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Microbial Properties Depending on Fertilization Regime in Agricultural Soils with Different Texture and Climate Conditions: A Meta-Analysis

Ding Yuan [1,†], Yi Hu [1,†], Shengnan Jia [1], Wenwen Li [1], Kazem Zamanian [1,2], Jiangang Han [3,4,5,*], Fan Huang [1,6] and Xiaoning Zhao [1,*]

[1] School of Geographical Sciences, Nanjing University of Information Science and Technology, Nanjing 210044, China
[2] Institute of Soil Science, Leibniz University of Hannover, Herrenhäuser Straße 2, 30429 Hannover, Germany
[3] College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China
[4] Co-Innovation Center for the Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China
[5] National Positioning Observation Station of Hung-tse Lake Wetland Ecosystem in Jiangsu Province, Hongze 223100, China
[6] School of Social Development and Management, Hunan Women's University, Changsha 410004, China
* Correspondence: hanjiangang76@126.com (J.H.); jasminezxnsx@msn.com (X.Z.)
† These authors contributed equally to this work.

Abstract: Over-fertilization has a significant impact on soil microbial properties and its ecological environment. However, the effects of long-term fertilization on microbial properties on a large scale are still vague. This meta-analysis collected 6211 data points from 109 long-term experimental sites in China to evaluate the effects of fertilizer type and fertilization duration, as well as soil and climate conditions, on the effect sizes on various microbial properties and indices. The organic fertilizers combined with straw (NPKS) and manure (NPKM) had the highest effect sizes, while the chemical fertilizers N (sole N fertilizer) and NPK (NPK fertilizer) had the lowest. When compared with the control, NPKM treatment had the highest effect size, while N treatment had the lowest effect size on MBN (111% vs. 19%), PLFA (110% vs. −7%), fungi (88% vs. 43%), *Actinomycetes* (97% vs. 44%), urease (77% vs. 25%), catalase (15% vs. −11%), and phosphatase (58% vs. 4%). NPKM treatment had the highest while NPK treatment had the lowest effect size on bacteria (123% vs. 33%). NPKS treatment had the highest while N treatment had the lowest effect sizes on MBC (77% vs. 8%) and invertase (59% vs. 0.2%). NPKS treatment had the highest while NPK treatment had the lowest effect size on the Shannon index (5% vs. 1%). The effect sizes of NPKM treatment were the highest predominantly in arid regions because of the naturally low organic carbon in soils of these regions. The effect sizes on various microbial properties were also highly dependent on soil texture. In coarse-textured soils the effect sizes on MBC and MBN peaked sooner compared with those of clayey or silty soils, although various enzymes were most active in silty soils during the first 10 years of fertilization. Effect sizes on microbial properties were generally higher under NPKM and NPKS treatments than under NPK or N treatments, with considerable effects due to climate conditions. The optimal field fertilizer regime could be determined based on the effects of fertilizer type on soil microorganisms under various climate conditions and soil textures. This will contribute to the microbial biodiversity and soil health of agricultural land. Such controls should be used for adaptation of fertilization strategies to global changes.

Keywords: long-term fertilization; microbial properties; soil degradation; agricultural sustainability; climate change

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1. Introduction

Microorganisms play important roles in soil biochemical processes, such as decomposition of organic material, nutrient cycling, and biotransformation of organic pollutants [1,2]. Even though the microbial biomass constitutes only a small portion of the soil, approximately 0.5–6.0% [3,4], it is considered a sensitive indicator of soil health and quality [5]. Moreover, the microbial biomass responds dynamically to management practices [6]. Especially in cropping systems, microorganisms are strongly affected by fertilization management and subsequent acidification. Acidification increases fungal abundance and composition by stimulating heterotrophic nitrification [7,8].

The rapid development of agriculture to feed the world's population increasingly depends upon the input of nitrogen (N) fertilizers. Studies have confirmed that irrational fertilization induces acidification [8,9] affecting the structure of the soil microbial community [10]. In 2019, the average N fertilizer per unit area of cropland in China reached 200 kg ha^{-1}, which is almost three times the average N fertilization rate in the world (70 kg ha^{-1}) [11]. Meanwhile, the rapid expansion of cultivated land area under human activities is causing some fragile ecosystems in China to face climate change; for example, the cultivated land area upstream of Tarim River expanded almost three times from 1997 to 2019 [12]. In a meta-analysis based on more than 100 datasets from long-term trials with annual upland crops from around the world, Geisseler and Scow (2014) found that mineral fertilizers increase the microbial biomass carbon content by an average of 15.1% compared with an unfertilized control [13]. In addition, a literature review by Allison and Martiny (2008) found that 84% of the 38 analyzed studies reported that microbial community composition is sensitive to the rate of nitrogen (N), phosphorus (P), and potassium (K) fertilization [14].

Long-term fertilization changes soil chemical properties, regulates the storage and transformation of soil nutrients, and can change the pH of the soil [8]. Any change in soil properties directly affects the microbial biomass, activity, and community structure [7]. Long-term application of mineral N, for instance, reduces soil microbial activity [15], while adding manure, plant residues, and other organic fertilizers maintain soil fertility and the stability of microbial systems [16]. Straw enrichment increases the content of soil organic matter, and improves the reproduction of microorganisms and microbial diversity [17]. This subsequently leads to better nutrient cycling and increases the fertility and productivity of the soil.

Soil moisture content is among properties that are usually controlled via land management. Water stress, for example, decreases the fungal "individuals" and the interactions among fungal populations. Water stress decreases fungal germination, radial growth, sporulation, and mycelial cord development [18]. However, a fungal community is more drought-tolerant than bacteria because their hyphae can transfer moisture from water-filled micropores [19,20]. Additionally, moisture-related differences between microbial communities in the same soil can be due to the adaptability of microorganisms that survive frequent natural wet–dry conditions [21,22]. The history of wetting–drying cycles and the dominant soil moisture regime are also determining factors. For example, microbial biomass carbon (MBC) shows an increase following soil rewetting in arid regions [23,24]. These varying results are all attributed to variations in soil properties, especially the moisture content and soil chemistry, which are directly or indirectly controlled by fertilization management. However, the response of soil microbial biomass and community composition to various fertilization management regimes remains uncertain.

The effects of fertilizers on soil microorganisms are uncertain due to specific geographical conditions, type of fertilizer, as well as soil physical conditions, particularly the particle size distribution, i.e., soil texture. To evaluate the influences of fertilizers on soil microbial properties, a meta-analysis was conducted to address the following objectives: (1) to find out the main factors controlling various soil microbial properties, depending on fertilizer type (chemical and organic); (2) to reveal the controlling effects of management duration under various climatic conditions on soil microorganisms; and (3) to provide the best fertilization strategies for sustainable agriculture practices.

2. Material and Methods

2.1. Data Sources

This study focuses on the major cropland areas in China. Data were collected from journal articles published in Elsevier Science Direct (https://www.sciencedirect.com/) (accessed on 7 April 2021) and from China National Knowledge Infrastructure (https://www.cnki.net/) (accessed on 8 April 2021) (1978–2021) with the keywords "fertilization, soil microorganisms, enzymatic activity". We have also considered studies cited in the references of these articles. We selected studies that met the following criteria: (1) the experimental sites were located in China; (2) the study location (site name, latitude, and longitude) was clear; (3) climatic conditions (mean annual temperature (MAT) and mean annual precipitation (MAP)) and soil texture information were available; (4) fertilization management measures, including cultivation duration and fertilization regimes, were given; and (5) the study was conducted with side-by-side comparisons of the control (CK) and treatments (e.g., chemical N or NPK fertilizers and organic fertilizers, i.e., manure and straw).

Using the above criteria, a total of 434 papers were collected, and 6211 data points were analyzed. According to the humidity index (HI = MAP/(MAT + 10)), the climatic conditions of 109 fertilization experimental stations in China were classified as arid regions ($\mathrm{HI} \leq 25$), semi-arid regions ($25 < \mathrm{HI} < 50$) and humid regions ($\mathrm{HI} \geq 50$) (Figure 1).

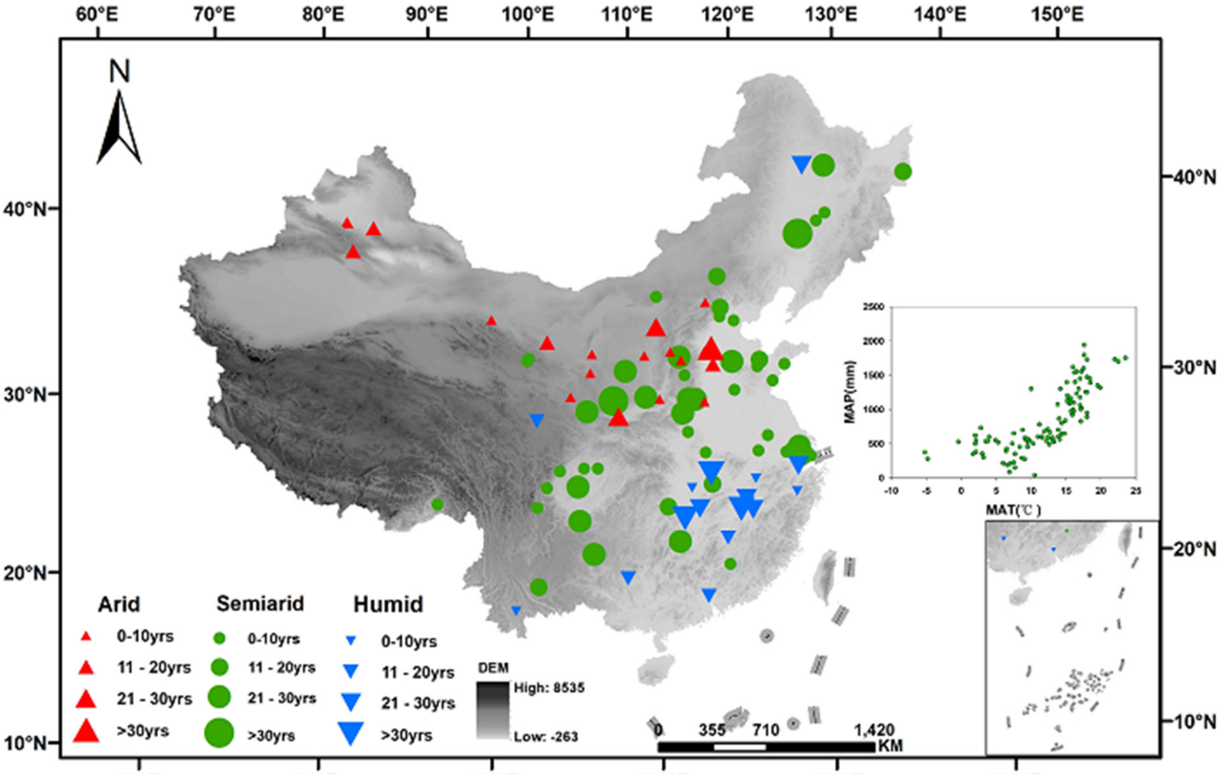

Figure 1. One hundred and nine long−term fertilization experimental stations in China, presented according to humidity index (HI = MAP/MAT + 10) and cultivation duration. triangles: arid ($\mathrm{HI} \leq 25$), circles: semi−arid ($25 < \mathrm{HI} < 50$), inverted triangles: humid ($\mathrm{HI} \geq 50$). The size of symbols represents cultivation duration. The digital elevation model (DEM) is a digital simulation of the terrain using limited terrain elevation data. The map is based on the standard map released by the Ministry of Natural Resources of the People's Republic of China (No. GS (2019)1822).

2.2. Data Analysis

2.2.1. Meta-Analysis

A random-effects meta-analysis was conducted to calculate the response of soil microbial properties under control (no fertilizer) compared with fertilization treatments [25]. The formula of the logarithm of response ratio (lnRR) for every soil microbial property is:

$$\mathrm{lnRR} = \ln\left(\frac{x_f}{x_c}\right) = \ln\left(x_f\right) - \ln(x_c) \tag{1}$$

where x_c and x_f are the value of control (no fertilizer) and fertilization treatments, respectively [26]. The results were also expressed as a percent change by using the conversion equation [27]: $\%\mathrm{change} = \left(\mathrm{e}^{\mathrm{lnRR}} - 1\right) \times 100\%$.

The variance (v) of RR was calculated as:

$$\mathrm{v} = \frac{\mathrm{s_f}^2}{\mathrm{n_f X_f}^2} + \frac{\mathrm{s_c}^2}{\mathrm{n_c X_c}^2} \tag{2}$$

where $\mathrm{n_f}$ and $\mathrm{n_c}$ are the sample sizes of the fertilization treatments and control, respectively, and $\mathrm{s_f}$ and $\mathrm{s_c}$ are the corresponding SDs [28]. When the studies had no SD or SE values, 1/10 of the mean was assigned to the SD [29].

The weighted effect size ($\overline{RR}$) was calculated from the RR of individual pairwise comparison between the fertilization treatments and control:

$$\overline{RR} = \frac{\sum_{i=1}^{m} w_i(RR_i)}{\sum_{i=1}^{m} w_i} \tag{3}$$

where m is the number of comparisons in the group, and w_i is the weighting factor of the ith experiment in the group; w_i was calculated as follows:

$$w_i = \frac{1}{v_i} \tag{4}$$

v_i is the variance (v) of the ith experiment. The standard error of ($\overline{\mathrm{RR}}$) was calculated as follows:

$$\mathrm{s}\left(\overline{\mathrm{RR}}\right) = \sqrt{\frac{1}{\sum_{\mathrm{i}=1}^{\mathrm{m}} \mathrm{w_i}}} \tag{5}$$

$$95\%\mathrm{CI} = \ln\overline{\mathrm{RR}} \pm 1.96\mathrm{s}\left(\overline{\mathrm{RR}}\right) \tag{6}$$

We also used the percentage change transformed from $\overline{\mathrm{RR}}$ to better visualize and explain the response of selected variables to the control:

$$\text{Effect size } (\%) = \left(\mathrm{e}^{\overline{\mathrm{RR}}} - 1\right) \times 100\% \tag{7}$$

Because the metabolism of microorganisms is affected by various factors, especially temperature and humidity, we proposed that the response ratio differs in various regions. To verify this hypothesis, the HI (humidity index) (Equation (8)) was used to classify fertilization experimental stations into three groups with HI ranges of ≤ 25 (LHI), 25–50 (MHI) and ≥ 50 (HHI).

$$\mathrm{HI} = \frac{\mathrm{MAP}}{\mathrm{MAT} + 10} \tag{8}$$

MAP and MAT in Equation (8) denote mean annual precipitation and mean annual temperature, respectively [26].

2.2.2. Statistical Analysis

In order to analyze the relationship between the fertilization regime and duration with microbial traits, the processed data were statistically analyzed using SPSS Statistics 22.0. One-way ANOVA was used and multiple comparisons between treatments were performed using the Least Significant Difference (LSD) method and then the *t*-test ($p < 0.05$ and $p < 0.01$). Linear regression analysis and correlation analysis were also performed to distinguish the changes of microbial properties in response to fertilization regime and duration as well as to climate conditions. Graphing was undertaken using SigmaPlot 14.0 and Origin 2019.

3. Results

3.1. The Effect of Fertilization Regime on Soil Microbial and Chemical Properties

There was a significant increase in the effect sizes of fertilization on microbial properties (28–73%) and chemical properties (27–96%), except for the Shannon index (3%), catalase (2%), C:N ratio (−7%), and pH (−23%) (Figure 2). The effect sizes of MBC (microbial biomass carbon) and MBN (microbial biomass nitrogen) increased 35% and 63%, respectively. The effect size of PLFA (phospholipid fatty acid) increased 37%. The effect sizes of three main microbial communities, i.e., bacteria, fungi, and *Actinomycetes* showed significant increases of 65%, 62%, and 73%, respectively. Urease, invertase and phosphatase activities increased significantly with effect sizes of 47%, 39% and 28%, respectively. The effect sizes of SOC (soil organic carbon), TN (total nitrogen) and AN (alkali−hydrolysable nitrogen) increased by 27%, 30%, and 37%, respectively. The effect size of phosphorus dramatically increased with the value of 96% for AP (available phosphorus), and 58% for TP (total phosphorus).

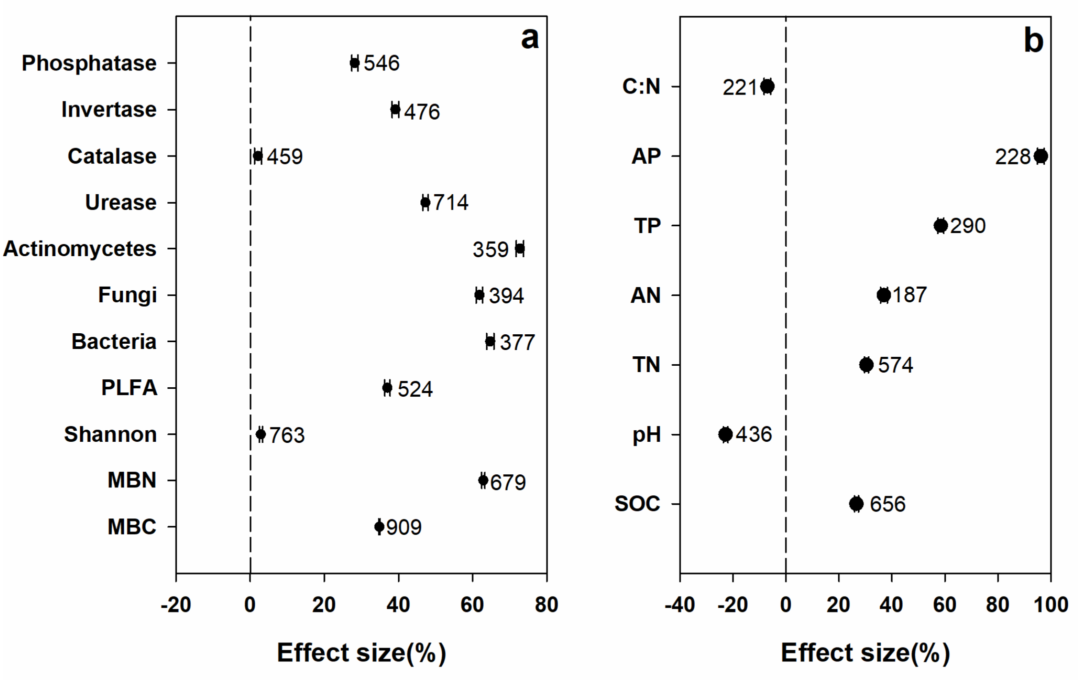

Figure 2. The effect sizes of fertilization on soil microbial properties (MBC, MBN, the Shannon index, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase and phosphatase) (**a**) and soil chemical properties (SOC, pH, TN, AN, TP, AP, and C:N ratio) (**b**) by meta−analysis. The numbers approximating the black dots are the data numbers. MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; PLFA: phospholipid fatty acid; SOC: soil organic carbon; TN: total nitrogen; AN: alkali−hydrolysable nitrogen; AP: available phosphorus; and TP: total phosphorus.

3.2. The Effect of Fertilization Regime on the Soil Microbial Properties

The chemical fertilizers combined with manure (NPKM) or straw (NPKS) had higher effect sizes compared with chemical fertilization using NPK (the phosphorus, potassium, and nitrogen fertilizer) or N (sole nitrogen fertilizer) on MBC, MBN, the Shannon index, PLFA, urease, and invertase (Figure 3). The NPKM fertilizer had the highest effect sizes on MBN (111%), PLFA (110%), bacteria (123%), fungi (88%), *Actinomycetes* (97%), urease (77%), catalase (15%), and phosphatase (58%). The NPKS fertilizer had the highest effect sizes on MBC (77%), the Shannon index (5%), and invertase (59%). The sole N fertilizer had the lowest effect sizes on MBC (8%), MBN (19%), PLFA (−7%), fungi (43%), *Actinomycetes* (44%), urease (25%), catalase (−11%), invertase (0.2%), and phosphatase (4%). In comparison, the NPK fertilizer had the lowest effect size on bacteria (33%) and the Shannon index (1%) (Figure 3).

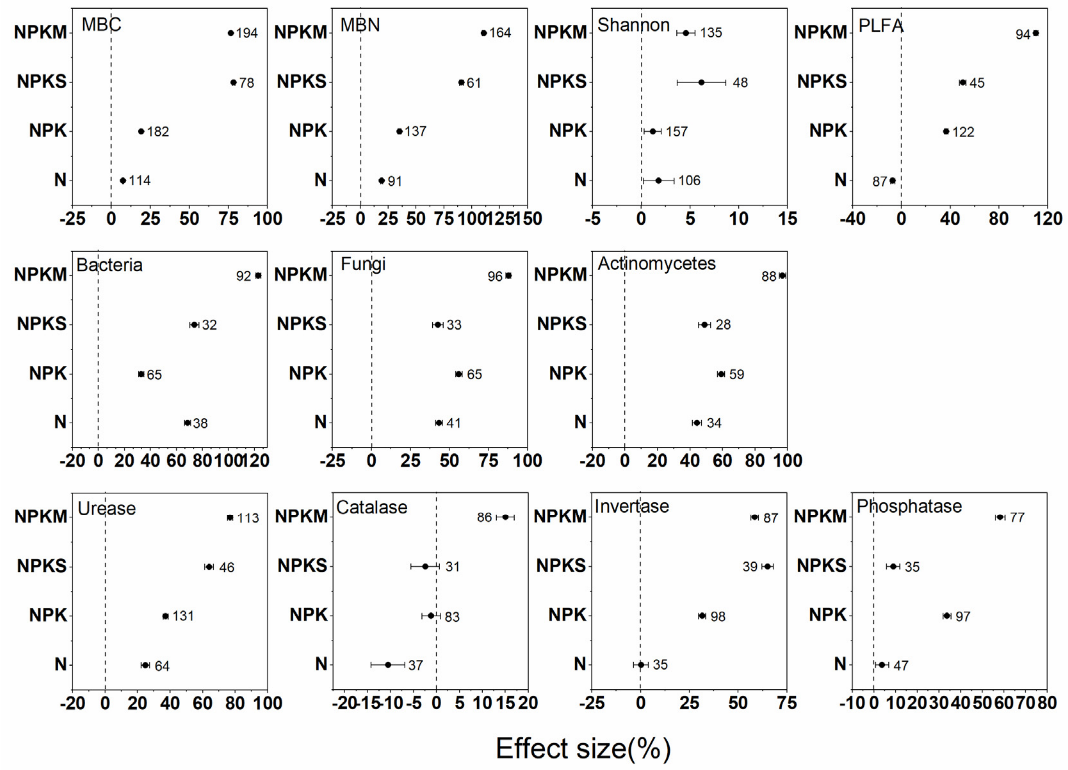

Figure 3. The effect size of fertilization on MBC, MBN, Shannon index, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase and phosphatase depending on four main fertilizer regimes, i.e., N, NPK, NPKS, NKPM. The numbers approximating the black dots are the data numbers. MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; PLFA: phospholipid fatty acid; N: sole nitrogen fertilizer; NPK: the phosphorus, potassium, and nitrogen fertilizer; NPKS: NPK combined with straw; NPKM: NPK combined with manure.

3.3. Climate Controls on the Effects of Fertilizer Type on Soil Microbial Properties

The effect sizes on MBC, MBN, and PLFA, respectively, were the highest under NPKM treatment in arid regions (125%, 154%, and 181%) followed by HHI (55%, 150%, and 100%), and were the lowest under sole N fertilizer treatment (0.02%, −0.05%, and 11%). The sole N fertilizer had the lowest effect size of those used in semi−arid (9%, 23%, and −11%) and humid regions (4%, 14%, and −9%), respectively. The NPKS fertilizer had the highest effect size of those used in humid regions (70%, 100%, and 30%), respectively. The effect size of the NPKS fertilizer on MBC and MBN were in second place following NPKM treatment (69% and 56%) in humid and arid regions (71% and 72%) (Figure 3: MBC, MBN). The effect size of the Shannon index was the highest under NPKS treatment in semi−arid land (10%).

The NPKM fertilizer had the highest effect sizes on bacteria, fungi, and *Actinomycetes*, with a change of 142%, 97% and 118%, respectively, in semi−arid regions (Figure 4). The NPKS fertilizer had the highest effect sizes on bacteria (149%) and *Actinomycetes* (95%) in arid regions, followed by NPKM treatment (137%, 80%) (Figure 4: bacteria, *Actinomycetes*); however, the NPKS fertilizer had the lowest effect size (−24%) on fungi (Figure 4: fungi). In MHI, The NPKS treatment had the highest effect sizes on MBC (81%), MBN (99%) and the Shannon index (7%). In HHI, NPKM treatment had the highest effect size on fungi (74%), however NPK treatment had the lowest (22%). In humid regions, the NPKS fertilizer had the highest effect sizes on bacteria (35%, 34%) and *Actinomycetes* (186% NPK) but NPKM treatment had the lowest effect sizes (Figure 4: bacteria, *Actinomycetes*).

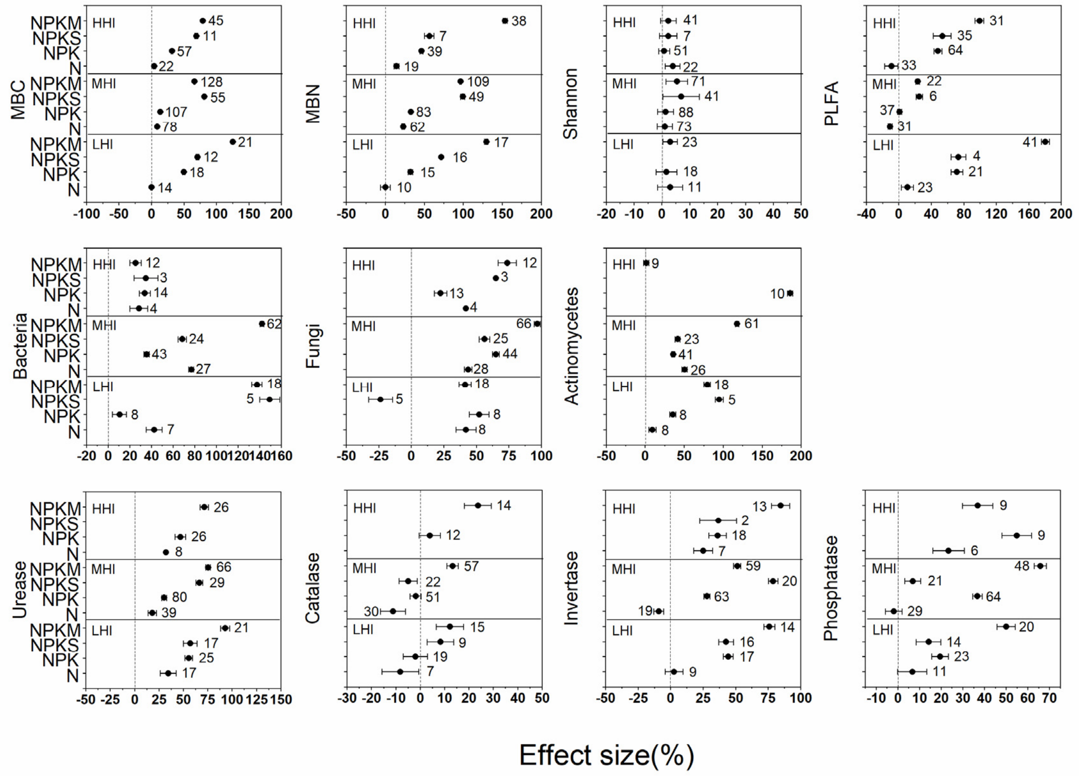

Figure 4. The effect sizes of fertilization on soil microbial properties (MBC, MBN, the Shannon index, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase, and phosphatase) by meta−analysis according to humidity index of four main fertilizer regimes (N, NPK, NPKS, and NKPM). The numbers approximating the black dots are the data numbers. N: sole nitrogen fertilizer; NPK: the phosphorus, potassium, and nitrogen fertilizer; NPKS: NPK combined with straw; NPKM: NPK combined with manure; LHI: arid regions (HI $\leq$ 25); MHI: semi−arid regions (25 < HI < 50); HHI: humid regions (HI $\geq$ 50).

The lowest effect sizes of urease and catalase activities were observed under sole N fertilizer treatment in arid (35%, −8%), semi−arid (18%, −11%), and humid regions (32%, n.d.); however, the highest effect sizes were observed under NPKM treatment in arid (93%, 12%), semi-arid (75%, 13%), and humid regions (71%, 24%) (Figure 4: urease, catalase). The sole N fertilizer had the lowest effect size on invertase in semi−arid (−9%), arid (2.6%) and humid regions (25%) (Figure 4: invertase). NPKM treatment had the highest effect size on invertase in arid (76%) and humid (85%) regions, but NPKS treatment had the highest effect size in semi-arid regions (79%). In arid and semi−arid regions, respectively, the sole N fertilizer had the lowest effect size on phosphatase (6.6%, −2%) but NPKM had the highest effect size (50%, 66%). NPKS treatment had the lowest effect size on phosphatase in arid (14%) and semi−arid regions (7%) (Figure 4: phosphatase).

3.4. The Effect of the Fertilization Duration on Soil Microbial Properties Depending on Fertilizer Type and Climate

The NPKM fertilizer had an effect size on microbial properties (MBC, MBN, the Shannon index, PLFA, bacteria, *Actinomycetes*, urease, catalase, invertase, and phosphatase) throughout years of cultivation, however most of the values of sole N fertilizer remained low (Figure 5). MBC displayed a hump−shaped tendency in effect sizes under NPKS treatment in semi−arid and humid regions, with peak values of 141% (11–15 years) and 126% (16–20 years), respectively. The sole N fertilizer had negative effects on the percentage change of PLFA (−23% MHI, −51% HHI) with 21–25 and 26–30 years of cultivation, while effect sizes of NPK and NPKS treatments increased gradually with years of cultivation.

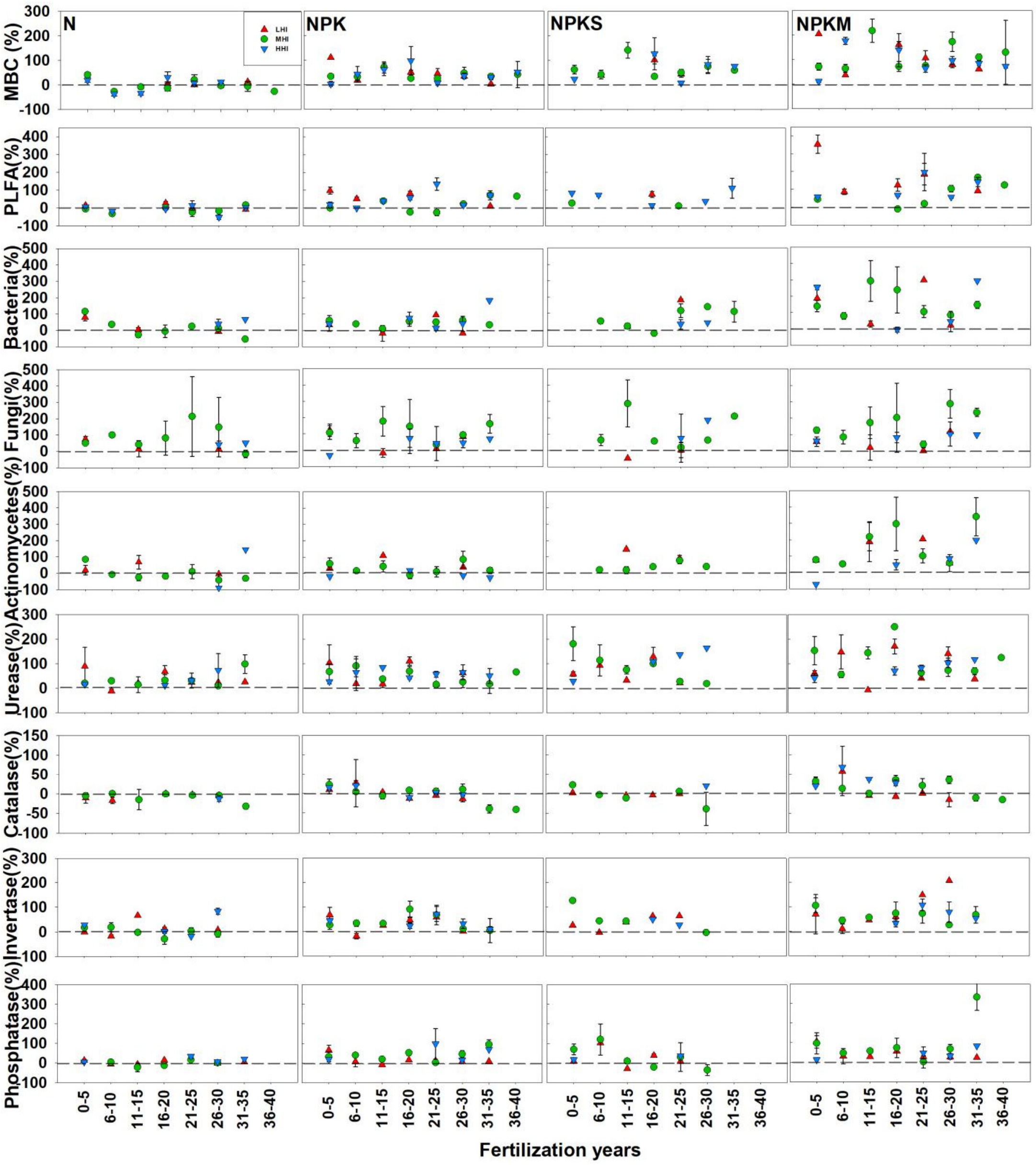

Figure 5. The effect sizes of fertilization duration on soil microbial properties (MBC, MBN, the Shannon index, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase, and phosphatase) with different HI (red triangle: HI ≤ 25; green circle: 25 < HI < 50; blue inverted triangle: HI ≥ 50) and four main fertilizer regimes (N, NPK, NPKS, and NKPM). N: sole nitrogen fertilizer; NPK: the phosphorus, potassium, and nitrogen fertilizer; NPKS: NPK combined with straw; NPKM: NPK combined with manure.

Bacteria and *Actinomycetes* gradually decreased in arid (from 80% to −7%, 19% to −4%) and semi−arid regions (from 116% to −54%, 84% to −32%) within 40 years cultivation using N sole fertilizer. Bacteria and *Actinomycetes* increased in semi−arid regions (from 53% to 111%, 21% to 41%) under NPKS treatment. The effect size of fungi under N sole fertilizer treatment peaked (214%) after 21–25 years cultivation in semi−arid regions; the effect size was 288% under NPKM treatment after 26–30 years.

The effect size of urease decreased (from 181% to 19%) in semi−arid regions and increased (from 28% to 164%) under NPKS treatment in humid regions within 30 years cultivation. The effect size of catalase decreased under N (from −5% to −32%), NPK (from 23% to −41%), NPKS (from 22% to −39%), and NPKM treatments (from 31% to −16%) in semi−arid lands during 30 years cultivation. The effect size of invertase reached a peak value (91%) after 16–20 years under NPK treatment in semi−arid regions, and decreased (from 127% to −4%) under NPKS treatment within 30 years cultivation. Phosphatase increased gradually under NPK treatment (from 33% to 95%) and decreased under NPKS treatment (71% to −36%) with years of cultivation.

3.5. The Effects of Fertilization on Microbial Properties Depending on Soil Texture

Clay loam had the largest effect sizes on microbial properties (MBC, MBN, the Shannon index, PLFA, bacteria, fungi, and *Actinomycetes*) with years of cultivation, but sandy loam had the lowest values (MBC, MBN, the Shannon index, PLFA, bacteria, fungi, and *Actinomycetes*) (Figure 6). The effect size on catalase had the lowest value (−80.49%) 11 years after fertilization in clay loam soils.

In most cases, the effect size on microbial properties peaked earlier in sandy loam compared with clay and silt loam. The effect size on MBC peaked after 15 years of fertilization in sandy loam, but only peaked after 20 years of fertilization in silty (546%) and clay loam (443%) (Figure 6). The effect size on MBN peaked after 18 years in sandy (629%) and silt loam (515%), but only peaked in clay loam (645%) after 28 years. Meanwhile, the effect size on PLFA peaked before 5 years fertilization in sandy loam (385%), but peaked after 23 years in silty loam (315%) and 22 years in clay loam (507%). The silty loam resulted in the highest effect sizes on urease (550%), catalase (179%), invertase (233%), and phosphatase (200%) before 10 years cultivation (Figure 6). The clay loam resulted in the highest effect sizes on urease (250%), catalase (93%), invertase (250%), and phosphatase (331%) after 20 years cultivation.

There was a wide range in the effect size (between the highest and lowest values) on microbial properties in most cases. The effect size on the Shannon index had a wider range in clay loam (from −51% to 89%) with the peak value after 19 years, than in sandy loam (from −27% to 29%) with the peak value after 5 years. The effect size on urease had the widest range in silty loam (from −60% to 550%) but the smallest range in clay loam (from −87% to 250%). The changes in bacteria, fungi, and *Actinomycetes* had the widest ranges in clay loam, with the smallest ranges in sandy loam soils.

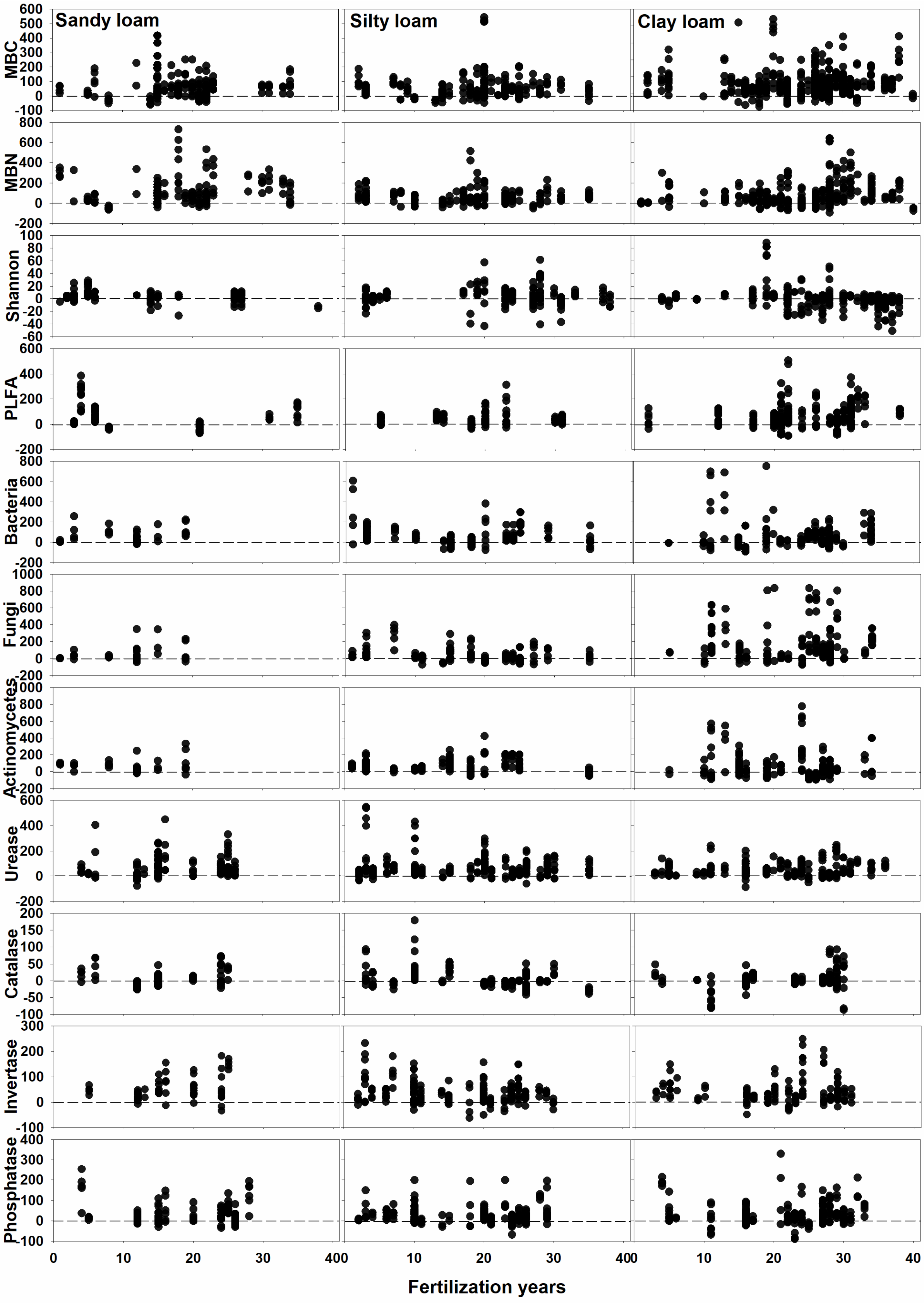

Figure 6. The effects of fertilization on microbial properties (MBC, MBN, Shannon index, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase, and phosphatase) depending on soil texture (sandy loam soils, silty loam soils, clay loam soils) with fertilization duration.

4. Discussion

4.1. The Effect of Long−Term Fertilization on Microbial Properties

Both long−term NPKM and NPKS treatments are conducive to improving MBC and MBN [30,31]. The slow release of nutrients from the added organic compounds provides a more stable habitat for microorganisms [32]. Long−term application of combined organic and inorganic fertilizers like NPKM and NPKS increase the richness index of the soil microbial community, and the Shannon Index, because the soil nutrient status and energy level improves and becomes more sufficient [30]. In addition, the acid neutralization capacity of soil increases, which directly improves the microorganisms' habitat [33].

The reduction in catalase activity (Figure 2) is due to soil acidification during long-term fertilization (ΔpH = −23%). As pH decreases, catalase activity also decreases and when pH < 5.0, catalase activity is almost completely lost [34]. Due to long-term fertilization, the regulation of nutrient storage and transformation [8] decreases the catalytic efficiency of enzymatic reactions, leading to a reduction in microbial activity [35]. Irrational fertilization, especially when employing ammonium-based fertilizers such as urea, accelerates soil acidification [36–39]. Therefore, sole N application had the lowest effect size on the microbial properties (MBC, MBN, PLFA, urease, catalase, invertase, and phosphatase) [40].

Nitrogen fertilization leads to changes in enzyme activity [41]. Compared with other fertilizers, urease showed the lowest effect under sole N fertilizer treatment such as urea (Figure 3). This is because urease activity was positively correlated with SOC [42]. In contrast, combined application of chemical and organic fertilizers provides a more balanced soil fertility, with a slow mineralization rate of organic N. Furthermore, organic fertilizers contain certain amounts of enzymes and urease among them [43].

4.2. The Effect of Climate and Fertilization Duration on Microbial Properties

The pronounced increase in MBC in arid regions was higher than in other regions under NPKM treatment (Figures 4 and 7) because of naturally low SOM content and N availability [24,44,45] after adding the organic fertilizer, allowing microorganisms to obtain sufficient nutrients and promoting the rapid propagation and growth of microorganisms. Therefore, fertilization applications in low−fertility agroecosystems have a more pronounced effect on soil nutrients which affect microorganisms. Increased belowground biomass and root exudation thus promote the reproduction of microorganisms and increase in microbial biomass [46,47].

The effect size of MBC showed a decreasing trend after an initial increase during the fertilization years (Figures 5 and 6). This is because of the gradual decrease in organic carbon mineralization rate over time in the agroecosystem [48]. Meanwhile, after long-term fertilization, the initially significant increase in urease activity became stable after 20 years of cultivation under NPKS and NPKM treatments. This may be due to a lack of correlation between organic fertilization and urease activity [49].

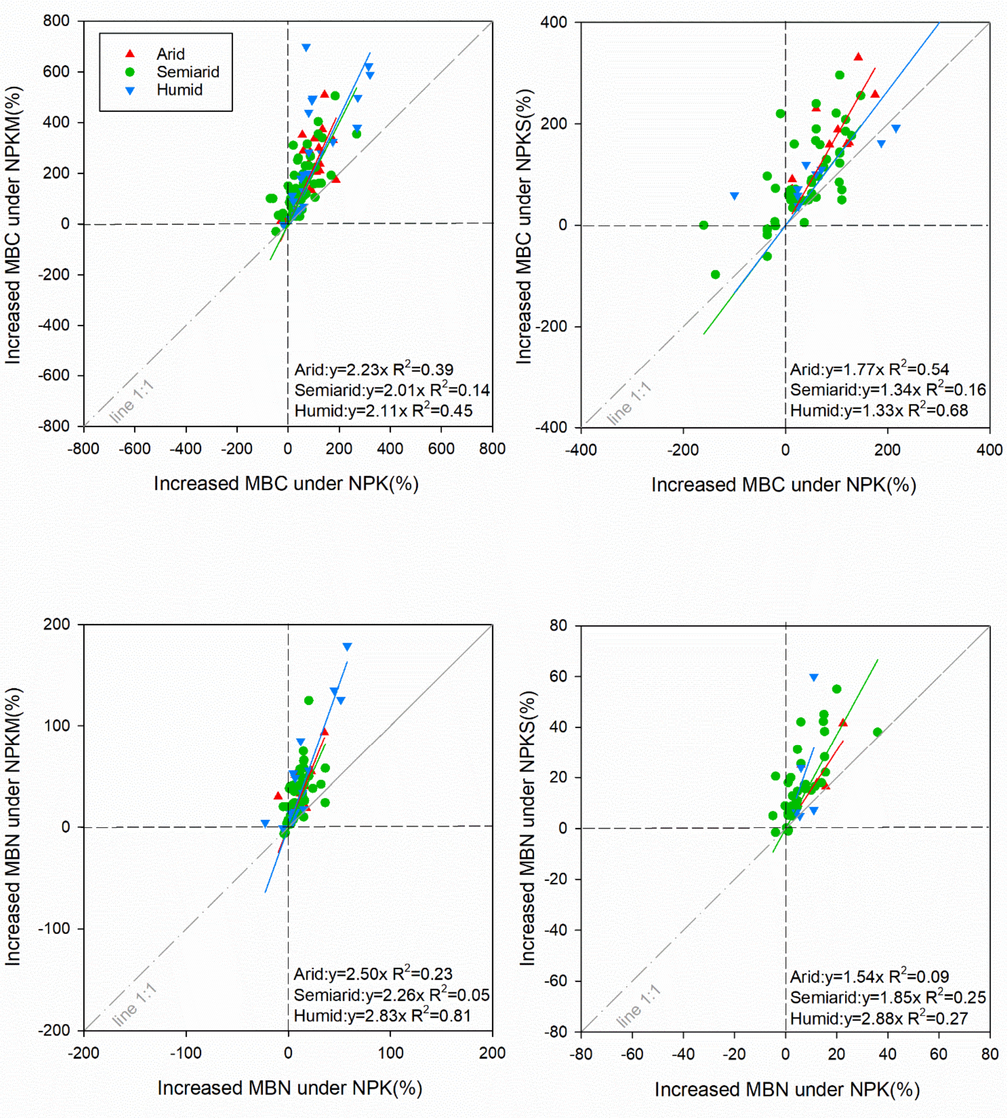

Figure 7. The linear relationships (y = ax) between the percentage changes of increased MBC and MBN under NPK and NPKM treatments depending on the HI (humidity index). The dashed lines show 1:1 ratios and colored lines reflect the linear regressions. All regression lines are significant at $p < 0.05$.

4.3. The Effects of Soil Texture on Microbial Properties

The effect sizes on MBC and MBN peaked earlier in sandy loam soils (Figure 6) because of a lower level of nutrient content [8,50]. Moreover, in coarse-textured soils, MBC, despite faster conversion of C and N, accounts for a lower proportion of SOC than fine-textured soils [51]. This leads to a greater plant response to fertilizers [52], as well as an increase in biomass production and C input to the soil in the short term. Moreover, particulate organic carbon in large aggregates of coarse—textured soils is easily decomposable [53], and so the MBC in such soils reaches its peak earlier after fertilization [54].

The largest increase in values of MBC, MBN, the Shannon index, PLFA, bacteria, fungi, and *Actinomycetes* with years of cultivation in fine—textured soils such as clay loam (Figure 6) is because of enhanced water and nutrient retention [55,56]. Moreover, soil organic matter stability and soil resistance to erosion and nutrient loss [50,57] are other controlling factors in fine—textured soils, which affect microbial activity and microbial diversity [58,59] when compared with coarse—textured soils. However, the activity level of urease in clay soils showed a lower level of growth (Figure 6). This is because anaerobic conditions are more common in clay soils, resulting in the production and accumulation of ammonium, thereby inhibiting urease activity [60]. Compared with silty soils, the increase in enzyme activity in clay soils during the early stage of cultivation is not as obvious due to anaerobic conditions, which may be caused by the water stagnation, and adsorbed enzymes on the surfaces of clay particles that reduce their activities [61,62] (Figure 6).

4.4. Best Field Strategies to Improve Soil Microbial Properties despite Long−Term Fertilization

Chemical fertilizers combined with organic residues or manure, in contrast to sole application of chemical fertilizers, are the most reasonable fertilization strategy, which have a significant positive effect on soil microbial indices (MBC, MBN, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase, and phosphatase) (Figure 3). Soil MBC increased by a factor of 2.23 during NPKM treatment and 1.77 during NPKS treatment in arid regions compared with NPK treatment, and MBN increased by a factor of 2.83 during NPKM treatment and 2.88 during NPKS in humid regions compared with NPK (Figure 7). MBC and MBN are more responsive microbial indicators to fertilization under NPKS and NPKM treatment (chemical combined with straw and organic fertilizers), especially in arid and semi−arid regions with humidity index of 20−40(NPKS) and 30−50(NPKM) (Figures 4, 7 and 8), depending on soil texture (Figure 6). This is because in arid regions drought increases the concentration of soil soluble carbon, thus providing a higher MBC conversion rate, but decreases the nitrification rate of soil microorganisms, especially bacteria, thus providing a lower MBN conversion rate [63]. Adjusting the amount of fertilizer applied according to soil texture and considering the climate increases nutrients and water use efficiency [64] by improving nutrient cycling via a microorganism's biomass [65] (Figures 4 and 6). Because of the pronounced effects of soil texture on microbial properties (Figure 6), employing amendments such as biochar or the addition of sand to clayey soils [66,67], which can improve soil structure and pore architecture as well as water use efficiency, are suitable management options for improving microbial properties. In addition, based on an appropriate reduction of fertilization, optimal irrigation and reduced tillage can further increase soil organic matter and crop yield [68], and promote sustainable agricultural development.

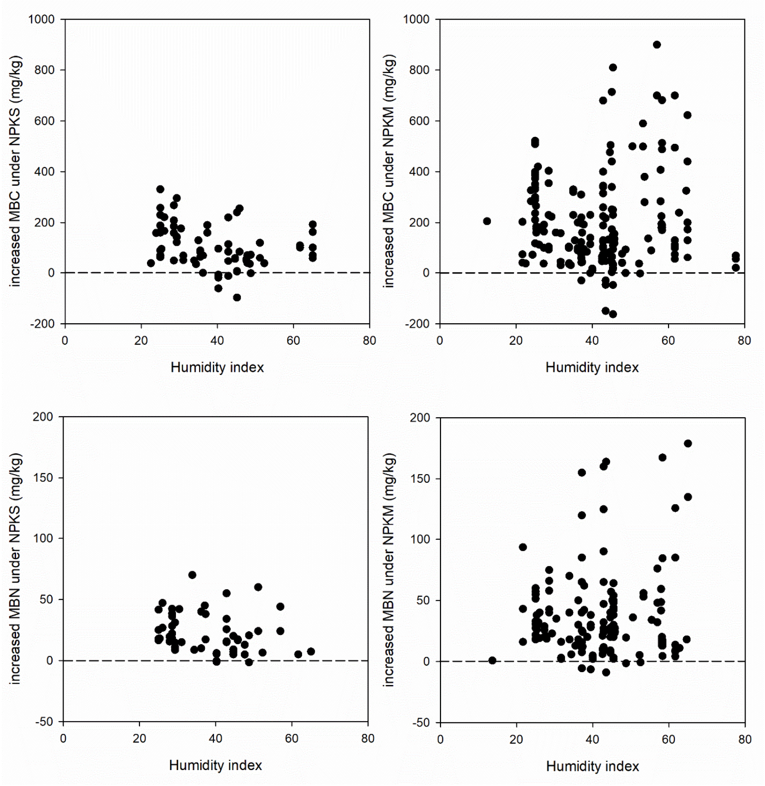

Figure 8. The relationships of MBC and MBN with HI (humidity index) under combined application of chemical and organic fertilizers, i.e., NPKS and NPKM. The dashed lines represent x = 0.

5. Conclusions

Overuse of chemical fertilizers leads to inefficient use of nutrients, soil pollution, greenhouse gas emissions, and eutrophication that threatens the sustainability of agriculture. Improving soil microbial properties can be a practical strategy to mitigate these consequences of long-term fertilization. In this meta-analysis, we used 6211 data points from 109 long-term experimental stations in China to comprehensively examine the effects of fertilization on soil microbial properties. We have analyzed changes in microbial properties including MBC, MBN, the Shannon index, PLFA, bacteria, fungi, *Actinomycetes*, urease, catalase, invertase, and phosphatase depending on fertilizer regimes, climate, cultivation duration and soil texture. The chemical fertilizers combined with straw (NPKS) and manure (NPKM) had the highest effect sizes, while chemical fertilizers N (sole N fertilizer) and NPK (NPK fertilizer) had the lowest. Effects on microbial properties were evident more quickly in coarse-textured soils than in fine-textured soils and the effects of fertilization were more pronounced in arid and humid regions than in semi-arid and sub-humid areas. In conclusion, as a win–win field management strategy, combined use of chemical and organic fertilizers is the best way to alleviate agricultural soil deterioration through improving its microbial properties. This is highly relevant when we also consider environmental changes that threaten long-term sustainable cultivation and food production.

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