



Research

Biodiversity to fertilization—Article

Responses of Soil Bacterial Diversity to Fertilization Are Driven by Local Environmental Context Across China



Youzhi Feng^a, Manuel Delgado-Baquerizo^b, Yongguan Zhu^{c,d}, Xiaozeng Han^e, Xiaori Han^f, Xiuli Xin^a, Wei Li^g, Zhibing Guo^h, Tinghui Dangⁱ, Chenhua Li^j, Bo Zhu^k, Zejiang Cai^{l,m}, Daming Liⁿ, Jiabao Zhang^{a,*}

^a State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

^b Department of Physical, Chemical, and Natural Systems, Pablo de Olavide University, Sevilla 41013, Spain

^c State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

^d Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

^e National Observation Station of the Hailun Agroecology System, Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 130102, China

^f College of Land and Environment, National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources, Shenyang Agricultural University, Shenyang 110866, China

^g Crop Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230001, China

^h Soil and Fertilizer Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230001, China

ⁱ Institute of Soil and Water Conservation, Northwest A&F University, Chinese Academy of Science & Ministry of Water Resources, Yangling 712100, China

^j Fukang Station of Desert Ecology, Key Laboratory of Oasis Ecology and Desert Environment, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

^k Key Laboratory of Mountain Environment Evolution and Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu 610041, China

^l Qiyang Agroecosystem of the National Field Experimental Station, Yongzhou 426199, China

^m National Engineering Laboratory for Improving the Quality of Arable Land, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

ⁿ Jiangxi Institute of Red Soil, Nanchang 331717, China

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ABSTRACT

Soil microbial diversity is extremely vulnerable to fertilization, which is one of the main anthropogenic activities associated with global changes. Yet we know little about how and why soil microbial diversity responds to fertilization across contrasting local ecological contexts. This knowledge is fundamental for predicting changes in soil microbial diversity in response to ongoing global changes. We analyzed soils from ten 20-year field fertilization (organic and/or inorganic) experiments across China and found that the national-scale responses of soil bacterial diversity to fertilization are dependent on ecological context. In acidic soils from regions with high precipitation and soil fertility, inorganic fertilization can result in further acidification, resulting in negative impacts on soil bacterial diversity. In comparison, organic fertilization causes a smaller disturbance to soil bacterial diversity. Despite the overall role of environmental contexts in driving soil microbial diversity, a small group of bacterial taxa were found to respond to fertilization in a consistent way across contrasting regions throughout China. Taxa such as *Nitrosospora* and *Nitrososphaera*, which benefit from nitrogen fertilizer addition, as well as *Chitinophagaceae*, *Bacilli*, and phototrophic bacteria, which respond positively to organic fertilization, could be used as bioindicators for soil fertility in response to fertilization at the national scale. Overall, our work provides new insights into the importance of local environmental context in determining the responses of soil microbial diversity to fertilization, and identifies regions with acidic soils wherein soil microbial diversity is more vulnerable to fertilization at the national scale.

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* Corresponding author.

E-mail address: jbzhang@issas.ac.cn (J. Zhang).

1. Introduction

By regulating nutrient cycling, food production, and ecosystem sustainability in natural and cropland ecosystems [1–3], soil microbial diversity is a fundamental driver of ecosystem functioning. Such a diversity is extremely vulnerable to fertilization [4], which is considered to be one of the most important anthropogenic drivers impacting terrestrial ecosystems and causing global changes [5,6]. For example, nutrient-heavy inputs have led to multiple side-effects, such as escalating production costs, heavy reliance on nonrenewable resources, water contamination, and soil degradation, with global consequences [7]. In the past decade, the influences of fertilization on soil microbial diversity have been frequently reported [8–10], although these investigations are mainly conducted at small (local and/or field) scales. It has been found that the application of organic fertilization can have different influences even in the same fertilization regime, depending on the soil characteristics. For example, organic fertilization causes an increase in diversity in acidic soils [11], a decrease in alkaline soils [12], and no impact in pH-neutral soils [13]. The responses of soil microbial diversity have also been found to be dependent on fertilization type (i.e., inorganic vs organic fertilization) [6,8]. Although investigations have been conducted on the large-scale response of soil microbial diversity to nutrient amendments [4,14], these studies mainly focus on natural ecosystems over relatively short time periods (less than four years). Thus, a comprehensive understanding of long-term and large-scale effects is basically lacking. The direction and magnitude of the responses of soil microbial diversity to long-term fertilization remain largely undetermined across contrasting ecological contexts (e.g., climates and soil types). In this study, we hypothesize that local ecological context—including the site-dependent environmental conditions of the climate [15,16] and soil properties [17]—ultimately controls the responses of soil microbial diversity (i.e., richness, community composition, and species level) to fertilization across large-scale environmental gradients [4,14]. In short, we started with the belief that soil microbial diversity might not be equally vulnerable to fertilization along the gradient of ecological contexts. It is also possible, however, that a small subset of microbial taxa may respond to fertilization in a relatively consistent manner. If so, this response is likely to be associated with the direct impact of nutrient and/or carbon additions on “opportunistic” (i.e., taxa with positive responses) or “sensitive” (i.e., those with negative responses) microbial taxa, which are early bioindicators of response to fertilization regimes. Assessing the role of ecological context in controlling the responses of soil microbial diversity to fertilization across a national scale and identifying those taxa that consistently respond are fundamental in order to accurately predict changes in the distribution of soil microbial diversity, as well as the associated functions of these taxa, in a globally changing world.

To accomplish our goals, we collected surface bulk soils from ten 20-year fertilization field experiments in croplands (mainly two crops, wheat and maize) across China (Fig. S1 and Table S1 in Appendix A). Our choice of experimental stations was based on two reasons: ① Over-fertilization is one of the most important concerns affecting Chinese cropland ecosystems. For example, today in China, the nitrogen (N) application rates for wheat and maize can be as high as $283 \text{ kg}\cdot\text{hm}^{-2}\cdot\text{a}^{-1}$ and $402 \text{ kg}\cdot\text{hm}^{-2}\cdot\text{a}^{-1}$, respectively [18]. ② Lands for these two crops cover a great majority of agroecological areas across China (24.5 million hm^2 of wheat (accounting for 19.6%) and 42.4 million hm^2 of maize (34.0%) in 2017) [19]. Each experimental station had several fertilization regimes over 20 years (Fig. S1 and Table S2 in

Appendix A): control (without fertilizer), inorganic fertilization with nitrogen–potassium (NK) and/or nitrogen–phosphorus–potassium (NPK), and organic plus inorganic fertilization (organic manure amendments (OM)) and/or NPKM (OM plus NPK). These experimental sites cover most climate zones, soil characteristics, and agricultural regimes in China (Fig. S1). Thus, this investigation provides a unique opportunity to evaluate the role of ecological context, in association with contrasting local environmental conditions in terms of soil properties and climate, in regulating the responses of soil microbial diversity to fertilization at the national scale. To this end, we used amplicon sequencing to investigate the responses (i.e., directions and magnitudes) of bacterial richness, community composition, and the relative abundances of common abundant taxa (top 10% of taxa in terms of relative abundance) to experimental fertilization. We focused on bacterial communities for two main reasons: ① Bacteria are the most diverse and abundant organisms on Earth; and ② bacterial communities are the fundamental engines for soil fertility, soil health, and plant productivity in agroecosystems [2].

2. Material and methods

2.1. Information on the long-term fertilization experiments

Detailed information on the ten long-term fertilization experimental stations is presented in Fig. S1 and Table S1. These stations are: Fukang (FK), starting from 1987; Fengqiu (FQ) from 1989; Changwu (CW) from 1984; Yingting (YT) from 1980; Yangliu (YL) from 1981; Mengcheng (MC) from 1982; Hailun (HL) from 1978; Shenyang (SY) from 1979; Jinxian (JX) from 1986; and Qiyang (QY) from 1990. The main fertilization treatments were: ① control, no fertilizer; ② NK applied as urea and potassium sulfate, no superphosphate; ③ NPK applied as urea, superphosphate, and potassium sulfate; ④ NPKM (half N applied as compost, the other half, as well as phosphate (P) and potassium (K), from chemical fertilizers); and ⑤ OM (i.e., total N at the same rate as in NPK treatment but from compost, plus chemical P and K fertilizers as in NPK treatment).

2.2. Soil sampling and chemical measurements

Surface bulk soils were sampled from ten long-term fertilization experimental stations across China after harvesting in 2015. For each triplicate plot of YT, YL, CW, SY, MC, QY, and JX and each quadruplicate plot of FQ, FK, and HL, two composite samples were independently taken from each plot. One composite sample was generated by homogenizing ten random soil cores with a depth of 10 cm. All the tools used were previously disinfected with 75% ethanol. A total of 284 soil samples were collected for downstream chemical and molecular analyses. The samples were placed in sterile plastic bags and transported to the laboratory at 4°C within one week. Sub-samples for biological assays were sieved (2 mm mesh size) and stored at -40°C for DNA extraction. For chemical assays, soil samples were air dried and sieved through a 100-mesh sieve to determine the soil pH values, the contents of soil organic matter (SOM), dissolved organic carbon (DOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), available potassium (AK), nitrate (N in the form of $-\text{NO}_3^-$), and ammonium (N in the form of $-\text{NH}_4^+$), according to the protocols provided by Lu [20]. Detailed information on soil chemical properties is given in Table S2.

2.3. Soil DNA extraction

For each soil sample, genomic DNA was extracted from 0.5 g of soil using a FastDNA SPIN Kit for soil (MP Biomedicals, CA), following the manufacturer's protocol. The extracted soil DNA was dissolved in 50 μL of Tris-EDTA buffer, quantified by spectrophotometry, and stored at $-40\text{ }^{\circ}\text{C}$ until further use.

2.4. Preparation of amplicon libraries and high-throughput sequencing

The bacterial community was characterized using 16S amplicon sequencing. For each DNA extract, the primer set 519F/907R was used to amplify approximately 400 bp of bacterial 16S rRNA gene V4–V5 fragments [21,22]. The oligonucleotide sequences included a 5-bp barcode fused to the forward primer. Polymerase chain reactions (PCRs) were carried out in 50- μL reaction mixtures with the following components: 4 μL ($2.5\text{ mmol}\cdot\text{L}^{-1}$ each) of deoxynucleoside triphosphates; 2 μL ($10\text{ mmol}\cdot\text{L}^{-1}$ each) of forward and reverse primers; 2 U of Taq DNA polymerase (TaKaRa, Japan), and 1 μL of template containing approximately 50 ng of community DNA as a template. Negative controls were always run with sterile double distilled water (ddH₂O) as the template. Thirty-five cycles ($95\text{ }^{\circ}\text{C}$ for 45 s, $56\text{ }^{\circ}\text{C}$ for 45 s, and $72\text{ }^{\circ}\text{C}$ for 60 s) were performed, with a final extension at $72\text{ }^{\circ}\text{C}$ for 7 min. The bar-coded PCR products from all of the samples were purified using a QIAquick Gel Extraction kit (QIAGEN, Germany), and then normalized in equimolar amounts. Next, they were prepared using the TruSeq DNA Sample Prep LT Kit and sequenced using the MiSeq Reagent Kit (600 cycles) following the manufacturer's protocols. The bacterial 16S rRNA gene sequences for this study have been deposited in the DNA Databank of Japan (DDBJ) database (accession no. PRJDB9137).

2.5. Processing high-throughput sequencing data

Raw sequence data were assembled with FLASH [23] and processed with the UPARSE algorithm [24]. Primers were trimmed with Cutadapt (Version 1.9.2) [25]. Sequences with an average quality score of below 25 and a length of less than 300 bp were discarded, and chimeras were filtered by UPARSE. Operational taxonomic units (OTUs) were delineated using a 97% similarity threshold, and the taxonomy was then determined by RDP classifier v2.12 for bacteria at a confidence threshold of 80%, using the taxonomy version of RDP 16S rRNA training set 16 [26]. OTU representative sequences were aligned using PyNASt against the GreenGene database (v 13_8) [27], and a phylogenetic tree was then constructed using FastTree [28]. In total, we obtained 20 294 908 bacterial 16S rRNA gene reads, with between 40 691 and 158 122 reads per sample, and a median value of 68 470 reads per sample. Because an even depth of sampling is required for alpha (α) diversity and beta (β) comparisons, samples were randomly rarified to 40 000 reads per sample for downstream analyses.

2.6. Changes in bacterial richness, community composition, and bioindicators in response to fertilization regimes

The influence of fertilization regimes on bacterial richness (i.e., the number of OTUs) was expressed as an index of effect size using the natural logarithm of the response ratio (lnRR). The lnRR was calculated based on the ratio of the bacterial richness in an organic plus inorganic fertilized group (OM and/or NPKM, hereafter termed "organic fertilization") or in an inorganic fertilized group (NK and/or NPK) to that of the control group without fertilization within each experimental site or across all experimental sites, using the R (version 3.3.1; R Development Core Team 2016) package

"Metafor" [29]. Thus, the lnRR values generated a summary of the outcomes to determine the responding magnitudes and directions of bacterial richness to fertilization regimes.

Shifts in community taxonomic composition were calculated by means of Bray-Curtis dissimilarities, which were then visualized using non-metric multidimensional scaling (NMDS) plots and evaluated by permutational multivariate analysis of variance (PERMANOVA) tests [30]. The F model values derived from PERMANOVA within each experimental site and across all sites characterized the degrees of shifts in bacterial taxonomic communities under fertilization.

To reveal the common abundant taxa (the top 10% OTUs accounting for 87.5% of the total reads) responding to fertilization at a large scale, we classified four categories based on the changing pattern of the lnRR of the relative abundance of each taxon to fertilization [31,32]. Across at least nine sites, consistently positive responders (i.e., with lnRR values greater than zero) were classified as "opportunistic," while consistently negative responders (i.e., with lnRR values less than zero) were termed "sensitive." In addition, taxa without changes in relative abundances (i.e., with lnRR values overlapping zero) in each site were termed "tolerant," and those without consistent changing patterns across sites were termed "context-dependent." The phylogeny of the bacterial taxa and each taxon's assigned life strategy under fertilization were drawn using the Interactive Tree of Life (iTOL) webtool (<https://itol.embl.de>).

2.7. Statistical analysis

The data were expressed as the means with standard deviation (SD), and different letters were used to indicate significant differences between different samples. Differences in fertilization were evaluated with one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's honestly significant difference (HSD) tests. Pearson and mantel analyses were respectively conducted to correlate community (i.e., richness and composition) to environmental variables [33]. $P < 0.05$ and $P < 0.01$ respectively denote significant and highly significant differences between samples. To determine the direct and indirect effects of environmental variables on the lnRR of bacterial richness, shifts in community composition, and changes in common abundant taxa, structural equation models (SEM) were conducted and tested using AMOS 20.0 (SPSS Inc.). A maximum likelihood estimation method was used to compare the SEM models with the observations. Model adequacy was determined by χ^2 tests, comparative fit index (CFI), goodness-of-fit index (GFI), and root square mean errors of approximation (RSMEA). Adequate model fits are indicated by nonsignificant χ^2 , high CFI, high GFI (>0.9), and low RSMEA (<0.05).

3. Results and discussion

3.1. Ecological context determines the responses of soil bacterial diversity to fertilization

We first investigated the responses of bacterial richness (i.e., the number of phylotypes of bacteria) to fertilization in the soils from ten experimental sites across China (Fig. 1(a)). These sites included contrasting climates (e.g., different mean annual temperature (MAT) and mean annual precipitation (MAP)) and soil properties (e.g., soil pH) (Table S1), as well as both inorganic and organic fertilization regimes (Table S2). Our results showed that both the direction and the magnitude of the responses of bacterial richness (calculated by the lnRR-response ratio) to fertilization were dependent on ecological context (i.e., were dependent on the site and fertilization regime (Fig. 1(a) and Fig. S2 in Appendix A) and

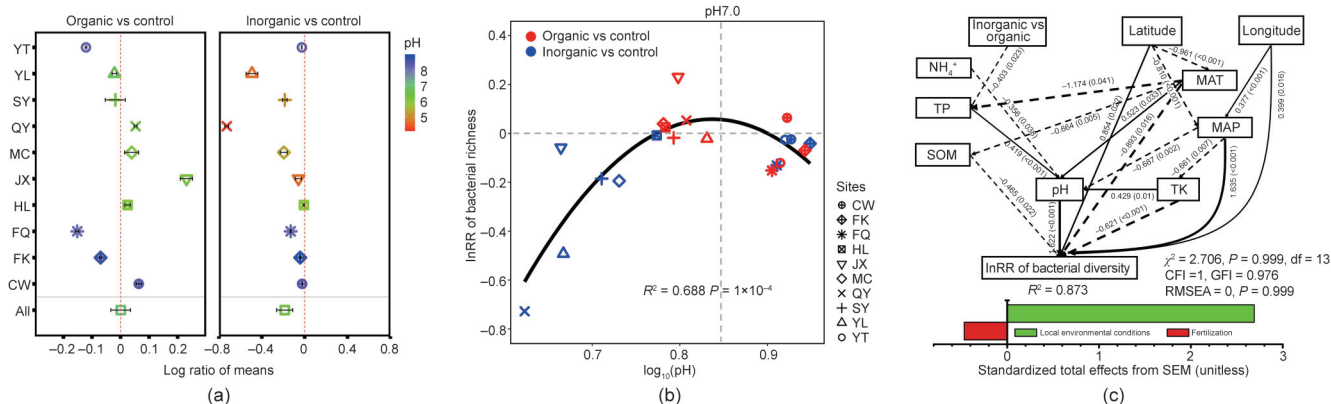


Fig. 1. (a) Effects of organic plus inorganic (NPKM and/or OM) and inorganic (NPK and/or NK) fertilization on bacterial richness (unitless) (calculated by the InRR of bacterial richness) in ten experimental sites across China: FK, FQ, CW, YT, YL, MC, HL, SY, JX, and QY. The horizontal error bars are 95% confidence intervals for effect sizes within each site and across the ten experimental sites. The sites have been color-coded according to soil pH gradient. (b) Correlation between InRR (under inorganic or organic fertilization) and soil pH (\log_{10} value) across ten experimental sites. (c) Direct and indirect effects of latitude, longitude, climate, soil properties, and inorganic vs organic fertilization on the InRR of bacterial richness across ten experimental sites. The inset bar graph presents the standardized total effects (direct plus indirect effects) of fertilization and local environmental conditions derived from the SEM on InRR. Numbers adjacent to lines indicate the effect size of the relationship. Continuous and dashed lines indicate positive and negative relationships, respectively. The width of the lines is proportional to the strength of the path coefficients. R^2 denotes the proportion of variance explained. df indicates degree of freedom.

Life strategy	Organic	Inorganic
Opportunistic	2.3%	0.2%
Sensitive	0.3%	0.7%
Tolerant	7.8%	12.6%
Context dependent	89.6%	86.5%

	Organic	Inorganic
Acidobacteria	13.4%	11.4%
Actinobacteria	11.7%	8.5%
Bacteroidetes	9.6%	7.0%
Chloroflexi	4.4%	8.7%
Firmicutes	4.4%	3.8%
Planctomycetes	7.6%	7.8%
Alphaproteobacteria	9.0%	10.3%
Betaproteobacteria	4.4%	2.5%
Deltaproteobacteria	5.8%	5.4%
Gammaproteobacteria	3.5%	5.4%
Others	26.2%	29.1%

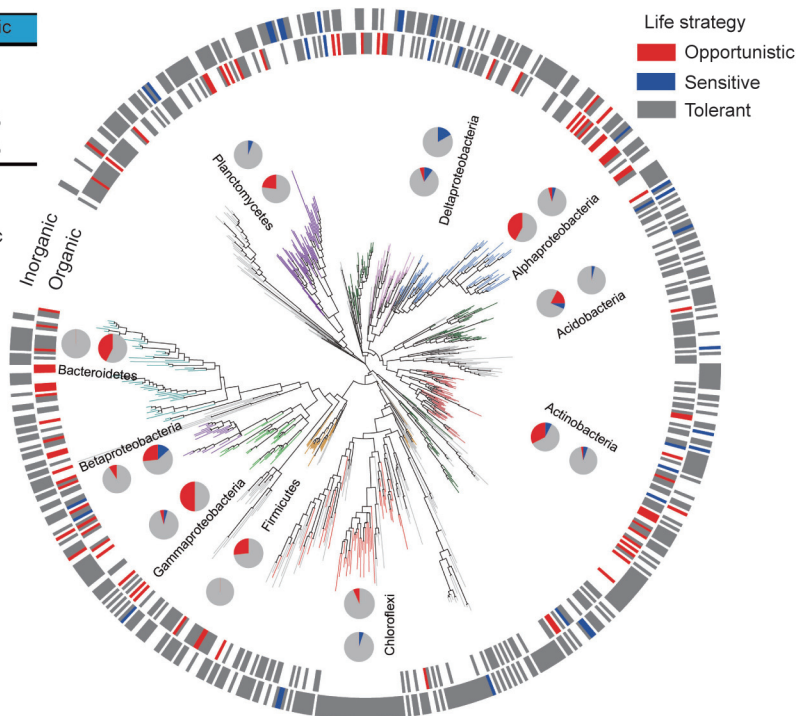


Fig. 2. Phylogeny of bacterial taxa, and each taxon’s assigned life strategy (colors in wider rings, corresponding to Fig. S6) under inorganic (outer wide ring) and organic (inner wide ring) fertilization. The inset table presents the percentages of four life strategies under fertilization. The values behind the phyla are the percentages (in total OTUs) of opportunistic, sensitive, and tolerant taxa under organic and inorganic fertilization, respectively. Phyla are indicated by branch colors. Pie charts show the distribution of life strategies within the most dominant phyla under inorganic (outer pie charts) and organic (inner pie charts) fertilization. Opportunistic and sensitive taxa respectively consist of the subset of taxa with consistently positive and negative responses to fertilizations (across at least nine sites); tolerant taxa are those that consistently show no response to fertilization (across at least nine sites). Taxa with inconsistent responses (i.e., context-dependent taxa) were excluded from the phylogenetic statistical analysis.

were associated with key local soil properties, climate, and fertilization regimes (Figs. 1(b) and (c)). These results provided the evidence that soil bacterial richness is not equally affected by fertilization across China. Our findings are inconsistent with the observations of Leff et al. [4] and Ramirez et al. [14] on the consistent responses of microbial diversity to inorganic nutrient amendments. Possible reasons for these inconsistencies might include important methodological differences: ① temporal

frameworks (<4 years vs 20 years of nutrient additions); ② nutrient input types (e.g., inorganic vs inorganic and organic); and ③ ecosystem types (e.g., natural ecosystems vs agroecosystems). The higher temporal and nutrient type resolutions of our study make our findings more comprehensive in comparison with previous works. It is well known that climate conditions (e.g., MAT and MAP) [34,35] and soil properties [16,17] drive soil microbial community diversity, structure, and

inorganic (t -test $P = 0.43$) or organic fertilization (t -test $P = 0.48$) across China. In summary, our findings suggest that soil microbial diversity is not affected similarly by fertilization in the investigated sites; it is far more vulnerable to fertilization in acidic (vs alkaline) soils; and it is more sensitive to inorganic fertilization than to organic fertilization at the national scale (as shown in the inset bar graphs in Fig. 1(c) and Fig. S4).

3.2. Only a small subset of bacterial taxa show consistent responses to fertilization

We then evaluated the responses of common dominant microbial phylotypes (top 10% taxa (Fig. S5 in Appendix A) in terms of relative abundance and accounting for 87.5% of all 16S rRNA gene reads, following the standard of Delgado-Baquerizo et al. [32], to over two decades of fertilization (Fig. 2). These common taxa were categorized into four groups based on their adaptive strategies to fertilization ($\geq 90\%$ of field experiment sites) (see Section 2.6): ① opportunistic taxa, composed of the subsets of taxa with consistently positive responses to fertilization; ② sensitive taxa, composed of the subsets of taxa with consistently negative responses to fertilization; ③ tolerant taxa, comprising those taxa with a consistent lack of response to fertilization; and ④ context-dependent taxa, including bacterial taxa with inconsistent responses to fertilization across multiple experimental sites. We noticed that a small subset of opportunistic taxa (2.3% and 0.2% for organic and inorganic fertilizations, respectively) and sensitive taxa (0.3% and 0.7%) consistently responded to fertilization across multiple experimental sites (Fig. 2 and Table S11 (in Appendix A)). The emergence of opportunistic taxa, compared with the control without fertilization, is likely to be directly driven by nutrient and/or carbon additions linked to fertilization regimes. Unlike bacterial richness and community composition, our SEM results indicated that the total effects accumulated from two decades of organic and inorganic fertilization were more important than ecological context, including local environmental conditions in terms of climate (e.g., MAT and MAP) and soil properties (e.g., N and SOM), in controlling the relative abundances of opportunistic taxa (Figs. 3(a) and (b)). The opportunistic taxa were mainly dominated by Proteobacteria and Bacteroidetes (Fig. 2 and Fig. S6 (in Appendix A)). Many members within these two phyla are potential copiotrophs [46], and thus might have competitive advantages in eutrophic environments under fertilization. Other examples of opportunistic taxa included *Nitrosospora* and *Nitrososphaera*, which could benefit from the addition of N from fertilization, and *Chitinophagaceae* (Bacteroidetes), *Bacilli* (Firmicutes), and phototrophic bacteria (*Rhodospirillum rubrum*, *Rhodospirillaceae*, and *Rhodospirillales* within Proteobacteria) (Fig. 2), which were all found to respond positively to exogenous carbon resource inputs from organic fertilization and in arable soils with high fertility [8,47–49]. Thus, it is reasonable to associate the desirable responses of these opportunistic taxa with the high soil fertility, regardless of the local ecological context. This information could be a powerful tool to determine the best agricultural practices. Sensitive taxa included members of Acidobacteria and Actinobacteria (Fig. 2 and Fig. S6), which are often classified as oligotrophic taxa [14,46]. In addition, taxa related to N cycling within Nitrospirae, Planctomycetes, and Proteobacteria (e.g., *Rhizobiales*, *Myxococcales*, and *Burkholderiales* [50]; Fig. 2 and Fig. S6) were classified as sensitive due to their response to the addition of N from fertilization. Such taxa can be used as early-warning indicators of the negative impacts of agricultural practices on soil fertility. Taken together, our findings suggest that these taxa that are opportunistic and sensitive to fertilization hold potential for use as bioindicators of soil fertility in response to anthropogenic activities at the national scale.

4. Conclusions

In summary, based on a national-scale investigation of ten 20-year experimental sites, our findings provide novel evidence that the local environmental context determines the responses of soil microbial diversity to fertilization. We demonstrate that soil microbial diversity is not equally vulnerable to fertilization across China. At this scale, soil microbial diversity is far more vulnerable to fertilization in acidic soils, in which fertilization results in further acidification, and to inorganic fertilization compared with organic fertilization. Only a very small subset of microbial taxa was found to consistently respond to fertilization across very different ecological contexts. These findings were integrated in order to better predict future potential changes in and the distribution of soil microbial diversity in a changing world [1]. They further advance the existing theoretical framework on the importance of local ecological context in controlling the responses (e.g., magnitude and direction) of soil microbial diversity to anthropogenic alterations at the national scale [4,14].

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Authors' contributions

Jiabao Zhang, Manuel Delgado-Baquerizo, Yongguan Zhu and Youzhi Feng developed the ideas in this article. Jiabao Zhang and Yongguan Zhu designed the experimental survey. Xiaozeng Han, Xiaori Han, Xiuli Xin, Wei Li, Zhibing Guo, Tinghui Dang, Chenhua Li, Bo Zhu, Zejiang Cai and Daming Li conducted the field samplings. Youzhi Feng, Manuel Delgado-Baquerizo, Yongguan Zhu and Jiabao Zhang analyzed the data. Youzhi Feng, Manuel Delgado-Baquerizo, Yongguan Zhu and Jiabao Zhang wrote the manuscript with contributions from all the co-authors.

Compliance with ethics guidelines

Youzhi Feng, Manuel Delgado-Baquerizo, Yongguan Zhu, Xiaozeng Han, Xiaori Han, Xiuli Xin, Wei Li, Zhibing Guo, Tinghui Dang, Chenhua Li, Bo Zhu, Zejiang Cai, Daming Li, and Jiabao Zhang declare that they have no conflict of interest or financial conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.eng.2021.09.012>.

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