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# The Effects of Five-Year Biosolid Application on the Diversity and Community of Soil Arthropods

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**Abstract:** Land application of biosolids is a beneficial form of management, although heavy metal contamination is a major concern. Biosolid application can shape the abundance, species richness, and community structure of arthropods, which are important regulators of soil processes. We investigated the effect of the five-year (2012–2017) application of domestic biosolids at 0, 15, 30, and 45 ton $ha^{-1}$ on the soil properties, enzyme activity, heavy metal concentrations, abundance, and diversity of soil arthropods in degraded sandy soil. The results showed that the application of a high amount of biosolids resulted in an increase in soil organic carbon of 2.6 times and in the water content of 2.8 times compared with CK (no biosolids). The total metal concentrations of Cr, Ni, Cu, Zn, Cd, and Pb increased by 6.6%, 3.2%, 6.6%, 7.7%, 13.3%, and 22.5%, respectively, compared with CK in soil ($p > 0.05$). The activities of seven enzymes, which mainly participate in carbon (C), nitrogen (N), phosphate (P), and sulfur (S) transformation, increased by 1.53%~122.7%, indicating that the soil function did not change under biosolid application. The number of individual arthropods collected from a square meter of soil changed from 0 to 2560. The total abundance of arthropods increased from 1.2 to 4 times under biosolid application ($p < 0.05$), but biosolid application had no effects on simple measures of richness and diversity (Shannon–Weaver index). Multivariate ordination techniques showed a significant shift of the arthropod community structure under biosolid application due to differing responses of several taxa to the biosolids. Redundancy analysis highlighted the influential role of soil chemical properties (soil organic C, total N, water content, microbial biomass, and pH) and cadmium in shaping the soil arthropod structure. These results suggest that long-term biosolid application with limited heavy metal concentrations does not have detrimental effects on soil arthropods or microbial-related soil function.

**Keywords:** biosolids; soil arthropod; enzyme activity; agricultural land



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## 1. Introduction

Land application of biosolids (treated sewage sludge) is an economical and beneficial form of management because it improves soil fertility and soil structure [1,2]. However, heavy metals and pathogens in biosolids cause major concerns for sustainable management. Therefore, permissible concentrations of the heavy metals and/or permitted loading rates in biosolids have been established. Nevertheless, concerns about farmland application of biosolids having adverse effects on soil organisms and soil function continue. Therefore, further data from field applications are needed, especially under long-term experimental conditions [3].

Soil fauna, as an important indicator of soil quality, may be affected by biosolid application. In a short-term experiment, biosolid application resulted in an increase in earthworm biomass [4], as well as in the abundance of nematodes and earthworms [5]. On the contrary, Coors et al. [2] found that biosolid application has no effect on fauna (nematodes, earthworms, and enchytraeids) structure and function [2]. Hout et al. [6] further explained that the high organic matter in biosolids, which enhances heavy metal immobilization, may

compensate for the negative effect of metals on fauna (microarthropods and macroinvertebrates) [6]. In fact, the effect of heavy metal contamination on soil fauna is contradictory as well. For example, van Gestel et al. [7,8] found that heavy metals sprayed on soil impact nematodes, earthworms, and enchytraeids; on the contrary, Creamer et al. [9,10] found that heavy metal pollution does not have a long-term effect on nematode assemblages or invertebrates. In terms of long-term experiments, only a few have shown weak evidence of the negative impact of liquid or dry biosolids on earthworms and nematodes [2,11]. However, the positive or negative effects of biosolids can be specific to certain taxonomic groups; therefore, further field experiments are needed for other fauna taxa, i.e., arthropods, which have high diversity and abundance and play a crucial role in driving ecosystem processes [12,13].

Soil-dwelling arthropods maintain the soil structure, regulate soil porosity (which affects root growth, hydrological processes, and aeration), affect C dynamics, and play a key role in nutrient cycling [14]. Soil microarthropods, as the main body of arthropods, not only directly affect the decomposition rate of organic matter by fragmentation and fecal production, but also indirectly affect the decomposition rate by controlling primary (bacteria and fungi) and secondary (nematodes and protozoa) decomposers. A few studies have been carried out focusing on soil arthropods in forest soil under litter control conditions [15,16]. However, to the best of our knowledge, the impact of long-term biosolid application upon a farmland community of soil arthropods remains largely unknown.

To address these, we examined the effects of five-year biosolid application on soil properties, soil arthropod community, and enzyme activity. We hypothesized that domestic biosolid application would not have a negative effect on arthropod diversity or enzyme activity. The present study comprised four parts: First, monitoring of soil fertility and heavy metal accumulation in soil and crop grain; second, monitoring of changes of various arthropod taxa in abundance, species richness, and species composition; third, monitoring of the soil enzyme activity in the C, N, P, and S cycles to determine the impact of heavy metals on soil function; fourth, explaining the relationship between soil properties and arthropod community structure to determine the key factors affecting the structure of arthropods.

## 2. Materials and Methods

### *2.1. Study Site*

A five-year (2012–2017) field experiment was carried out at the Kaifeng Academy of Agriculture and Forestry (34.77° N, 114.27° E) in Henan Province, China. The five-year mean temperature and rainfall of the site were 15.38 °C and 467.1 mm, respectively. The soil texture was sandy loam (Fluventic Ustochrept), composed of 73.9% sand, 10.3% silt, and 15.8% clay. The characteristics and total heavy metal concentrations of the topsoil (0–20 cm) are shown in Table 1.

**Table 1.** Physicochemical properties and total concentrations of heavy metals in the topsoil and biosolids.

| Properties | Unit | Soil | Biosolids |
|---|---|---|---|
| Water content | % | 5.87 | 34.99 |
| pH | | 8.42 | 8.05 |
| Soil organic carbon | g $kg^{-1}$ | 7.05 | 130.64 |
| Total nitrogen | g $kg^{-1}$ | 0.44 | 17.61 |
| Total phosphate | g $kg^{-1}$ | 0.33 | 9.86 |
| Total potassium | g $kg^{-1}$ | 4.3 | 13.9 |
| Cr | mg $kg^{-1}$ | 28.71 | 200.49 |
| Ni | mg $kg^{-1}$ | 12.31 | 22.25 |
| Cu | mg $kg^{-1}$ | 9.77 | 164.35 |
| Zn | mg $kg^{-1}$ | 22.90 | 338.24 |
| Cd | mg $kg^{-1}$ | 0.14 | 1.31 |
| Pb | mg $kg^{-1}$ | 13.95 | 22.25 |

### 2.2. Experimental Setup

Domestic sewage sludge was collected from a municipal wastewater treatment plant in Kaifeng (where industrial wastewater was separated from domestic wastewater), and aerobically composted for 15 days at 55–60 °C, mixed with peanut shells and maize stalks at a ratio of 10:1:1. A composting agent that included *Bacillus subtilis*, *Aspergillus niger*, and *Sporotrichum thermophile* was inoculated to speed up the process. The properties of the biosolid are listed in Table 1.

Four biosolids treatments were carried out under the same mineral fertilization: (1) CK, which received no biosolids; (2) BS1, which received 15 ton $ha^{-1}$ of biosolids; (3) BS2, which received 30 ton $ha^{-1}$ of biosolids; (4) BS3, which received 45 ton $ha^{-1}$ of biosolids per season. The experiment was carried out using a randomized block design with three replications. Each plot (2 × 2.5 m) was surrounded by a 1 m high concrete wall. Chemical fertilization followed local farmers' practice: Nitrogen was applied at 225 kg $ha^{-1}$ as urea, $P_2O_5$ at 86 kg $ha^{-1}$ as monoammonium phosphate, and $K_2O$ at 113 kg $ha^{-1}$ as potassium chloride per season, respectively. The cropping system was winter wheat and summer maize per year. All fertilizers and biosolids were applied on the soil surface and rotary tilled into an arable layer before crop planting. The irrigation followed local farmers' practice: Three times for winter wheat and one time or no irrigation for summer maize.

### 2.3. Soil and Arthropod Sampling

Before the maize harvest on 1 October 2016 and 2017, three soil quadrats (20 × 20 cm) in each plot were excavated for macrofauna collection by hand sorting. Four soil cores (2 cm diameter and 10 cm depth) were pooled together to collect meso/micro-fauna via the Berlese–Tullgren method. Upon returning from the field, we immediately transferred the soil samples into Berlese–Tullgren funnels and irradiated the samples with 25 W electric bulks for three days. Arthropods were collected into vials containing 80% ethanol. Soil arthropods were identified up to the family level, except Acariformes (order level), according to the "Pictorial Keys to Soil Animals of China" [17] and counted under a microscope. Another 10 soil cores were pooled together for the enzyme activity, microbial biomass, and physicochemical property analyses.

### 2.4. Soil Enzyme Activity

Nine enzyme activities participating in the C (β-glucosidase, β-cellobiohydrolase, and N-acetyl-glucosaminidase), N (urease and arylamidase), P (phosphatase), and S (sulfatase) cycles were analyzed, including phenol oxidase and peroxidase. The activities of six fluorometric enzymes (except urease, phenol oxidase, and peroxidase) were analyzed using the microplate fluorometric protocol [18]. In brief, acetate buffer was added to fresh soil and stirred with a magnetic stirrer to maintain a uniform suspension. Then, the buffer, sample suspension, references, and substrates were dispensed into a 96-well microplate following the order described by DeForest [19]. The fluorescence was quantified using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo) with 365 nm excitation and 450 nm emission filters. For non-fluorometric enzymes, phenol oxidase and peroxidase were measured using L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate in a 96-well microplate [19]. The activities were measured at 450 nm with a spectrophotometer. Urease activity was measured at 578 nm with a spectrophotometer after fresh soil was incubated with urea solution and citrate buffer.

### 2.5. Soil Properties

Soil organic C and total N were measured using dry combustion (Elementar, Elementar UK LTD, South Manchester, UK), total P using the alkali fusion method, soil pH using a pH meter (1/5 water), total K and total heavy metals (Cu, Zn, Cd, Pb, Ni, and Cr) using an HF-$HClO_4$ microwave digester (Mars 6, CEM Corporation, Matthews, NC, USA) and analysis via ICP-MS (Agilent 7600, Santa Clara, CA, USA), soil microbial biomass

using the chloroform fumigation method, and soil water content using the 105 °C oven-drying method.

### *2.6. Statistics*

The Fisher's least significant difference (LSD) test was used to evaluate the differences in soil properties, heavy metal concentrations, and enzyme activity between biosolids treatments after ANOVA showed that the differences in the mean values among the treatments were significant. Arthropod diversity indices, including the Shannon–Wiener and Pielou indices, were calculated to indicate variations in the alpha diversity. Ordination diagrams for the community structure of soil arthropods were created using principal components analysis (PCA) to study variations in the beta diversity. Population data were log-transformed to decrease the variance between samples. Then, the relationship with soil properties was analyzed using redundancy analysis (RDA). Statistical analyses were performed with SigmaPlot 12.0 (Systat Software, Inc., Chicago, CA, USA). Both PCA and RDA were performed with CANOCO 4.5 (Microcomputer Power, Ithaca, NY, USA).

## 3. Results

### *3.1. Soil Properties and Total Heavy Metal Concentrations*

The soil organic C, nutrient contents, microbial biomass C, and water content significantly increased, while the soil pH decreased by 9.9% under biosolid application ($p < 0.05$; Table 2). A greater quantity of biosolids had a much stronger positive or negative (pH) effect on the soil properties.

**Table 2.** Soil properties after five-year biosolid application (mean ± standard deviation).

| Treatments | SOC g·kg$^{-1}$ | TN g·kg$^{-1}$ | MBC mg·kg$^{-1}$ | MBC/SOC mg g$^{-1}$ | Available P mg·kg$^{-1}$ | Available K mg·kg$^{-1}$ | pH | Moisture % |
|---|---|---|---|---|---|---|---|---|
| CK | 5.67 ± 1.04 b | 0.50 ± 0.07 d | 5.43 ± 0.71 b | 1.17 ± 0.18 b | 30.82 ± 4.25 d | 13.37 ± 1.15 d | 9.02 ± 0.16 c | 3.62 ± 0.61 b |
| BS1 | 10.34 ± 0.94 a | 0.96 ± 0.12 c | 15.29 ± 1.65 b | 1.48 ± 0.09 b | 61.10 ± 3.97 c | 30.03 ± 3.78 c | 8.61 ± 0.18 bc | 5.49 ± 1.18 b |
| BS2 | 12.30 ± 2.63 a | 1.24 ± 0.16 b | 60.78 ± 8.24 a | 4.94 ± 0.21 a | 78.34 ± 3.48 b | 48.35 ± 2.90 b | 8.19 ± 0.14 ab | 9.80 ± 0.71 a |
| BS3 | 14.10 ± 4.02 a | 1.52 ± 0.09 a | 71.22 ± 9.63 a | 4.78 ± 0.37 a | 89.66 ± 5.36 a | 63.35 ± 7.63 a | 8.13 ± 0.12 a | 10.97 ± 1.12 a |

Note: Different letters mean significant differences at the 5% level. CK, received no biosolids. BS1, BS2, and BS3: Biosolids applied at a rate of 15, 30, and 45 ton ha$^{-1}$ per season, respectively. SOC, soil organic carbon; TN, total nitrogen; MBC, microbial biomass carbon; P, phosphate; K, potassium.

Although a large number of biosolids were applied every year, the heavy metal concentrations in domestic biosolids were much lower than the loading rate per year permitted by EPA, USA (Table 3). Therefore, the total heavy metal concentrations in the soil did not increase significantly after the five-year biosolid application (Table 3). According to the Class II Standards of Chinese Soil Environmental Quality (GB15618- 1995), the land can be used as an agricultural field. However, the heavy metal concentrations showed an increasing trend under the application of a higher amount of biosolids.

**Table 3.** Total heavy metal concentrations in the soil after five-year biosolid application (mean ± standard deviation).

| | Cr | Ni | Cu | Zn | Cd | Pb |
|---|---|---|---|---|---|---|
| CK (mg·kg$^{-1}$) | 35.92 ± 1.80 a | 14.55 ± 0.36 a | 15.47 ± 0.84 a | 45.39 ± 0.34 a | 0.15 ± 0.02 a | 11.43 ± 2.16 a |
| BS1 (mg·kg$^{-1}$) | 38.02 ± 2.19 a | 14.15 ± 0.39 a | 17.46 ± 0.92 a | 49.58 ± 2.05 a | 0.15 ± 0.016 a | 11.96 ± 0.48 a |
| BS2 (mg·kg$^{-1}$) | 35.22 ± 2.37 a | 13.82 ± 0.79 a | 13.83 ± 1.92 a | 43.19 ± 4.96 a | 0.16 ± 0.04 a | 11.43 ± 2.69 a |
| BS3 (mg·kg$^{-1}$) | 38.31 ± 4.35 a | 15.02 ± 1.64 a | 16.49 ± 1.87 a | 48.91 ± 5.37 a | 0.17 ± 0.02 a | 14.01 ± 2.25 a |
| EPA loading rate * | - | 420 | 75 | 140 | 1.9 | 15 |
| Loading rate of BS3 # | 5.88 | 21 | 9.55 | 19.61 | 0.08 | 1.24 |
| GB15618-1995 + | 250 | 60 | 100 | 300 | 0.60 | 350 |

CK, received no biosolids. BS1, BS2, and BS3: Biosolids applied at a rate of 15, 30, and 45 ton ha$^{-1}$ per season, respectively. Different letters indicate significant differences between treatments (Fisher's LSD test). * EPA-permitted loading rate (kg ha$^{-1}$ y$^{-1}$), §503.13, USA; # loading rate of BS3 under 90 ton/year (two seasons) and a water content of 35% (kg ha$^{-1}$ y$^{-1}$); + Class II standards of Chinese Soil Environmental Quality: If the heavy metal concentrations (mg kg$^{-1}$) in the soils are lower than this standard, the soils can be used in an agricultural field.

The heavy metal concentrations in the grains of maize and wheat showed similar trends to that in soil (Table 4). They accumulated more in the grains under the application of a higher amount of biosolids. However, the concentrations were much lower than those permitted by the Chinese Standards for Quality Control of Agricultural Product.

**Table 4.** Heavy metal concentration in the grain of wheat and maize after five-year biosolid application (mean ± standard deviation).

| | | Cr | Ni | Cu | Zn | Cd | Pb |
|---|---|---|---|---|---|---|---|
| Wheat | CK (mg·kg$^{-1}$) | 0.12 ± 0.01 b | 0.11 ± 0.02 b | 1.47 ± 0.27 b | 10.93 ± 0.52 c | 0.004 ± 0.000 b | 0.01 ± 0.00 b |
| | BS1 (mg·kg$^{-1}$) | 0.15 ± 0.02 a | 0.06 ± 0.00 b | 1.61 ± 0.22 b | 12.00 ± 1.74 bc | 0.004 ± 0.000 ab | 0.02 ± 0.00 b |
| | BS2 (mg·kg$^{-1}$) | 0.16 ± 0.01 a | 0.10 ± 0.01 b | 1.85 ± 0.16 b | 15.26 ± 0.66 ab | 0.004 ± 0.000 ab | 0.03 ± 0.00 a |
| | BS3 (mg·kg$^{-1}$) | 0.19 ± 0.04 a | 0.16 ± 0.01 a | 1.96 ± 0.18 a | 16.49 ± 0.82 a | 0.005 ± 0.000 a | 0.03 ± 0.00 a |
| Maize | CK (mg·kg$^{-1}$) | 0.13 ± 0.03 c | 0.09 ± 0.01 b | 1.14 ± 0.11 b | 13.36 ± 0.90 b | 0.004 ± 0.000 b | 0.01 ± 0.00 a |
| | BS1 (mg·kg$^{-1}$) | 0.17 ± 0.01 bc | 0.17 ± 0.02 a | 1.47 ± 0.17 ab | 17.84 ± 0.05 a | 0.005 ± 0.000 b | 0.01 ± 0.00 a |
| | BS2 (mg·kg$^{-1}$) | 0.23 ± 0.02 a | 0.19 ± 0.01 a | 1.63 ± 0.18 a | 18.62 ± 1.53 a | 0.006 ± 0.001 ab | 0.01 ± 0.00 a |
| | BS3 (mg·kg$^{-1}$) | 0.22 ± 0.02 ab | 0.21 ± 0.02 a | 1.91 ± 0.07 a | 20.15 ± 0.41 a | 0.008 ± 0.001 a | 0.01 ± 0.00 a |
| Standards of Agricultural Product * | | 1 | 0.4 | 10 | 50 | 0.1 | 0.2 |

CK, received no biosolids. BS1, BS2, and BS3: Biosolids applied at a rate of 15, 30, and 45 ton ha$^{-1}$ per season, respectively. Different letters indicate significant differences between treatments (Fisher's LSD test). * Chinese Standards for Quality Control of Agricultural Product.

### *3.2. Soil Arthropod Diversity and Community Structure*

Individual arthropods collected under biosolid application changed from 0 to 2560 per square meter (Table 5), and these arthropods belonged to 6–11 families in 2016 and 10–12 families in 2017. Most of the arthropods were of the meso/micro size. Among the meso/micro size, Prostigmata, Mesostigmata, and Oribatida were the dominant groups at the family level, and contributed 27%, 27%, and 24% to the total soil arthropods, respectively. The total number of individual arthropods in the arable layer increased under biosolid application compared with CK (Table 5). There were no effects of biosolid application on arthropod diversity (Shannon–Weaver index), evenness (Pielou index), species richness, or dominant groups (Table 5). However, the arthropod community structure was altered by the long-term application of a high amount of biosolids according to PCA (Figure 1, Left). The RDA ordination diagrams show that the arthropod taxa were significantly related to the soil properties (soil organic C, total N, microbial biomass C, water content, and pH) and cadmium concentration (Figure 1, Right).

### *3.3. Soil Enzyme Activity*

Soil enzymes are proxies for microbial activity and ecosystem function. Six enzyme activities, related to the C, N, and P cycles, increased by 29.45%~122.70%, 57.17%~116.86%, and 1.53%~10.61%, respectively ($p < 0.05$), compared with CK. Meanwhile, the activity of phenol oxidase and peroxidase, which are produced under stress conditions, decreased by 28.51%~35.40% and 31.09%~43.98%, respectively, compared with CK ($p < 0.05$) (Figure 2).

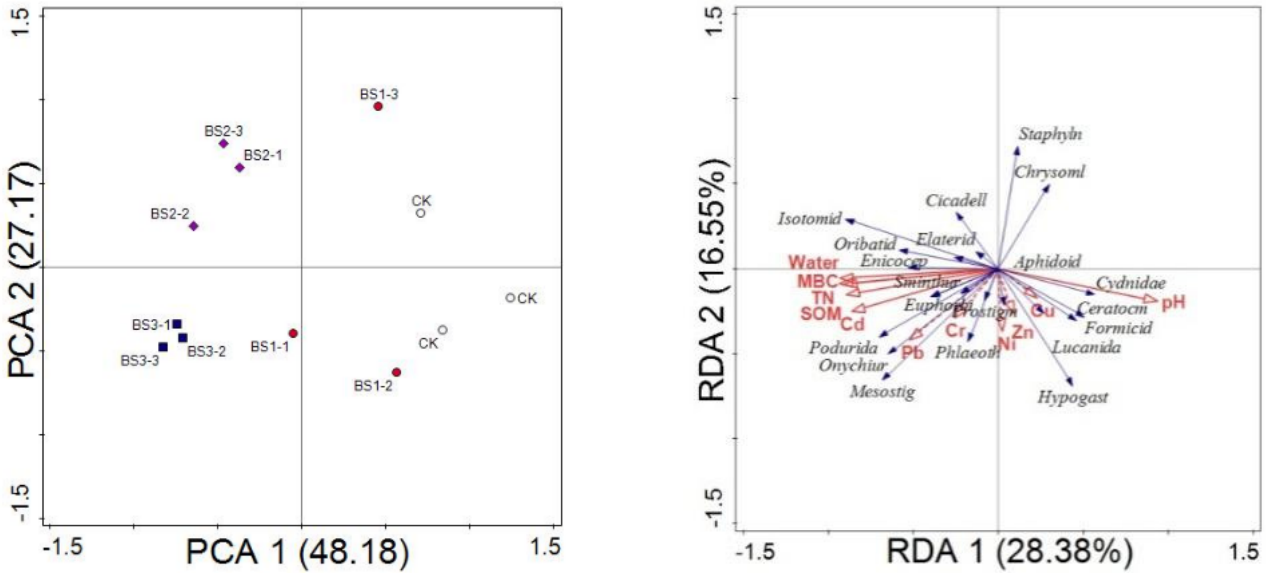


**Figure 1.** Principal component analysis ordination diagram for soil arthropod groups under different amounts of applied biosolids (**Left**). CK, without biosolids. BS1, BS2, and BS3: Biosolids applied at a rate of 15, 30, and 45 ton ha$^{-1}$ per season, respectively. Redundancy analysis of the arthropod groups with soil properties and total heavy metal concentrations (**Right**). SOM, soil organic matter; TN, total nitrogen; MBC, microbial biomass carbon. Dashed lines indicate non-significance.

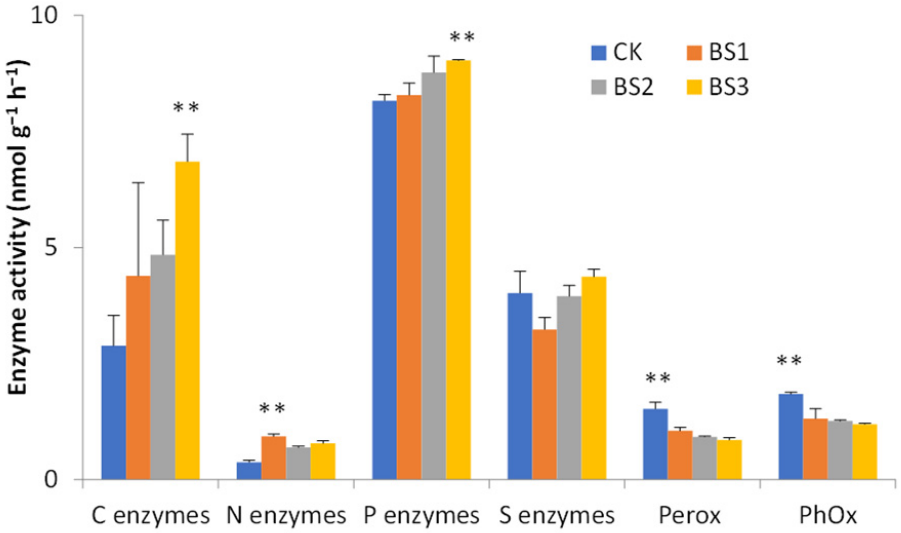


**Figure 2.** Soil enzyme activity under five-year biosolid application. CK, without biosolids. BS1, BS2, and BS3 refer to biosolids inputs of 15, 30, and 45 ton ha$^{-1}$ per season. The double asterisks above the columns indicate significant differences between treatments (Fisher's LSD test).

**Table 5.** Mean densities and mean numbers of the taxonomic groups of soil macro- and meso/micro-arthropods in samples from biosolids treatments (mean ± standard deviation).

| Order | Family | Size | Guild | 2016 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | CK | BS1 | BS2 | BS3 | CK | BS1 | BS2 | BS3 |
| Acariformes | Prostigmata | Meso/micro | S | **480 ± 418** | **1024 ± 1531** | **928 ± 454** | **1828 ± 1320** | **544 ± 111** | **896 ± 653** | **928 ± 474** | **832 ± 985** |
| | Mesostigmata | Meso/micro | S | **320 ± 277** | **1216 ± 770** | **1216 ± 529** | **2560 ± 617** | **608 ± 443** | 192 ± 333 | 384 ± 418 | **1088 ± 617** |
| | Oribatida | Meso/micro | S | **192 ± 254** | 256 ± 200 | **416 ± 388** | 320 ± 293 | **384 ± 96** | **736 ± 733** | **2208 ± 1854** | **2240 ± 2134** |
| Diplura | Japygidae | Meso/micro | O | | | | | | | | 32 ± 55 |
| Collembola | Onychiuridae | Meso/micro | Ph | | 128 ± 222 | 96 ± 96 | 192 ± 96 | 32 ± 55 | **384 ± 584** | **576 ± 584** | 480 ± 384 |
| | Hypogastruridae | Meso/micro | F | 96 ± 0 | 96 ± 96 | | 32 ± 55 | 288 ± 144 | 192 ± 254 | | 128 ± 111 |
| | Sminthuridae | Meso/micro | Ph | | | | | | | | 32 ± 55 |
| | Isotomidae | Meso/micro | F | | | 32 ± 55 | 128 ± 147 | 32 ± 55 | 64 ± 55 | **672 ± 692** | **1088 ± 55** |
| | Entomobryidae | Meso/micro | F | | | | | | | | |
| | Poduridae | Meso/micro | O | | | 64 ± 111 | 64 ± 111 | | 32 ± 55 | | 288 ± 166 |
| Coleoptera | Staphylinidae | Macro | S | 64 ± 111 | | | 0 | | 32 ± 55 | 32 ± 55 | |
| | Scarabaeidae | Macro | F | | | | 32 ± 55 | | | | |
| | Elateridae | Macro | S | | | 32 ± 55 | | | | | |
| | Chrysomelidae | Macro | Pr | 32 ± 55 | | | | | | | |
| Hymenoptera | Formicidae | Macro | O | 32 ± 55 | | 32 ± 55 | | 32 ± 55 | | | |
| Hemiptera | Ceratocombidae | Macro | Ph | 32 ± 55 | | | | 64 ± 111 | | 32 ± 55 | |
| | Enicocephalidae | Macro | Ph | | | | | | | 32 ± 55 | |
| | Cydnidae | Macro | Ph | 32 ± 55 | | | | | | | |
| | Cicadellidae | Macro | Ph | | | 32 ± 55 | | | | | |
| Diptera | Phoridae | Macro | S | | | | | | | | 32 ± 55 |
| Coleoptera larvae | Lucanidae | Macro | Ph | | 32 ± 55 | | | | | | |
| | Elateridae | Macro | Ph | | | 96 ± 96 | 64 ± 111 | | | | 32 ± 55 |
| Thysanoptera | Phlaeothripidae | Macro | Ph | 32 ± 55 | | | | | | 32 ± 55 | 64 ± 111 |
| Homoptera | Aphidoidea | Macro | Ph | | | 64 ± 111 | | 32 ± 55 | | | |
| | Euphorbiaceae | Macro | Ph | | | | 32 ± 55 | | | | |
| Total macro-arthropods | | | | 224 ± 111 a | 32 ± 55 a | 256 ± 111 a | 128 ± 147 a | 128 ± 147 a | 32 ± 55 a | 128 ± 222 a | 128 ± 111 a |
| Total meso/micro-arthropods | | | | 1088 ± 454 b | 2720 ± 2064 ab | 2752 ± 388 ab | 5120 ± 1832 a | 1888 ± 839 a | 2496 ± 1414 a | 4768 ± 2274 a | 6208 ± 3354 a |
| Total abundance of soil arthropod | | | | 1312 ± 388 b | 2752 ± 2117 ab | 3008 ± 388 ab | 5248 ± 1707 a | 2016 ± 926 a | 2528 ±1441 a | 4896 ± 2164 a | 6336 ± 3412 a |
| Species richness | | | | 10 | 6 | 11 | 10 | 9 | 8 | 9 | 12 |
| Species diversity (Shannon–Weaver index) | | | | 1.28 ± 0.19 a | 0.89 ± 0.48 a | 1.34 ± 0.45 a | 1.20 ± 0.26 a | 1.45 ± 0.27 a | 1.52 ± 0.45 a | 1.40 ± 0.22 a | 1.68 ± 0.13 a |
| Species evenness (Pielou index) | | | | 0.76 ± 0.10 a | 0.66 ± 0.31 a | 0.73 ± 0.08 a | 0.66 ± 0.08 a | 0.86 ± 0.04 a | 0.82 ± 0.17 a | 0.77 ± 0.09 a | 0.81 ± 0.08 a |

CK, without biosolids. BS1, BS2, and BS3: Biosolids applied at a rate of 15, 30, and 45 ton $ha^{-1}$ per season, respectively. In the Guild column: S, saprozoic; O, omnivores; Ph, phytophage; F, fungivore forms; Pr, predators. ANOVA analysis showed that the differences in the mean values among the treatments were not significant for species diversity (Shannon–Weaver index; $F = 0.893$ and $p = 0.485$) and species evenness (Pielou index; $F = 0.893$ and $p = 0.485$). The bold values refer to species accounting for more than 1% of the total arthropods in each treatment. Different letters indicate significant differences between treatments at $p<0.05$ level (Fisher's LSD test).

## 4. Discussion

### *4.1. Response of Soil Arthropods to Biosolid Application*

Five-year biosolid application resulted in a significant increase in soil organic C, total N, and water content. Heavy metals also accumulated within the soil and crop grains, but the increment was not significant compared with CK. Biosolid application significantly increased the abundance of arthropods but had no influence on species diversity or taxonomic richness. This is similar to the results of manure application in cropland or litter manipulation in forest systems [15,20]. Meanwhile, the arthropod community composition, which is significantly related to organic matter decomposition [21], was altered due to differing responses of several taxa to biosolid application or litter control (Figure 1; [15]). RDA analysis showed that soil chemical properties were the main reason for the change in community composition. If the variation in the arthropod community is related to heavy metal contamination, the decomposition rate of organic material will decrease [22]. However, our results showed that C-, N-, and P-related enzyme activity increased significantly under biosolid application. Therefore, we can infer that the high organic matter in biosolids may strongly adsorb metals, and thus decrease the detrimental effects of heavy metals on fauna and soil function. This is supported by Hout et al. [6,23]; they found that despite soil being heavily polluted by Fe, Pb, Zn, Cd, and Cu, mesofauna communities were well-developed in a settling pond of ironworks under forest cover. Furthermore, the abundance, richness, and diversity of Collembola (the dominant group in the pond) were comparable to those observed in forested ecosystems [24]. The abundance of macrofauna in the pond was also comparable to that in natural forests [25]. Further analysis showed that the heavy metal concentrations had no significant effect on Collembolan community composition, but influenced macroinvertebrates [6].

The impact of heavy metals on soil biota is strongly related to their bioavailability [6], which depends on the soil properties and sensitivity [26], adaptation [27], and avoidance strategy [28] of the species. This means that under biosolid application, soil fauna, on the one hand, may adapt to or avoid those sites where heavy metals accumulate slowly, and on the other hand, the high organic matter content in biosolids can decrease the mobility and bioavailability of metals by integration of said metals into organo-mineral associations [6]. Therefore, biosolid application not only increases soil fertility but also decreases the mobility and bioavailability of metals. In addition, the very low concentration of heavy metals in domestic biosolids compared with the Standards published in China and the USA or references in metal contamination experiments [29] may be one of the reasons for the lack of a detrimental effect of heavy metals on arthropod species and abundance.

### *4.2. Relationship between Arthropods and Soil Properties*

Our results showed that there were relatively small species of fauna in degraded sandy soil, and the dominant species (Acariformes) increased significantly under biosolid application. This is supported by Swift [30–32], who concluded that communities of soil animals in agricultural soils are characterized by small species with high reproductive rates under different tillage, fertilizer, and irrigation/rain-feed conditions. Furthermore, species richness and diversity index (Pielou index) are positively related to soil organic C and negatively related to soil pH [32]. However, in this study, the species richness and diversity index (Pielou index) did not change under biosolid application (Table 5), although the soil organic C content increased 2.6 times and the soil pH decreased by 9.9%. This is in agreement with the results obtained from litter manipulation experiments, in which the removal or double input of litter had no effect on arthropod richness and diversity in a tropical rainforest [15].

According to RDA, we found that the main arthropod abundance and community structure were controlled by soil organic C, total N, and water content. This result is comparable to that of Xu et al. [2,20,23,31], who proposed that soil moisture and/or soil organic C are the key factors affecting the abundance and composition of soil fauna. However, arthropod species differed in their responses to chemical factors; for example, six

out of the seven main arthropod taxa were positively related to soil nutrient conditions, while only Hypogastruridae was positively related to pH (Figure 1). This result is similar to that of Marian et al. [2,21], who found an abundance of some fauna (e.g., Oribatida) positively related to soil organic C, while other faunae (e.g., Collembola) were strongly correlated with the sand content, which is negatively correlated with soil organic C.

### *4.3. Fauna as an Indicator of Soil Quality Changes*

The abundance and composition of fauna are sensitive to land management and are involved in many soil functions; therefore, they are thought to be useful indicators of soil quality changes [33]. However, different species of soil fauna are used as indicators of soil quality. In agricultural land (upland, paddy, and vegetable land), the Nemata and earthworm populations are used as indicators of soil quality due to their ability to regulate microorganism density and their interaction with the microorganisms, which can increase soil organic C [34,35]. However, under biosolid application, the abundance of Nemata (nematodes, enchytraeids, and rotifers) does not positively relate to soil organic C, and soil types have greater effects on total Nemata [10]. Soil invertebrates, which are abundant and relatively easy to sample, are used as an indicator of soil quality [23]. After comparing three indicators (ratio of Acarina/Collembola, invertebrates' diversity and QBS), Santorufo et al. [23] proposed that QBS, which is mainly based on the presence/absence of micro-arthropod groups (does not include abundance) and their adaptation of soil environment, is most appropriate for soil quality assessment in metal-contaminated soil. Soil macrofaunae—which are relatively simple to measure, ubiquitous, and familiar to farmers—are also used as an early indicator of soil quality changes [36]. In purple cropland, soil fauna community indices (density, group diversity, and total individuals of soil fauna community) and the number of individuals of Nemata, Lumbricida, Isotomidae, and Oribatida have been suggested as indicators of changes in soil fertility due to their close relationship with soil organic matter [20]. Yan et al. [33] found that an index of soil fauna communities (FAI, including abundance, diversity of the community, and functional traits of the community) can be used to evaluate changes in soil fertility and soil quality. In sandy loam soil, we found that the abundance of dominant arthropod species was positively related to soil organic C, but diversity was not associated with soil organic C under biosolid application. Thus, simple measures of abundance or diversity are not informative for soil quality changes in agricultural fields. Therefore, further studies are needed to determine which soil fauna and parameters related to soil fauna should be used as indicators of soil quality changes.

## 5. Conclusions

Five-year domestic biosolid application increased the soil organic matter and water content by more than two times in degraded sandy soil. At the same time, heavy metals accumulated in the soil and grains of summer maize and winter wheat. However, heavy metal concentrations were much lower than the standards for agricultural fields and grain products. In addition, the microbial enzyme activity controlling C, N, and P cycling increased significantly under biosolid application. These results suggest that long-term biosolid application with limited heavy metal concentrations does not have a detrimental effect on soil arthropods and soil function.

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