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Declining soil pH reduces microbial biomass-driven soil multifunctionality

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ABSTRACT

Soil acidification due to climate and anthropogenic changes persistently threatens biodiversity and biomass, the essential drivers of ecosystem multifunctionality. However, the influence of a sustained reduction in soil pH on the regulatory role of microbial communities in ecosystem multifunctionality has not yet been assessed. Here, we investigated the critical pH thresholds at which microbial biomass becomes a key determinant of soil multifunctionality (SMF) based on a large-scale paddy field study ($n = 429$) and a global dataset ($n = 35{,}641$). We found that when the soil pH was <5, microbial biomass (i.e., bacterial or fungal) was significantly positively correlated with the soil SMF, representing a critical threshold for microbial biomass regulation of ecosystem multifunctionality. We further predicted the global pattern of the microbial drivers of SMF under soil acidification scenarios over the next 50 years. Our results indicate that as soil acidification continues, the global area of biomass-mediated SMF will increase by approximately 14 % by 2070. Our results highlight that due to ongoing acidification, biomass reduction will cause accelerated losses in global SMF.

1. Introduction

Soil multifunctionality (SMF) is a composite concept that reflects the ability of a soil ecosystem to perform multiple functions simultaneously and is used to evaluate ecosystem value (Manning et al., 2018; Zhao, 2024). Multiple soil indicators, including soil nutrient status, biodiversity, and other soil properties, can be used to measure multifunctionality (Hu et al., 2021). The soil microbiota (i.e., bacteria and fungi) is reported to regulate ecosystem multifunctionality by participating in terrestrial ecosystem functions, such as carbon (C) dynamics, nutrient cycles, and plant productivity (Delgado-Baquerizo et al., 2018; Manning et al., 2018; Schuldt et al., 2018; Wardle et al., 2004). Although interrelated, the associations between microbial diversity, biomass, and SMF are discordant and context dependent. For example, numerous studies have demonstrated that in grassland, forest, and dryland ecosystems, bacterial and fungal communities exert positive impacts on multifunctionality (Delgado-Baquerizo et al., 2016; Hu et al., 2021; Ma et al., 2022), while in semiarid grasslands and subtropical forests, the effects on multifunctionality are negative (Bardgett and van der Putten, 2014; Wardle et al., 2004). Consequently, it is crucial to investigate the regulatory factors that shape the interactions between microbial communities (i.e., bacterial and fungal diversity and biomass) and SMFs.

Soil pH is a critical and robust predictor of microbial biomass and diversity in terrestrial ecosystems (Schuldt et al., 2018; Wagg et al., 2019; Wang et al., 2020). For example, in the pH range from 4.0 to 8.3, the diversity and relative abundance of bacteria are positively responsive to increasing pH, while fungi are less responsive (Wang et al., 2020). In comparison, a century-old experiment conducted in cropland showed that low pH favoured fungal growth and that high pH favoured bacterial growth (Wagg et al., 2019). Additionally, soil pH was found to have threshold effects on microbial diversity, microbial composition, and co-occurrence structure between bacteria and fungi (Shi et al., 2021). Although there are varying findings about how microbes react to pH shifts, these studies confirmed that soil pH dominates the associations

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between microbial diversity, biomass, and the functions of soil nutrient cycles (Fierer and Jackson, 2006; Rousk et al., 2009, 2010). Recent studies have shown that soil acidification with reduced soil pH may potentially reduce soil C and nitrogen (N) stocks by constraining microbial diversity or biomass (Crowther et al., 2019; Huang et al., 2019). However, it remains unclear whether pH affects the associations between microbial diversity, biomass, and SMF and therefore may change along pH gradients.

Here, we hypothesize that the existence of pH thresholds affects the associations between microbial diversity, biomass, and SMF. Since soil acidification reduces the functional redundancy of microbial communities (Huang et al., 2021; Malik et al., 2018), we predicted that in acidic soil environments, a biomass-driven SMF would be more pronounced. To test our hypotheses, we collected a total of 429 paddy soil samples with pH values ranging from 3.6 to 8.7 across 13 regions in China's main growing regions. For a comprehensive understanding, we measured the soil microbial diversity and biomass, as well as eleven soil variables that indicate the nutrient pools, representing matter and energy stocks of soil C, N, phosphorus (P), and potassium (K) (Hu et al., 2021).

In addition to field studies, we used a published global microbial biomass dataset to assess the associations between microbial biomass and the SMF in diverse ecosystems worldwide (Meng et al., 2019). Additionally, we predicted changes in the risk of a reduced global SMF over the next 50 years under persistent pH changes due to altered environmental factors (e.g., continuous nitrogen fertilizer application) based on random forest machine learning methods. Our results highlight that due to ongoing acidification, biomass reduction will cause accelerated losses in global SMFs.

2. Materials and methods

2.1. A field study of constructed rice paddies in China

In total, 429 soil samples (39 paddy soil sites × 11 replicates) were collected from a broad spatial extent in 13 regions of China (Fig. 1a, Supplementary Table 1). These regions, including Hailun, Changchun, Shenyang, Yuanyang, Fengqiu, Lin'an, Quzhou, Zixi, Jian'ou, Changting, Hengyang, Qingxin and Haikou, encompass a substantial geographical range from 110°10′ E to 126°14′ E in longitude and 19°32′ N to 46°58′ N in latitude. The great geographical differences in the mean annual temperature (MAT) ranged from 1.5 °C to 23.8 °C, and the mean annual precipitation (MAP) ranged from 399 mm to 2216 mm (Supplementary Table 1). In each region, three rice fields located within 20 km were chosen to maintain a consistent climate, soil type, and agricultural practices. Eleven soil samples were collected from a 100 m × 100 m plot using a spatially explicit 'L-shaped' sampling design at each site. Soil samples from each sampling point were mixed from five soil cores (2.5 cm in diameter) from the upper 15 cm. Living plant material was manually removed. The soil samples were transported to the laboratory on dry ice, and physicochemical variables were measured immediately. Subsamples were stored at −80 °C to measure the soil microbial variables.

2.2. Soil physicochemical analysis

In the field study, eleven variables were measured as crucial indicators of soil nutrient pools: total phosphorus (TP), available phosphorus (AP), total nitrogen (TN), dissolved organic nitrogen (DON), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), dissolved total nitrogen (DTN), available potassium (AK), total potassium (TK), dissolved organic carbon (DOC), and soil organic carbon (SOC) (Supplementary Table 1). TP was measured using HF-$HClO_4$ (Jackson, 1958), and AP was determined using the molybdenum blue method with sodium bicarbonate (Olsen et al., 1954). The TN, NH_4^+-N, and NO_3^--N contents were quantified using the Kjeldahl method (Page, 1982). DTN was measured with a spectrophotometer following oxidation with sodium hydroxide-potassium persulfate. The DON content was assessed using the difference between DTN and the sum of NO_3^--N and NH_4^+-N. TK was measured using HF-$HClO_4$ (Jackson, 1958), and AK was detected by atomic absorption spectrophotometry using ammonium acetate (Page, 1982). SOC was quantified by employing the potassium dichromate method during wet digestion (Nelson, 1982). DOC was measured using a total organic carbon analyser (Shimadzu Corporation, Kyoto, Japan). The soil pH was measured using a glass electrode, maintaining a water-to-soil ratio of 2.5:1. The soil cation exchange capacity (CEC) was determined using the sodium acetate method (Meimaroglou and Mouzakis, 2019).

2.3. Microbial biomass analysis

Soil microbial biomass was analysed using phospholipid fatty acid (PLFA) analysis (Fan et al., 2017). Briefly, 0.5 g of fresh soil was mixed with an extract of chloroform:methanol:phosphate buffer (1:2:0.8 *v*/v/v). The phospholipids were separated and extracted, and then methanol was added to form fatty acid methyl esters. The contents of various fatty acids were determined by gas chromatography. For indicating bacterial biomass, the PLFAs i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7t, 16:1ω7c, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 were used (Bastida et al., 2021). To indicate fungal biomass, the PLFA 18:2ω6 was used (Frostegard and Baath, 1996).

2.4. DNA extraction, PCR amplification, and amplicon sequencing

For field studies, genomic DNA was extracted from 2 g of soil sample by combining freeze grinding and sodium dodecyl sulfate for cell lysis. For bacteria, the primers 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTWTCTAAT) were used to amplify the V3-V4 region of the 16S rRNA gene (Degnan and Ochman, 2012). For fungi, the primers ITS1-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) were used to amplify the internal transcribed spacer 1 (ITS1) region of the rRNA gene (Kong, 2011). Polymerase chain reaction (PCR) amplification was performed in triplicate with a 20 μl reaction system containing 4 μl of 5 × FastPfu Buffer, 0.8 μl of each primer (5 μM), 2 μl of 2.5 mM dNTPs, 10 ng of template DNA, and 0.4 μl of FastPfu Polymerase on an ABI GeneAmp® 9700 (ABI, Foster City, CA, U.S.A.). The thermocycling conditions for bacteria were as follows: predenaturation at 95 °C for 3 min; 28 cycles of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C; and extension at 72 °C for 10 min. The thermocycling conditions for fungi were as follows: predenaturation at 95 °C for 3 min; 35 cycles of 30 s at 95 °C, 30 s at 59.3 °C, and 45 s at 72 °C; and extension at 72 °C for 10 min. Triplicate PCR products were mixed and purified. The pooled DNA was diluted to 2 nM and processed using a MiSeq benchtop sequencer (Illumina Inc., San Diego, CA, USA) following the manufacturer's instructions. The sequencing library was prepared with a TruSeq DNA kit. The library was sequenced using an Illumina MiSeq platform to generate 250 bp paired-end reads.

2.5. Sequence processing

The quality control of the raw sequencing reads was determined using the Trimmomatic (version 0.40) program. Poorly quality reads were removed by Btrim, and chimeras were detected by Uchime (Edgar et al., 2011). Unassembled reads were discarded. Operational taxonomic units (OTUs) were clustered at 97 % nucleotide identity by Usearch (version 7.1) and annotated with the taxonomy data available in the Ribosomal Database using a confidence threshold of 0.7. Bacterial taxonomic assignment was performed using the SILVA database (version 138) (Quast et al., 2012); fungal taxonomic assignment was performed using the UNITE database (version 8.2) (Kõljalg et al., 2013).

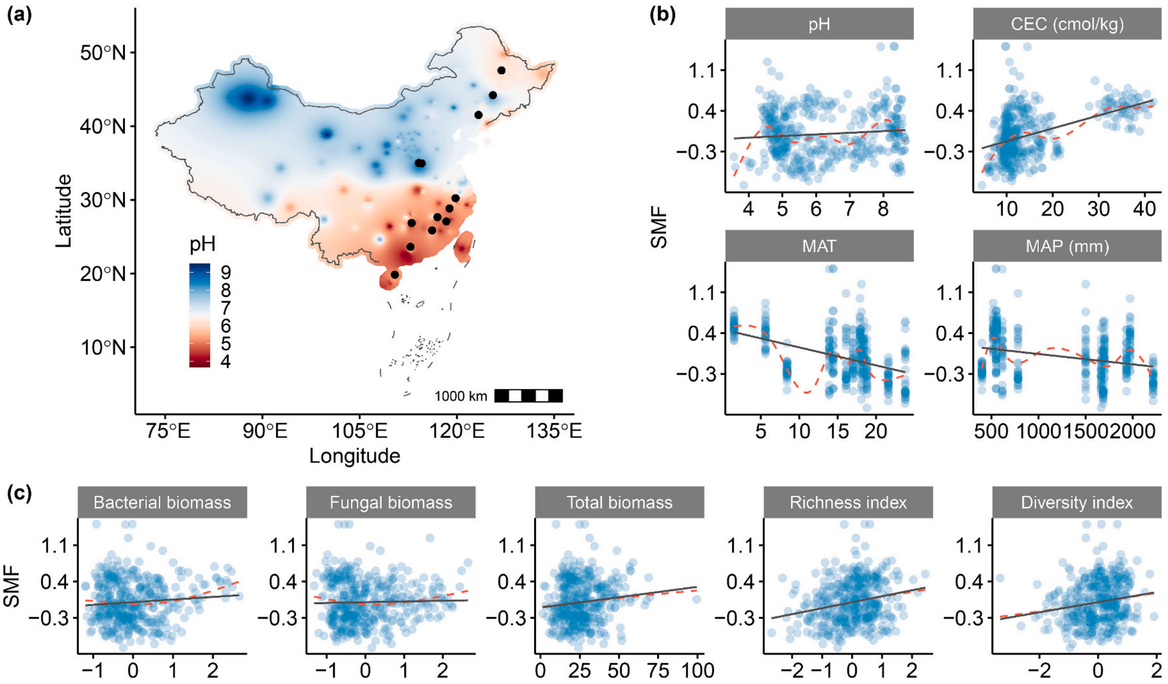

Fig. 1. Illustration of the sampling scheme and the relationships between different factors and soil multifunctionality. (a) The black dots represent 13 different regions. Three paddy fields were randomly selected in each region, and 11 samples were collected from each paddy field, for a total of 429 samples. Linear and nonlinear relationships between abiotic (b) and biotic (c) factors and soil multifunctionality (SMF). The solid black line represents linear models (LMs), and the red dashed line represents generalized additive models (GAMs).

2.6. *Assessment of soil multifunctionality*

Eleven variables, namely, TP, AP, TN, DON, NO_3^--N, NH_4^+-N, DTN, AK, TK, DOC, and SOC, were measured as crucial indicators of soil nutrient pools (Supplementary Table 1). These variables are regarded as equivalent to the storage of C, N, P, and K and serve as indicators of the availability of these elements to plants and microorganisms in terrestrial ecosystems (Delgado-Baquerizo et al., 2016). Briefly, these variables are considered individual soil functions that are commonly measured indicators of terrestrial multifunctionality (Delgado-Baquerizo et al., 2016; Fetzer et al., 2015; Hu et al., 2021), reflecting various functional categories, including soil properties and fertility, nutrient cycling, and climate regulation (Fetzer et al., 2015). We standardized each soil function by transforming its Z scores and averaging them to obtain the multifunctionality indices.

To avoid potential trade-offs and redundancy among individual soil functions (Hu et al., 2021), we calculated Pearson's correlation coefficients for a total of 66 pairs of soil functions. Among these, 29 pairs showed significant positive correlations (Supplementary Fig. 1), while the other combinations were independent, with none exhibiting significant negative correlations, indicating no trade-offs between them. Additionally, we identified soil function redundancy by distinguishing combinations of soil variables with strong correlations (Gamfeldt et al., 2008; Hu et al., 2021; Manning et al., 2018). However, our study revealed only one instance where the correlation between TP and AP had r value >0.7 (Supplementary Fig. 1), suggesting that the redundancy among the soil variables was relatively low.

2.7. *Moving-window analysis*

To evaluate how the microbial biomass-SMF relationships varied along pH gradients, we performed a moving-window analysis (Hu et al., 2021). Briefly, the linear mixed-effects model was constructed in a subset window of 100 samples with the lowest pH values, and this process was repeated for the remaining samples along the pH gradient. Within each window of 100 samples, the standardized coefficients were bootstrapped 500 times. A nonlinear regression was then fitted to these coefficients of diversity, biomass, and their correlations with pH across the gradient. The pH thresholds were identified for the changes in the coefficients between SMF, diversity, and biomass.

2.8. *Data processing and statistical analyses*

R (v 4.1.1; www.r-project.org/) and Python (v 3.12; www.python.org/) were used for all the data analyses and figures, with a significance level of $p < 0.05$ for all the statistical tests. Spearman correlation analysis was used to estimate the relationships between eleven soil variables and abiotic factors (i.e., pH, CEC, MAT, and MAP) and biotic factors (i.e., microbial diversity indices and microbial biomass). Furthermore, we constructed a linear mixed-effects model (Supplementary Table 2; Eq. (1)) to verify the correlations between multiple biotic and abiotic factors and the SMF:

$$\begin{aligned} \text{SMF} = {} & \text{pH} + \text{CEC} + \text{MAT} + \text{MAP} + \text{Biomass} + \text{Diversity} + \text{MAT} \times \text{MAP} \\ & + \text{pH} \times \text{Biomass} + \text{pH} \times \text{Diversity} + (1|\text{Region}) \end{aligned} \tag{1}$$

where "×" indicates an interaction term. To account for the similarities in soil among the 13 regions in the field study, we included "(1|Region)" as a random term.

The moving-window analysis showed a clear shift in the relationships between the SMF and microbial biomass at the three pH thresholds. We divided 429 soil samples from the field into three groups: samples with pH $<$ 5 (low pH, $n = 119$); samples with pH $>$ 6 (high pH, $n = 122$); and samples with pH 5–6 (moderate pH, $n = 188$). Linear regression analysis was performed to determine the relationships between each component of microbial diversity and biomass and SMF within the different pH ranges based on ordinary least squares (OLS) regression.

To verify the relationship between the SMF and microbial biomass at the global scale, we constructed a global database including global microbial biomass, MAT, MAP, pH, SMF and ecosystem type. The global microbial biomass and ecosystem data were obtained from He et al. ($n =$ 35,641) (He et al., 2020). The nutrient variables used to calculate the global SMF were obtained from the Global Soil Dataset for Earth System Modelling at a 10 km resolution. The MAT and MAP data were obtained from the WorldClim database (https://www.worldclim.org), which has a resolution of 1 km. After these efforts, we constructed a simplified linear mixed-effects model through Eq. (2) (Supplementary Table 3) and performed moving-window analysis to vary the associations among microbial biomass and SMF with pH:

$$\mathrm{SMF} = \mathrm{pH} + \mathrm{MAT} + \mathrm{MAP} + \mathrm{MAT} \times \mathrm{MAP} + \mathrm{Biomass} + \mathrm{pH} \times \mathrm{Biomass} + (1|\mathrm{Ecosystem\ Type}) \tag{2}$$

where "(1|ecosystem type)" indicates a random term to account for the similarities of soil among ecosystems in the global database.

To investigate the potential impact of soil pH reduction caused by fertilization on the relationship between biomass and SMF, we predicted global soil pH changes at the 0–15 cm depth after 50 years. Briefly, based on published global metadata from Zhao et al. (2022), we constructed a random forest model to estimate the factors (i.e., the duration of N application, nitrogen fertilizer application amount, initial soil pH, MAP, and MAT) that influence soil pH changes. We adjusted some of the primary hyperparameters to obtain the optimal model in the RF model. The model demonstrated the least mean square error (MSE = 0.11) with ntree = 337, mtry = 156, nodesize = 10, and maxnodes = 30. To ensure model reliability, five replications and tenfold cross-validation were carried out, resulting in a model MSE of 0.12, indicating no overfitting or underfitting problems. Finally, the pH was predicted based on the best model trained by the above method. The initial soil pH was obtained from the Global Soil Dataset for Earth System Modelling. The nitrogen fertilizer application rates were reported by the Food and Agriculture Organization (FAO) of the United Nations (https://www.fao.org) (FAO, 2021).

3. Results

3.1. Driving factors of soil multifunctionality in paddy fields in China

Our field analysis indicated that pH was the primary abiotic factor influencing individual soil functions (i.e., eleven soil variables) (Supplementary Fig. 2a). In comparison, the primary biotic factor affecting the function of the elemental cycle was microbial biomass (bacterial and fungal biomass) (Supplementary Fig. 2b). Subsequently, linear and generalized additive models showed that the SMF responded nonlinearly to most of the increasing abiotic and biotic variables (e.g., pH, CEC, and bacterial and fungal biomass) (Fig. 1b and c). This finding implies that interactions between different biotic and abiotic factors may affect their relationships with the SMF.

We then fitted a linear mixed-effects model to evaluate the relationships between multiple biotic and abiotic factors and the SMF (Supplementary Table 2). We found that the pH, CEC, MAT, and total microbial biomass were positively correlated ($p < 0.01$) with the SMF. In particular, total microbial biomass alone was positively associated with SMF, whereas the interaction between pH and total biomass was negatively associated with SMF ($p < 0.001$). Similarly, while the microbial diversity index alone showed a weak correlation with the SMF ($p =$ 0.08), the interaction term between the diversity index and pH was not correlated with the SMF ($p = 0.70$). These findings indicated that the associations between microbial community (i.e., diversity and biomass) and SMF may delicately shift with changes in pH.

3.2. Threshold effects of soil pH on associations between the microbial community and soil multifunctionality

To investigate the potential roles of soil pH in shaping the associations between SMF, microbial diversity, and biomass, we performed a moving-window analysis (Fig. 2). We observed that the positive relationship between SMF and microbial biomass decreased sharply in a linear pattern as the pH increased to 5 in paddy fields (Fig. 2a). When the pH increased from 5 to 6, the relationship shifted to a nonlinear pattern and maintained a positive association with the SMF. When the pH exceeds 6, the relationships between the SMF and microbial biomass become negatively correlated and gradually decrease with increasing pH. Moreover, the correlation coefficients between SMF and diversity with increasing pH showed similar but more subtle changes. Additionally, the relationship between the SMF and the microbial-pH interaction terms displayed consistent thresholds intervals with opposite patterns across the three pH ranges ($\mathrm{pH} < 5$, $5 \leq \mathrm{pH} \leq 6$, and $\mathrm{pH} > 6$), especially for the pH:biomass interaction term (Fig. 2b). The inverse patterns observed between SMF and biomass, as well as the pH:biomass interaction, can be attributed to the negative correlation between pH and total microbial biomass (Supplementary Table 4).

We determined whether a significant linear correlation existed between each variable of biomass/diversity and the SMF across the three pH groups (Supplementary Fig. 3). As expected, in samples with $\mathrm{pH} < 5$ ($n = 119$), OLS regression revealed a strong positive correlation between bacterial biomass and SMF (goodness of fit: $R^2 = 0.25$, significance: $p <$ 0.001; Spearman's correlation coefficient: $r = 0.59$, $p < 0.001$). However, there was no significant relationship between SMF and fungal biomass or diversity in samples with $\mathrm{pH} < 5$ ($p > 0.05$). In samples with pH values between 5 and 6 ($n = 122$), SMF was positively correlated with the microbial diversity index ($R^2 = 0.06$, $p < 0.05$; $r = 0.24$, $p <$ 0.01), while no relationship was detected with biomass ($p > 0.05$). In samples with $\mathrm{pH} > 6$ ($n = 188$), the SMF showed a notable negative correlation with fungal biomass ($R^2 = 0.02$, $p < 0.05$; $r = -0.17$, $p <$ 0.05) and a positive correlation with fungal diversity ($R^2 = 0.03$, $p <$ 0.05; $r = 0.18$, $p < 0.05$). These results agreed with our hypothesis that pH exerts threshold effects on the associations between microbial diversity, biomass, and SMF.

3.3. Global-scale impact of soil pH on biomass-multifunctionality relationships across ecosystems

Building upon our findings from region-scale field samples collected from artificial wetland ecosystems (i.e., paddy fields) in China (Fig. 2a and b), we further conducted a global meta-analysis to evaluate the effect of pH on biomass-multifunctionality relationships in different ecosystems (Fig. 2c and d). Given the significant impact of the interaction term of pH and microbial biomass ($p < 0.05$, Supplementary Table 3), a clear nonlinear association between microbial biomass and SMF as global soil pH increased was detected (Fig. 2c and d). Although the variation pattern indicated distinct pH thresholds (i.e., $\mathrm{pH} < 5$, 5–7.5, and $\mathrm{pH} > 7.5$), which differed from the pH range observed in the paddy field results, we still observed consistent patterns (Supplementary Fig. 4). Specifically, SMF had the strongest positive correlation with biomass in samples with $\mathrm{pH} < 5$; the positive relationship became weaker in samples with pH values between 5 and 7.5, and there was no positive correlation in samples with $\mathrm{pH} > 7.5$.

Notably, we found that the random effects of the model (i.e., ecosystem type) influenced the explanatory effects of different biochemical variables on SMF changes (Supplementary Table 3). Moreover, we verified whether ecosystem type may influence the pH–biodiversity–multifunctionality relationships in less and more acidic regions. We found that among the six ecosystems (Supplementary Table 5), including temperate coniferous forest, cropland, mixed forest, temperate broadleaf forest, pasture, and tundra, the positive correlations between SMF and bacterial and fungal biomass decreased with decreasing acidity. Notably, in natural wetland and tropical/subtropical forest systems, the positive correlation between SMF and bacterial and fungal biomass was strongest in samples with pH 5–7.5 and weakest or negative in samples with $\mathrm{pH} > 7.5$. Accordingly, these results support

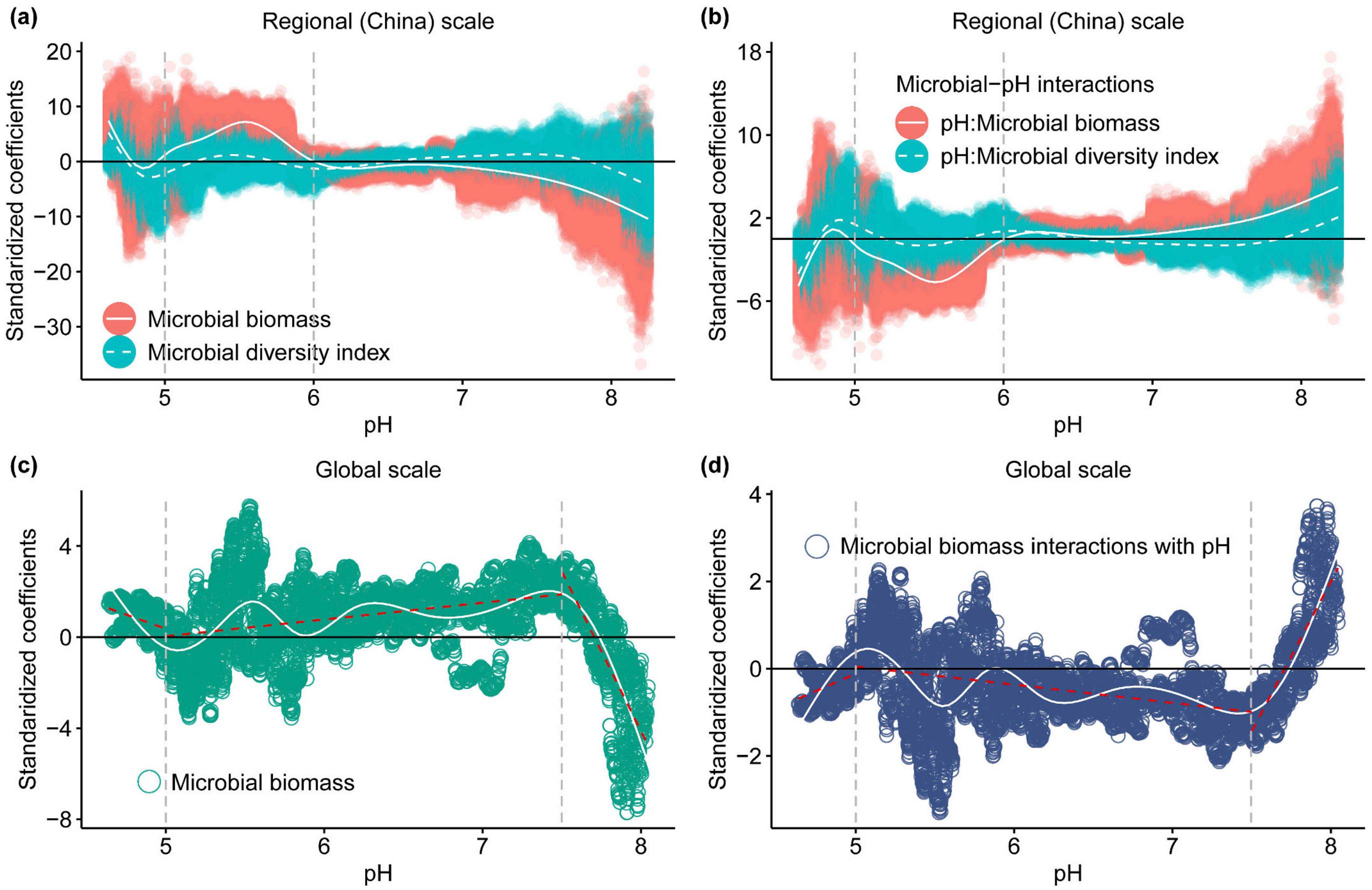

Fig. 2. Nonlinear variation in the relationship between soil multifunctionality and microbes with soil pH at the regional and global scales. The white trend line is a nonlinear curve between pH and the bootstrapped standardized coefficient obtained from the linear mixed-effects models. The red dashed line shows the linear regression analysis between standard coefficients and pH in different pH ranges (i.e., $pH < 5$, pH between 5 and 7.5 and $pH > 7.5$) in c and d. The dashed grey line represents the threshold pH values.

the existence of pH thresholds that affect biomass-SMF relationships, especially at $pH < 5$.

3.4. *Intensified soil acidification accelerates the loss of soil multifunctionality under continued soil acidification*

As reported by the FAO (https://www.fao.org) as the global N fertilization rate for 2019 across six continents (FAO, 2021), we predicted global soil pH changes at depths of 0–15 cm for the next 50 years. We included the duration of N application, nitrogen fertilizer application amount, initial soil pH, MAP, and MAT in the prediction model. We found that the duration and amount of N fertilizer applied are critical factors causing a decrease in pH at the global scale (importance = 49.5 % and 47.7 %, $p < 0.01$, $n = 441$; Supplementary Figs. 5, 6, and 7). As predicted, the global soil pH will change considerably in the next 50 years when temperature and precipitation are constant, especially in regions where the initial soil pH is <5 and >7.5 (Fig. 3a and b). Therefore, we categorized the risk of SMF loss under acidification conditions into three zones based on the discovered pH thresholds in this study: high ($pH < 5$), moderate (pH between 5 and 7.5), and low ($pH > 7.5$). Specifically, the area of regions with a $pH < 5$ was expected to increase by 13.54 %, while that of low-risk regions with a $pH > 7.5$ would decrease by 12.65 % (Fig. 3c). These results indicated that continued soil acidification may exacerbate the risk of biomass-driven loss of SMFs in the future.

4. Discussion

Our results demonstrated the threshold effects of soil pH on driving the associations between the microbial community and SMF at both regional and global scales. Specifically, below a pH threshold of 5, bacterial biomass becomes the primary biological indicator in paddy fields, while both bacteria and fungi drive soil multifunctionality across various ecosystems globally where the pH is below 5. This phenomenon may be attributed to the specific management practices and anaerobic conditions prevalent in paddy fields, which potentially favour bacterial activity over fungal activity (Liu et al., 2016; Wang et al., 2017). Given the greater aerobic tolerance of fungi than bacteria, fungi typically exhibit greater activity in drier environments, potentially diminishing their significance in flooded rice paddies (Wang et al., 2021). Therefore, at the global scale, although the threshold effects on multifunctionality are consistent, the response of microbial community dynamics to soil pH across different ecosystems exhibits a more complex pattern.

Slightly different pH thresholds were observed in paddy fields compared to global samples; for example, in paddy fields, the pH threshold ranges included <5, 5–6, and >6, whereas in global samples, the pH thresholds ranged from <5, 5–7.5, and >7.5. This discrepancy could be attributed to the diverse array of ecosystem types worldwide. Our regional-scale study exclusively included paddy fields, a particular type of wetland ecosystem. Moreover, rice cultivation areas in China are predominantly situated in regions characterized by acidic soils and facing challenges associated with soil acidification (Dong et al., 2022; Guo et al., 2018). Regions with $pH < 5$ typically have single or multiple attributes, such as humid climates, high organic C stocks, high

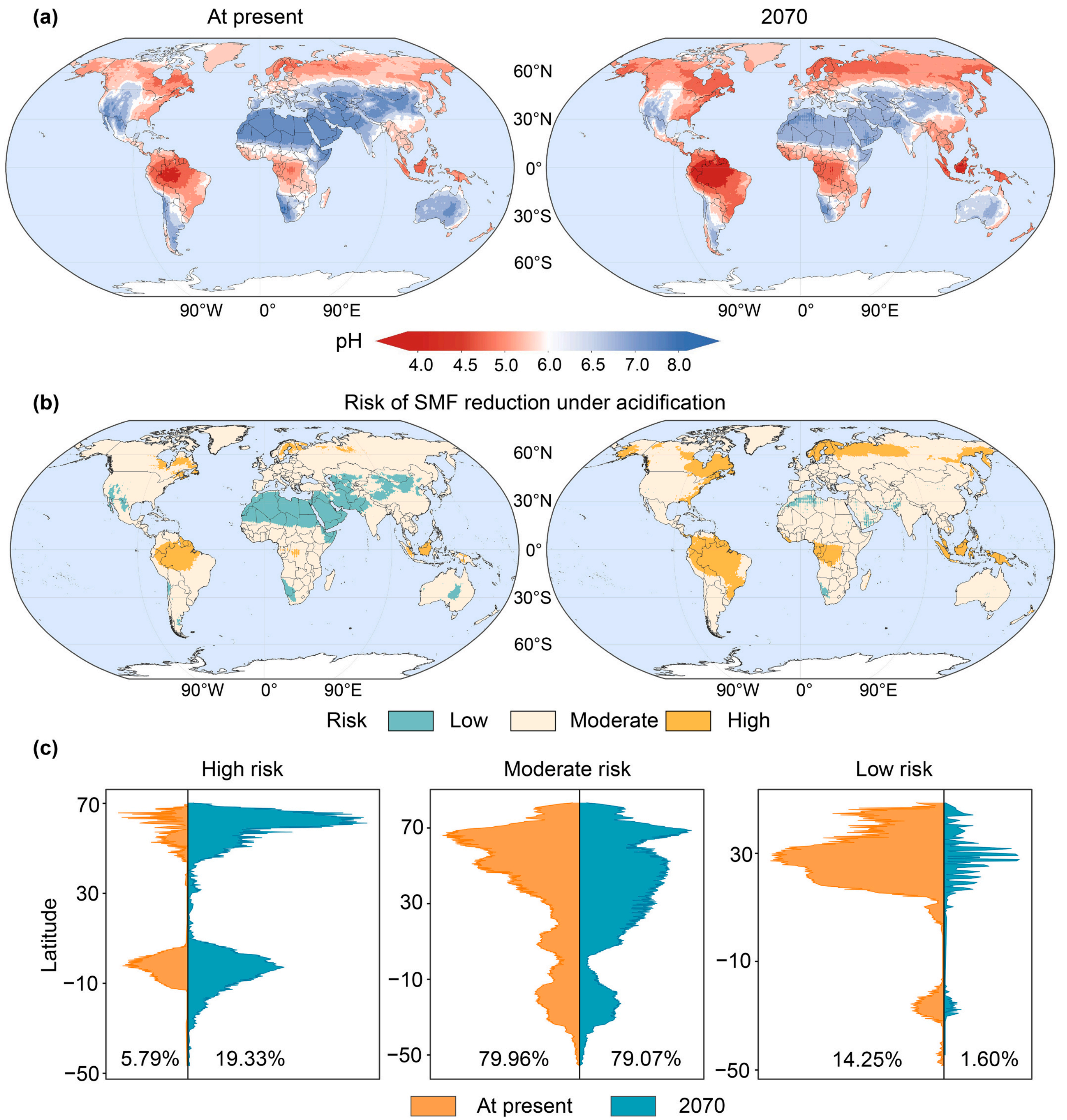

Fig. 3. Changes in global soil pH and the risk of reduced soil multifunctionality under acidification scenarios over the next 50 years. (a) Soil pH at 0–15 cm depth globally at present and in 2070 based on 58 kg·ha^{-1} of N fertilizer application when MAT and MAP are constant. (b) Changes in the risk of reduced soil multifunctionality (SMF). (c) Variations in the risk of reduced soil multifunctionality at different risk levels with latitude.

exchangeable aluminium ions, high nitrogen deposition, or high-intensity management practices such as excessive fertilization (Crowther et al., 2019). At the global scale, regions with pH < 5 were mainly distributed in boreal forests, natural wetlands, mixed forests, and tropical/subtropical forest systems (Supplementary Table 5). The regions with pH values between 5 and 7.5 had the most expansive distribution range, and there were no apparent commonalities among these regions. In contrast, regions with pH > 7.5 (Shangguan et al., 2014), such as shrubland, pasture, and desert ecosystems, typically have a dry climate. In these systems, aridity is explicitly a limiting factor and may decouple the positive microbe-SMF relationship (Delgado-Baquerizo et al., 2016). Moreover, the lower availability of soil micronutrients (e.g., Fe and Zn) in arid regions with high soil pH will force microbial communities to comprise more cooperators (Evans and Cushman, 2009). These findings provide crucial insights into ecosystem-specific characteristics and dynamic changes in microbial communities when maintaining the stability and functionality of soil ecosystems.

Moreover, as the pH shifts from acidic to alkaline, the abundance and function of different microbial groups (e.g., bacteria and fungi) diverge in their contributions to SMF. In acidic conditions ($pH < 5$), the positive correlation between SMF and microbial biomass, particularly bacteria, strengthened as pH decreased (Fig. 2, Supplementary Fig. 3). This suggested that bacterial adaptations to low pH environments play a role in organic matter decomposition and nutrient (e.g., N) cycling (He et al., 2024), which enhanced their positive association with SMF. Conversely, in alkaline soils, the slope of the relationship between microbial biomass and multifunctionality decreased sharply, indicating that microbial diversity (e.g., fungi) becomes more crucial to multifunctionality (Supplementary Fig. 3). Although the functional roles of individual fungal groups may be diluted, sustaining fungal diversity contributes to maintain functionality (Mori et al., 2016; Xue et al., 2023). These dynamic responses of SMF highlight that soil pH exerts differential impacts on microbial biomass, diversity, composition, and interactions across pH ranges (Rousk et al., 2010; Shi et al., 2021), potentially influencing nutrient biogeochemical cycling.

Accordingly, thresholds for soil pH have been demonstrated in many aspects of terrestrial ecosystems, such as microbial community structure, nutrient cycling, organic matter decomposition, and plant–microbe interactions (Fierer et al., 2012; Rousk et al., 2010). For example, when the pH in acidic soil increased to a threshold of $pH = 6.2$ due to anthropogenic changes, the acid retardation of microbial growth was alleviated following increased soil carbon decomposition (Malik et al., 2018). This may be because soil pH is considered a comprehensive indicator influenced by various factors, including soil properties, climate change, and soil management strategies (Malik et al., 2018; Slessarev et al., 2016), which directly affect crucial processes, such as nutrient availability, enzyme activities, and SOM decomposition (Feyissa et al., 2022; Malik et al., 2018). Furthermore, increasing the soil pH above certain thresholds can significantly alter the microbial enzyme activities that facilitate soil P and N release, enhancing plant growth and further influencing the microbial population structure (Aciego Pietri and Brookes, 2008; Geisseler and Horwath, 2008). This feedback loop suggests that slight pH adjustments could have cascading effects on ecosystem productivity and stability (Shi et al., 2021). Given the close relationships between various changes in microbial community composition and soil functions (e.g., C cycling (Fierer and Jackson, 2006; Rousk et al., 2010)), as well as multifunctionality (Zhao et al., 2024; Zheng et al., 2019), the results of previous reports showing that biomes and acidity regulate the community-level thermal response (MCTR) of microbes (i.e., bacteria and fungi) (Hijmans et al., 2005) are similar to those of this study. These results confirmed that in acidic environments, the total abundance of biological cells in a region, at the highest levels of ecological organization, ultimately leads to essential restrictions on the SMF (Fierer and Jackson, 2006). Our results underscore the importance of understanding how bacteria and fungi collaboratively influence soil multifunctionality, particularly when the pH is below 5, in response to the impacts of global environmental changes on ecosystem services. Therefore, we suggest that the threshold effect not only supports scientific theory but also provides practical references for global environmental policies and sustainable agricultural practices.

Numerous studies have demonstrated that N deposition and fertilization decrease the pH and thus reduce microbial biomass (Jia et al., 2020; Li et al., 2024). Here, we highlight the necessity of considering the role of microbes in ecological multifunctionality when predicting the ecological consequences of nitrogen deposition and agricultural activities such as N fertilization. This implies that the SMF will decrease with acidification, as the SMF is positively correlated with biomass in most regions (Supplementary Fig. 4). Moreover, the stronger dependence of multifunctionality on biomass in regions with $pH < 5$ implies that these regions will suffer a greater risk of reduced multifunctionality in soil acidification situations. Overall, these results demonstrated that a soil pH of 5 should be considered a critical threshold at which microbial biomass becomes a key determinant of SMF based on a large-scale field study and global dataset. Our study highlights the valuable concern that in acidic soils, SMFs may be more vulnerable to environmental disturbances, potentially posing a threat to ecosystem sustainability.

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Code availability

The code used for this work is available from the corresponding author on request.

CRediT authorship contribution statement

Meitong Jiang: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Weigen Huang:** Writing – original draft, Formal analysis, Data curation. **Jixian Ding:** Methodology, Investigation. **Zhiyuan Ma:** Writing – review & editing, Methodology. **Han Hu:** Methodology, Data curation. **Ruilin Huang:** Writing – review & editing, Formal analysis, Conceptualization. **Yunfeng Yang:** Writing – review & editing, Methodology. **Jizhong Zhou:** Writing – review & editing, Methodology. **Yuting Liang:** Writing – review & editing, Resources, Funding acquisition, Conceptualization.

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

The raw sequence data for bacteria and fungi are publicly available under the NCBI BioProject Accession numbers PRJNA562601 and PRJNA562792, respectively. The data collected in this study can be publicly accessed through the corresponding website links in the text.

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