van Bruggen *et al*., 2015)	Netherlands	Taxonomic	16S DGGE
(van der Bom *et al*., 2018)	Denmark	Taxonomic	16S seq
(Wang *et al*., 2018)	Heilongjiang, China	Taxonomic	16S seq
(Wei *et al*., 2008)	Heilongjiang, China	Taxonomic	16S DGGE
		Functional	Biolog Ecoplate
(Yao *et al*., 2018)	Inner Mongolia, China	Taxonomic	16S and 18S seq
(Zhong *et al*., 2010)	Jiangxi, China	Functional	Biolog Ecoplate

628 Table S2. Meta-analysis diagnostics.

Test	I^2 (%)	Q	df	P
H_{fun} NPK vs. CON	75.8	20.6	7	0.0044
H_{fun} ORG vs. CON	77.8	23.2	8	0.0031
H_{fun} ORG vs. NPK	76.6	36.9	8	<0.0001
H_{tax} NPK vs. CON	97.0	406.6	33	<0.0001
H_{tax} ORG vs. CON	97.0	231.2	34	<0.0001
H_{tax} ORG vs. NPK	97.6	601.4	44	<0.0001
$pH_{NPK} - pH_{CON}$ vs. pH_{CON}	98.5	1017.5	10	<0.0001
$pH_{ORG} - pH_{CON}$ vs. pH_{CON}	69.6	23.1	9	0.0059

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