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Assessment of Soil Health in Urban Agriculture: Soil Enzymes and Microbial Properties

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Abstract: Urban agriculture has been recently highlighted with the increased importance for recreation in modern society; however, soil quality and public health may not be guaranteed because of continuous exposure to various pollutants. The objective of this study was to evaluate the soil quality of urban agriculture by soil microbial assessments. Two independent variables, organic and inorganic fertilizers, were considered. The activities of soil enzymes including dehydrogenase, β-glucosidase, arylsulfatase, urease, alkaline and acid phosphatases were used as indicators of important microbial mediated functions and the soil chemical properties were measured in the soils applied with organic or inorganic fertilizer for 10 years. Fatty acid methyl ester analysis was applied to determine the soil microbial community composition. Relatively higher microbial community richness and enzyme activities were found in the organic fertilizers applied soils as compared to the inorganic fertilizers applied soils. Principal component analysis explained the positive influence of organic fertilizers on the microbial community. The application of organic fertilizers can be a better alternative compared to inorganic fertilizers for the long-term health and security of urban agriculture.

Keywords: urban agriculture; soil quality; soil enzyme; organic fertilizer; FAME

1. Introduction

The term urbanization refers to population increase in a certain area due to the movement of rural population to industrialized areas [1]. The World Health Organization estimated that 54% of the world population is concentrated in urban areas [2]. As urbanization intensified, the soils in urbanized areas suffered severe pollution problems due to residential and/or industrial and/or commercial activities. Urban soils show high unpredictable variability because of the continuous inflow of foreign materials (i.e., wastes and waste water releasing from industries and households,

atmospheric depositions, vehicle smokes, constructions, etc.) and soil disturbance, which leads to substantial changes in structures and functions of urban soils such as porosity, bulk density, acidity, water and nutrient cycling systems, etc. [3–5].

Urban soils may not be appropriate for agriculture because of improper soil physiochemical and biological properties such as high bulk density, low organic carbon (OC) and nutrient contents, low biota population or activity, and high possibility of containing high amounts of toxic pollutants (organic and inorganic) [5]. Nonetheless, urban agriculture produces various types of food which account for about 15% of the world's total consumption of food [6]. Hence, to ensure public health and food security, the assessment of soil quality in urban agricultural lands is necessary. Soil quality or soil health has been defined by United States Department of Agriculture (USDA) [7] as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans", and it is a result of balanced soil biological, chemical and physical properties. Urban soils are considered as low quality soil due to the subjecting of high land conversion practices as explained previously [8]. Both crop production and animal husbandry practicing in urban lands are considered urban agriculture [9]. In more details, "Urban agriculture is an activity that produces, processes, and markets food and other products, on land and water in urban and peri-urban areas, applying intensive production methods, and (re)using natural resources and urban wastes, to yield a diversity of crops and livestock. In a broad sense, it encompasses the entire area in which a city's sphere of influence (social, ecological, or economic) comes to bear daily or directly on its population" [10]. Here, we have focused only the crop producing urban agricultural lands, and refer to this as urban agriculture from this point onwards.

One of the most sensitive indicators for assessing the soil quality is identifying soil biological parameter such as microbial communities and activities because of its rapid reactivity or alteration against significant environmental changes [11,12]. The microbial biomass carbon, soil respiration, potential nitrogen (N) mineralization capacity, ATP content, soil fatty acid profiles, DNA characterization, and soil enzyme activities are widely-utilized biological indicators [13]. The extracellular enzymes produced by soil microorganisms and plant roots mediate organic matter decomposition and nutrient cycling in soil [14,15]. Organic fertilizers often enhance the activities of numerous enzymes and boost the soil biogeochemical cycles [16,17]. Applying inorganic fertilizers can also modify soil nutrient cycles for short-term and long-term periods [18]. Previous studies also have reported noticeable changes in soil microbial communities with respect to organic or inorganic fertilizer application [19,20]. Most organic fertilizers tend to increase the total soil microbial biomass once incorporated via the enhancement of specific groups of microbes inherent in a particular soil. However, the response of the microbial community structure to organic fertilizers depends on the variations of carbon (C) or quality of the organic fertilizers [21]. Microbial community alterations in soils after the addition of inorganic fertilizers also have been reported [22,23]. Reductions in dinitrogen fixers and mycorrhizae were observed following the applications of inorganic N and phosphorus (P) fertilizers, respectively [24,25]. However, inorganic N fertilization (e.g., urea) enhanced the organic matter turnover in soils having a high C/N ratio through the enhancement of microbial communities [26]. Moreover, the inorganic fertilizers which provide N, P and potassium (K) influence the soil enzyme activities [27,28]. Both enhancement and decline of microbial communities following inorganic fertilizer application have been observed [22,29]. However, urban agricultural soils receive least attention in reported studies [8]. As urban agricultural soils produced considerable amount of foods, the assessment of long-term impact of organic and inorganic fertilizer application on urban agricultural soils is important.

Understanding the microbial community composition and its physiological capacity is very important to understand the quality of urban agricultural soils [30,31]. However, the microbial community and their activity have barely been investigated as the assessment tools for urban agricultural soils, exposed to organic or inorganic fertilizer. This study was conducted to determine the impacts of long-term applications (i.e., >10 years) of organic and inorganic fertilizers on the

enzyme activity and microbial communities which may influence nutrient availability in the urban agricultural soils.

2. Materials and Methods

2.1. Field Sites

Seoul, the capital of the Republic of Korea, is one of the most developed, highly industrialized and densely populated cities in the world. It occupies an area of 605.25 km^2 with a population of over 10 million and a population density of ~16,700 persons km^{-2} [32]. The sixteen field sites near Seoul were selected as typical urban agriculture areas, and considered the type of fertilizers applied (i.e., organic fertilizer = 8 sites and inorganic fertilizer = 8 sites) when selecting the lands.

The field sites ranged from 12.6 to 14.7 m^2 and had the application of organic or inorganic fertilizers for >10 years. All soils were sandy loam textured, and cultivated with leafy vegetables (i.e., lettuce and Chinese cabbage) during the past 10 years (Table 1). The application amounts of N, P and K inorganic fertilizers were in the range of 2.4–2.7 $kg{\cdot}m^{-2}{\cdot}year^{-1}$, 0–12.6 $kg{\cdot}m^{-2}{\cdot}year^{-1}$, and 0–0.6 $kg{\cdot}m^{-2}{\cdot}year^{-1}$, respectively (Table 1). Commercially available plant material based composts in Seoul were used in organic fertilizer applied soils (9.4–10.5 $kg{\cdot}m^{-2}{\cdot}year^{-1}$).

2.2. Soil Sampling and Chemical Characterization

Topsoils (0–15 cm) from the selected 16 field sites were sampled due to the cultivation of shallow rooted crops. Random sampling was practiced, and (20 random samples per site) mixed well to make a composite sample per site (composite samples = 16). The soil samples were categorized into two groups of soils applied with organic (n = 8) and inorganic (n = 8) fertilizers over 10 years. Each eight sampled soils in one group were considered as pseudoreplicates as similar fertilizers and land management practices were applied during the past 10 years [33]. The sub-samples were air-dried and sieved to <2 mm for chemical and enzyme analyses, and other sub-samples were stored at −18 °C for fatty acid methyl ester (FAME) analysis.

Soil pH was measured in a ratio of 1:5 of soil to deionized water suspension using a digital pH meter (WTW inoLab pH7110, Darmstadt, Germany). The exchangeable cations (Ca^{2+}, K^+, Mg^{2+}, and Na^+) were extracted with 1 M ammonium acetate, pH 7 and then analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES, GBC Integra XL, Analytical Solutions Australia, Sydney, NSW, Australia). Soil organic matter was determined using the modified Walkey and Black method and the Tyurin titrimetric method with acid-wet oxidation and dichromate [34]. Soil P_2O_5 content was determined by the Lancaster method [35]. Kjeldahl distillation was used to analyze the available N [36].

2.3. Soil Enzymes

Six soil enzymes involved in the biogeochemical cycles of C (dehydrogenase, β-glucosidase), S (arylsulfatase), N (urease) and P (alkaline and acid phosphatases) were determined [37]. Soil dehydrogenase activity was measured using 2,3,5-triphenyltetrazollium chloride as the electron acceptor according to the method defined by Casida et al. [38]. Phosphatase, arylsulfatase and β-glucosidase activities were determined as described by Tabatabai [39], using *p*-nitrophenyl phosphate, *p*-nitrophenyl sulfate and *p*-nitrophenyl-β-D-glucopyranoside as substrates, respectively. Alkaline and acid phosphatase activities were determined at a pH of 11 and 6.5, respectively. Urease activity was measured using urea as the substrate [40].

Table 1. Urban agricultural lands used for the study.

Organic Fertilizer Applied Soils								
Site No.	**Texture**	**Land Preparation**	**Cultivations Period**	**Crop Cycles**	**Land Size (m^2)**	**Crops**	**Amount of Fertilizer Applied ($kg \cdot m^{-2} \cdot year^{-1}$)**	
							Compost (Plant Material Based)	
1	Sandy loam	Hand plough	April–October	4–5	12.8	Lettuce	10.2	
2	Sandy loam	Hand plough	April–October	6	12.9	Lettuce	10.5	
3	Sandy loam	Hand plough	April–October	5	14.7	Lettuce	9.8	
4	Sandy loam	Hand plough	April–October	6	13.9	Lettuce	9.4	
5	Sandy loam	Hand plough	April–October	6	12.3	Lettuce, Chinese cabbage	10.0	
6	Sandy loam	Hand plough	April–October	5	12.1	Lettuce	10.0	
7	Sandy loam	Hand plough	April–October	5	13.0	Chinese cabbage	10.2	
8	Sandy loam	Hand plough	April–October	5	13.6	Chinese cabbage	10.0	
Inorganic Fertilizer Applied Soils								
Site No.	**Texture**	**Land Preparation**	**Cultivations Period**	**Crop Cycles**	**Land Size (m^2)**	**Crops**	**Amount of Fertilizer Applied ($kg \cdot m^{-2} \cdot year^{-1}$)**	
							N (Urea)	**P (Superphosphate)**	**K (Potassium Sulfate)**
9	Sandy loam	Hand plough	April–October	5	14.2	Chinese cabbage	2.4	10.8	0.5
10	Sandy loam	Hand plough	April–October	5	12.9	Lettuce, Chinese cabbage	2.7	11.0	0.4
11	Sandy loam	Hand plough	April–October	5–6	13.1	Lettuce	2.5	12.6	0.0
12	Sandy loam	Hand plough	April–October	5	14.1	Lettuce	2.5	12.0	0.0
13	Sandy loam	Hand plough	April–October	5	12.8	Chinese cabbage	2.5	12.0	0.6
14	Sandy loam	Hand plough	April–October	6	14.6	Lettuce	2.4	11.0	0.0
15	Sandy loam	Hand plough	April–October	4–5	14.5	Lettuce	2.6	0	0.0
16	Sandy loam	Hand plough	April–October	5	14.2	Lettuce	2.7	12.4	0.0

2.4. Fatty Acid Methyl Ester Analysis and Identification of Microbial Fatty Acid Biomarkers

Microbial fatty acids were extracted from the soils by saponification and methylation to form FAMEs, as explained by Schutter and Dick [41]. The method involves using 3 g of <2 mm sieved freeze-dried soil added to a glass centrifuge tube and adding of 15 mL of 0.2 M KOH in methanol to release the lipids from the microbial membranes. The tubes were vortexed for 20 s and incubated in a water bath at 37 °C for 1 h. Then the samples were vortexed for 10 s at 10 min intervals to facilitate the liberation of the FAMEs followed by methylation for 1 h, and the samples were neutralized with 1.0 M acetic acid. Ten milliliters of HPLC-grade hexane were used to partition FAMEs into the organic phase. Partitioning was facilitated by vortexing for 60 s and centrifuging at 330 rpm for 20 min. Then, a 5 mL aliquot from the hexane layer was pipetted into a clean glass screw-top test tube. The extracted fatty acids were analyzed using a gas chromatograph (Agilent 6890 GC, Santa Clara, CA, USA) equipped with a flame ionization detector (with a MIDI Sherlocks microbial identification system; Microbial ID, Ins., Newark, NJ, USA). Schutter and Dick [41] explained the fatty acid nomenclature used to identify the fatty acids separated in the chromatogram. The suffixes "c" and "t" denote the *cis* and *trans* conformations, the prefixes "i" and "a" denote *iso*- and *anteiso*-branched fatty acids, and "*cy*" and "*Me*" denote *cyclopropane* and *methyl groups*, respectively.

Microbial fatty acid biomarkers identification was done based on Waldrop et al. [42]; gram-positive bacteria, a15:0, i15:0, i16:0, a17:0 and cy17:0; gram-negative bacteria, cy19:0 ω8c, sum in feature 3 (16:1ω7c/16:1ω6), sum in feature 5 (a18:0/18:2ω6,9c) and sum in feature 8 (18:1ω7c); actinomycetes, 10Me18:0; fungi, 18:1ω9c and arbuscular mycorrhizal fungi, 16:1ω5c. The relative abundance of each FAME biomarker or specific microbial group in the entire microbial community was calculated by the ratio of an amount of the FAME biomarker and the total amount of FAMEs [18]. The specific microbial groups (i.e., gram-positive and gram-negative bacteria, actinomycetes, fungi and arbuscular mycorrhizal fungi) were identified based on the detected fatty acids in the FAME analysis. The fatty acid biomarkers were also used to evaluate the diversity within the each microbial group in two treatments (i.e., organic fertilizer and inorganic fertilizer).

2.5. Statistics

The *t*-test was used to compare the effects of organic and inorganic fertilizers on soil microbial and chemical parameters. Pearson's correlation analysis was used to show correlations for the soil enzymes, FAMEs and soil chemical properties, and SAS statistical software (SAS Institute, Cary, NC, USA) was used. A principal component analysis (PCA) was performed to evaluate the behavior of the microbial community in response to the independent variables, organic and inorganic fertilizers using the R software [43–45].

3. Results and Discussion

3.1. Soil Chemical Properties

Soil organic matter content and all nutrients except Mg were significantly higher in the soils applied with organic fertilizers ($p < 0.05$; Table 2); however, no difference was found in Mg contents between the soils treated with organic and inorganic fertilizers ($p < 0.05$). Results showed that the organic fertilizers maintained the soil pH (i.e., 6.9) at an optimum level for soil nutrients availability, but not the inorganic fertilizers (i.e., 5.2). Most of the soil nutrients are available in the soils having a slightly acidic pH of 6.5 to a slightly alkaline pH of 7.5 [46]. Therefore, the application of organic fertilizer might be more beneficial to maintain the soil pH at a desirable level for soil nutrient availability. The application of inorganic fertilizers has been known to provide the readily available soil nutrients [47,48]. Contrarily, the long-term application of inorganic fertilizer reduced the soil nutrients availability in the evaluated urban agricultural soils (Table 2). This may be due to the transformation of available forms of nutrients into unavailable forms by the soil acidity, and the poor nutrients retention corresponding to the low organic matter content in inorganic fertilizer applied

soils [49]. The supplement of readily available nutrients (i.e., inorganic fertilizers) for a long-term tends to reduce the organic matter content in soil which causes in reduction of cation exchangeable sites. Therefore, soils ability to retain nutrients for plant growth get reduces and application of inorganic fertilizer in very short-terms is required to maintain the crop productivity [49]. Jiang et al. [50] also reported a reduction of nutrient retention ability and crop production with respect to long-term application of inorganic fertilizers in winter wheat–maize rotation systems compare to the application of organic fertilizers.

Table 2. Chemical properties for the urban soils applied with organic and inorganic fertilizers over 10 years. Data are presented as mean ± standard deviation.

Soil Property	Organic Fertilizer	Inorganic Fertilizer
pH (1:5 soil:deionized water)	6.7 ± 0.2	5.2 ± 0.1
EC ($dS \cdot m^{-1}$)	0.7 ± 0.2 *	0.3 ± 0.1
Organic matter ($g \cdot kg^{-1}$)	54 ± 14 *	17 ± 6
Ca^{2+} ($cmol \cdot kg^{-1}$)	10.9 ± 1.4 *	8.6 ± 1.2
K^{+} ($cmol \cdot kg^{-1}$)	1.6 ± 0.2 *	0.6 ± 0.0
Mg^{2+} ($cmol \cdot kg^{-1}$)	2.6 ± 0.3	2.6 ± 0.0
Na^{+} ($cmol \cdot kg^{-1}$)	0.7 ± 0.0 *	0.4 ± 0.0
P_2O_5 ($mg \cdot kg^{-1}$)	1831 ± 16 *	372 ± 8
NO_3-N ($mg \cdot kg^{-1}$)	96 ± 27 *	15 ± 12

Notes: * Indicates significant difference between organic and inorganic fertilizer at $p < 0.05$.

Organic fertilizer applied urban agricultural soils showed significantly high amount of Ca^{2+}, K^{+}, Mg^{2+}, P_2O_5, and NO_3-N compared to the inorganic fertilizer applied soils (Table 2). This may be due to the high nutrient retention ability of organic fertilizer applied soils corresponding to the high organic matter content (i.e., 54 $g \cdot kg^{-1}$) than that of in inorganic fertilizer applied soils [50]. Organic fertilizer applied soils showed sufficient K^{+}, and Mg^{2+} (Sufficient range, K: 0.4–0.6 $cmol \cdot kg^{-1}$, Mg: 0.5–2.5 $cmol \cdot kg^{-1}$) contents for plant growth; however, it was below the sufficient level in inorganic fertilizer applied soils [51]. Calcium does not show any deficiency in alkaline soils, but it can be deficient in acidic soils [49]. Hence, there is a risk of Ca deficiency in inorganic fertilizer applied soils due to the soil acidity. Organic fertilizer applied soils illustrated comparatively high amount of P than the inorganic fertilizer applied soils, however, the level of P in both soils was not in the sufficient range (Sufficient range, P: 30–50 $mg \cdot kg^{-1}$; P_2O_5: 4258.3–7097.2 $mg \cdot kg^{-1}$) [51]. Phosphorus is one of the limiting nutrients in both acidic and alkaline soils due to the fixation by the soil minerals [49]. The NO_3-N content in organic fertilizer applied soils was near to the sufficient level (i.e., 95.8 ± 27.4 $mg \cdot kg^{-1}$), but it was deficient (i.e., 15.4 ± 11.6 $mg \cdot kg^{-1}$) in inorganic fertilizer applied soils (Sufficient range NO_3-N: 100–250 $mg \cdot kg^{-1}$) [52]. Hence, when consider the plant nutrient availability organic fertilizer applied soils was in a better condition compare to the inorganic fertilizer applied soils.

3.2. Soil Microbial Community Structure

All microbial groups including gram-positive and gram-negative bacteria, actinomycetes, fungi and arbuscular mycorrhizal fungi (AMF) had significantly higher ($p < 0.05$) populations in the soils had organic fertilizers applied compared to the soils with inorganic fertilizers (Figure 1). The gram-positive bacterial markers predominately consist of branched fatty acids and a fatty acid with a cyclopropane group. The i16:0 and cy17:0 gram-positive bacterial markers were found at significantly higher ($p < 0.05$) levels in the soils with organic fertilizers than in the soils with inorganic fertilizers (Table 3). The gram-negative bacterial biomarkers showed no distinguishing pattern in response to both fertilizers. The AMF biomarker 16:1ω5c also did not respond to both fertilizers. However, the actinomycetes biomarker 10Me18:0 significantly increased ($p < 0.05$) in the soils with the addition of organic fertilizer as compared to the soils with that of inorganic fertilizer. The sum in the feature 5 (18:0 ante/18:2ω6,9c) fungal biomarker was also discernibly higher ($p < 0.05$) in the soils with organic fertilizers.

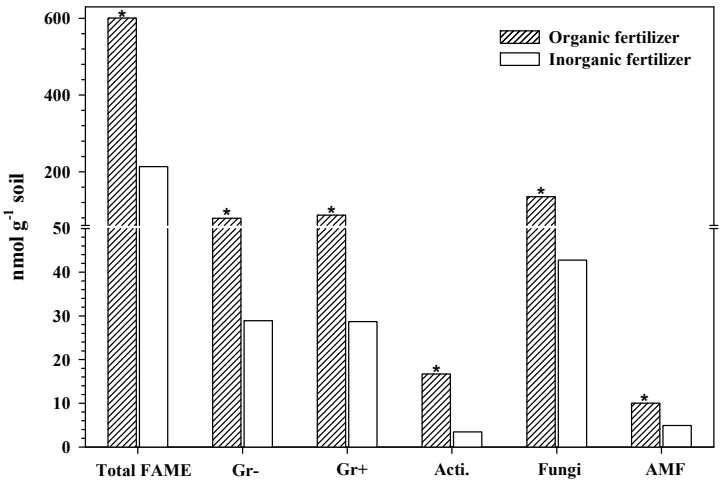

Figure 1. Microbial community composition of the urban soils applied with organic and inorganic fertilizers over 10 years. Gr-, Gr+, Acti. and AMF are gram-negative bacteria, gram-positive bacteria, actinomycetes, and arbuscular mycorrhizal fungi, respectively. Data are presented as mean values (Organic fertilizer = eight sites, inorganic fertilizer = eight sites). Stars above bars indicate the significant difference at $p < 0.05$.

The collective fatty acid biomarkers of each microbial group illustrated a shift of the microbial community with the additions of fertilizers (Table 3). The amounts of gram-positive and gram-negative bacteria, actinomycetes, fungi and AMF were significantly enhanced by the organic fertilizers when compared to the inorganic fertilizers. Moreover, the PCA trends were clear that the addition of organic fertilizers noticeably altered the richness of the microbial community in the soils and positively influenced different microbial groups' growth (Figure 2). Many studies reported that the addition of organic fertilizers can stimulate total microbial community by increasing a level of labile organic matter [53–56]. Nevertheless, it may not be easy to discover the exact quantity and composition of the organic fertilizers which could increase the soil microbial community [57].

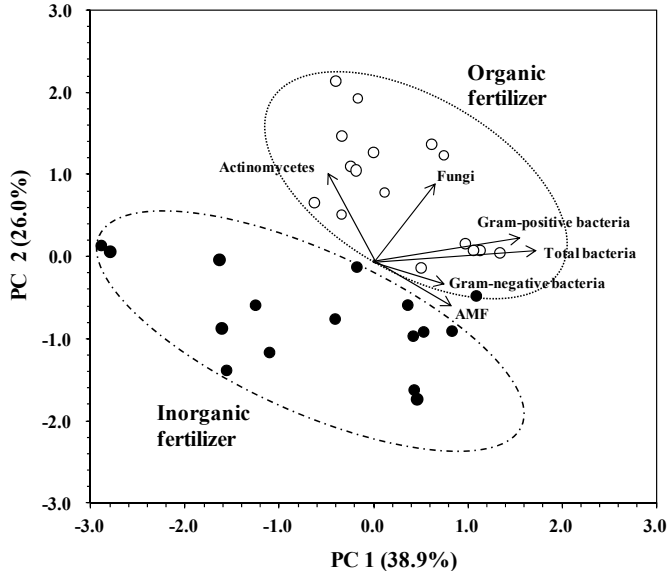

Figure 2. Ordination plot of the principal component analysis (PCA) based on the microbial communities' absolute abundance (nmol·g^{-1} soil) in the urban soils applied with organic and inorganic fertilizers over 10 years. PC1 and PC2 explain 38.9% and 26.0% of the variance, respectively. AMF stands for arbuscular mycorrhizal fungi.

Table 3. Changes for the relative abundance of biomarker fatty acids in the urban soils applied with organic and inorganic fertilizers over 10 years.

Specific Microbial Groups and Corresponding Fatty Acid Biomarkers	Organic Fertilizer	Inorganic Fertilizer
	nmol%	
Gram-positive bacteria		
a15:0	3.5 ± 0.9	3.5 ± 0.1
i15:0	4.6 ± 0.4	4.0 ± 0.8
i16:0	2.5 ± 0.1 *	2.1 ± 0.2
a17:0	1.8 ± 0.3	2.0 ± 0.3
i17:0	2.0 ± 0.5	1.7 ± 0.4
cy17:0	1.4 ± 0.1 *	1.2 ± 0.1
Gram-negative bacteria		
cy19:0ω8c	2.7 ± 0.2 *	2.0 ± 0.3
sum in feature 3 (16:1ω7c/16:1ω6c)	4.0 ± 0.4	5.3 ± 0.4 *
sum in feature 5 (a18:0/18:2ω6, 9c)	10.6 ± 1.0 *	8.7 ± 0.9
sum in feature 8 (18:1ω7c)	5.0 ± 0.2	5.1 ± 0.3
Actinomycetes		
10Me18:0	2.7 ± 0.8 *	1.6 ± 0.6
Fungi		
18:1ω9c	11.8 ± 1.1	11.2 ± 0.8
AMF †		
16:1ω5c	1.7 ± 0.1	2.2 ± 1.0

Notes: † arbuscular mycorrhizal fungi; * indicates significant difference between organic and inorganic fertilizer at $p < 0.05$.

3.3. Soil Enzyme Activities

All enzymes except acid phosphatases showed significantly higher activities in the soils with long-term application of organic fertilizers ($p < 0.05$) than the inorganic fertilizer application (Figure 3). The addition of organic fertilizers enhanced the alkaline phosphatase, β-glucosidase, arylsulfatase, urease and dehydrogenase activities by 41%, 26%, 47%, 39% and 41%, respectively, as compared to that of in inorganic fertilizers.

The demand for P on plant growth is related to the decomposition cycling of organic matter containing P which is one of the most limiting nutrients following N. Phosphatases hydrolyze the organic P to an inorganic form to make P plant available [58]. Phosphatases can be represented as acid or alkaline forms and their activities depend on soil acidity. Alkaline phosphatases activity is vital in alkaline and neutral soils while the acid phosphatases are readily activated in acidic soils [59]. The organic fertilizer applied soils in this study had neutral soil pH, and the activity of alkaline phosphatases was imperative for the soils treated with organic fertilizers rather than with inorganic fertilizers.

β-glucosidase plays a critical role in the decomposition of soil organic matter mainly derived from plant residues. It is involved in the final step of cellulose degradation by catalyzing the hydrolysis of β-D-glucopyranosides [60]. Since cellulose is the most abundant polysaccharides in the plant biomass, the degradation of cellulose by β-glucosidase activity facilitates the organic matter decomposition in the soils. Moreover, the change in β-glucosidase activity can be a very sensitive indicator determining the abundance of soil organic C [61].

Arylsulfatase is an important enzyme of soil S cycle. It hydrolyzes the S-esters and releases S in inorganic forms into soils [62], and the hydrolysis of urea is catalyzed by the urease and releases NH_4^+ ions as readily available plant nutrients. In general, high urease activity occurs in soils treated with animal manure as composts or organic fertilizers [63]. The activity of dehydrogenase also reflects the overall status of the microbial activity, degree of organic matter decomposition and nutrient availability in the soil. Therefore, the change in dehydrogenase activity can be a reliable indicator in determining changes in soil fertility, resulting from the biological oxidation of organic matter.

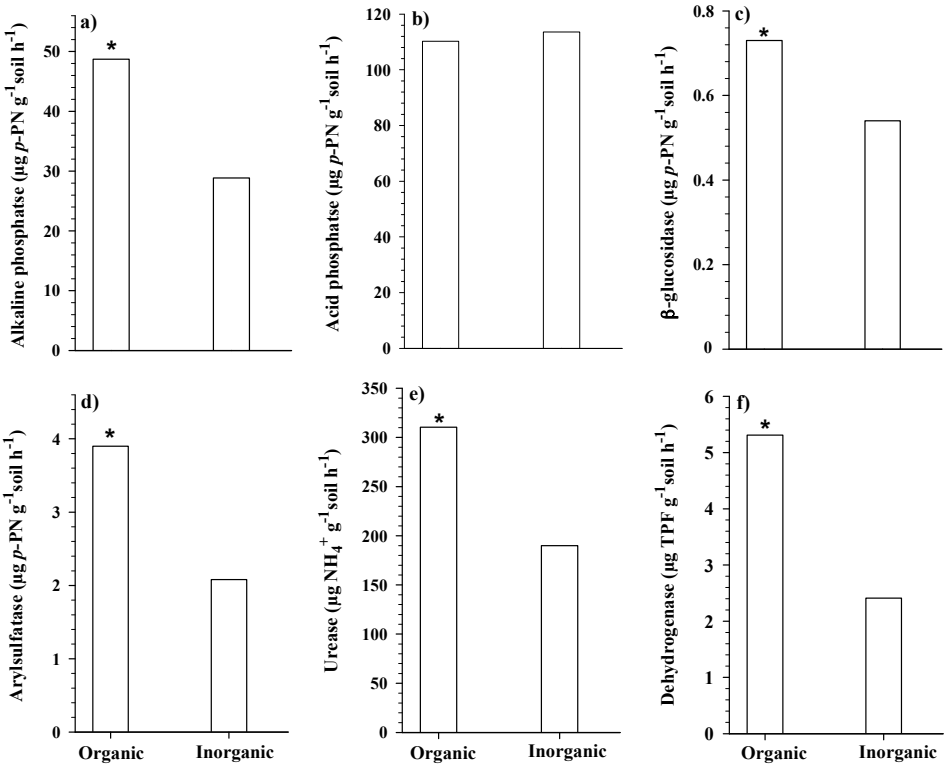

Figure 3. Soil enzyme activities of: (**a**) alkaline phosphate; (**b**) acid phosphate; (**c**) β-glucosidase; (**d**) arylsulfatase; (**e**) urease; and (**f**) dehydrogenase in the urban soils applied with organic and inorganic fertilizers over 10 years. Data are presented as mean values (Organic fertilizer = eight sites, inorganic fertilizer = eight sites). Stars above bars indicate the significant difference at $p < 0.05$.

In this study, all subjected enzymes showed better activity in the urban soils treated with organic fertilizers than in those treated with inorganic fertilizers. This implies that the long-term application of organic fertilizers would have an advantage in enhancing soil quality in the urban agricultural soils.

3.4. Relationships of Soil Quality Parameters

Alkaline phosphatase activity was significantly correlated with the gram-positive bacterial markers i15:0 ($r = 0.78$, $p < 0.001$) and cy17:0 ($r = 0.65$, $p < 0.05$), the gram-negative bacterial markers cy19:0ω8c ($r = 0.59$, $p < 0.05$), sum in feature 3 (16:1ω7c/16:1ω6c) ($r = -0.83$, $p < 0.005$), and the fungal marker sum in feature 5 (a18:0/18:2ω6, 9c) ($r = 0.69$, $p < 0.05$; Table 4). The activity of β-glucosidase was also positively correlated with the gram-positive bacterial marker i16:0 ($r = 0.84$, $p < 0.005$) and the gram-negative bacterial marker cy19:0ω8c ($r = 0.73$, $p < 0.05$). However, the gram-negative bacterial marker sum in feature 3 (16:1ω7c/16:1ω6c) was negatively correlated with all enzyme activities. Both fungal markers were positively correlated with arylsulfatase, urease, and dehydrogenase activities ($p < 0.05$–0.005) and the actinomycetes marker 10Me18:0 was also positively correlated with urease activity ($r = 0.75$, $p < 0.05$).

The Pearson correlation showed the significant relationships between the soil chemical properties and enzyme activities in the urban agricultural soils (Table 5). The relationship between soil enzymes and chemical properties did not behaved similarly in organic and inorganic fertilizer applied soils. All enzymes showed significant relationships with many soil chemical properties (all p values <0.05 or less) in organic fertilizer applied soils; however, this was not true in inorganic fertilizer added soils. Generally, organic fertilizers do not provide readily available nutrients; however, the increase of soil enzyme activities in soils with organic fertilizers stimulates the release of the available forms of nutrients slowly to the soil [37]. This may be the reason for the strong positive correlations of

soil organic matter, and soil nutrients with many soil enzymes in organic fertilizers added soils, as compared to the inorganic fertilizers added soils.

Table 4. Pearson correlation coefficients between soil enzyme activities and microbial fatty acids of the urban agricultural soils applied with organic and inorganic fertilizer over 10 years.

	Acid P †	Alkaline P ‡	β-Glucosidase	Arylsulfatase	Urease	Dehydrogenase
Organic fertilizer						
Gram-positive bacteria						
a15:0	0.25	−0.12	0.54	−0.06	0.16	0.23
i15:0	−0.23	0.78 **	0.29	0.71 *	0.37	0.09
i16:0	−0.44	0.40	0.84 **	0.29	0.28	0.17
a17:0	0.02	−0.24	−0.29	−0.30	−0.31	−0.02
i17:0	−0.36	0.45	0.02	0.39	0.09	0.17
cy17:0	−0.61 **	0.65 *	0.53	0.39	0.08	−0.16
Gram-negative bacteria						
cy19:0ω8c	−0.43	0.59 *	0.73 *	0.37	0.34	0.06
sum in feature 3 (16:1ω7c/16:1ω6c)	0.17	−0.83 **	−0.62 *	−0.94 ***	−0.80 **	−0.68 *
sum in feature 5 (a18:0/18:2ω6, 9c)	0.09	0.69 *	0.51	0.83 **	0.82 **	0.79 **
sum in feature 8 (18:1ω7c)	0.44	−0.11	0.01	−0.18	0.07	0.06
Actinomycetes						
10Me18:0	0.12	0.41	0.52	0.49	0.75 *	0.55
Fungi						
18:1ω9c	0.34	0.41	0.39	0.62 *	0.68 *	0.86 **
AMF §						
16:1ω5c	0.19	0.10	−0.45	0.15	−0.05	0.01
Inorganic fertilizer						
Gram-positive bacteria						
a15:0	0.36	−0.04	0.93	0.46	0.38	0.43
i15:0	−0.55	0.33	0.21	−0.77	−0.65	−0.87 *
i16:0	0.10	0.28	0.95 **	0.04	0.11	0.01
a17:0	−0.49	0.37	−0.18	0.48	−0.26	0.32
i17:0	−0.69	0.42	−0.36	0.31	−0.46	0.07
cy17:0	−0.79	0.59	0.22	−0.47	−0.76	−0.64
Gram-negative bacteria						
cy19:0ω8c	−0.45	0.53	0.25	−0.81 *	−0.53	−0.74
sum in feature 3 (16:1ω7c/16:1ω6c)	−0.73	0.38	0.06	−0.79	−0.81	−0.91 *
sum in feature 5 (a18:0/18:2ω6, 9c)	0.71	−0.32	0.11	0.83	0.80	0.96 **
sum in feature 8 (18:1ω7c)	0.25	0.35	0.91 *	0.40	0.36	0.35
Actinomycetes						
10Me18:0	0.80	−0.19	−0.03	0.31	0.80	0.66
Fungi						
18:1ω9c	0.68	−0.11	0.59	0.87 *	0.80	0.92 *
AMF §						
16:1ω5c	−0.44	0.49	0.42	−0.63	−0.49	−0.69

Notes: † acid phosphatases; ‡ alkaline phosphatases; § arbuscular mycorrhizal fungi; *, **, and *** indicate $p < 0.05$, $p < 0.005$ and $p < 0.0001$, respectively.

Previous studies found that soil enzyme activities were generally enhanced by organic fertilizers [16,64]. The change in soil enzyme activities has often been used to determine soil quality such as soil fertility, nutrient cycling potential, soil productivity, contamination degree, etc. [37]. Moreover, the type of fertilizer leads to the changes in the behavior of soil enzymes, thereby critically influencing the soil nutrient cycling and in-site fertility [13]. In the literature, the addition of inorganic fertilizers reduced soil nutrient availability and enzyme activity in a cycle of organic matter mineralization [65–67]. In this study, however, the individual fatty acid biomarkers did not show any distinguished pattern of soil enzyme activity as discussed previously. Based on the argument in a study by Burger and Jackson [68], this might have resulted from intensive agricultural practices rather than the influence of organic fertilizer application because of the extremely high sensitivity of soil microbes for the intensive agricultural practices.

Table 5. Pearson correlation coefficients between enzyme activities and soil chemical properties in the urban soils applied with organic and inorganic fertilizers over 10 years.

Enzyme Activities	pH	EC †	OM ‡	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}	P_2O_5	NO_3
Organic fertilizer									
acid P §	0.14	−0.08	0.17	0.06	−0.45	−0.83 *	0.01	0.67	−0.19
alkaline P ¶	−0.84 *	−0.97 **	0.99 **	0.96 **	−0.91 **	−0.41	0.95 **	0.42	−0.99 **
β-Glucosidase	0.38	0.6	−0.53	−0.54	0.2	0.07	−0.53	−0.42	0.56
arylsulfatase	−0.92 **	−0.98 **	1.00 ***	0.99 **	−0.93 **	−0.33	0.92 **	0.3	−0.99 ***
dehydrogenase	−0.79	−0.93 *	0.93 *	0.93 *	−0.76	−0.37	0.98 *	0.42	−0.94 *
urease	−0.42	−0.56	0.63	0.56	−0.83 *	−0.73	0.41	0.57	−0.64
Inorganic fertilizer									
acid P §	0.68	0.74	0.96 *	−0.68	0.85 *	0.66	0.45	0.70	−0.71
alkaline P ¶	−0.13	−0.21	−0.82 *	0.24	−0.29	0.00	−0.57	−0.59	0.22
β-Glucosidase	0.48	0.38	0.17	−0.35	0.31	0.76	0.37	−0.14	−0.34
arylsulfatase	0.90	0.88 *	−0.78 *	−0.89 *	0.78 *	0.73 *	0.78 *	−0.17	−0.88 *
dehydrogenase	0.97 **	0.98 **	−0.85 *	−0.98 **	0.95 **	0.82 *	0.56	0.05	−0.98 **
urease	0.83 *	0.87 *	−0.92 *	−0.81 **	0.93 *	0.77 *	0.49	0.49	−0.84 *

Notes: † electrical conductivity; ‡ organic matter; § acid phosphatases; ¶ alkaline phosphatases; *, **, *** indicate $p < 0.05$, $p < 0.005$ and $p < 0.0001$, respectively.

4. Conclusions

Long-term organic or inorganic fertilization has significant impacts on the soil microbes and their extracellular enzymes activities in urban agricultural soils. The addition of organic fertilizers tended to increase most enzyme activities and available nutrients in the soils, as compared to that of inorganic fertilizers. Total FAME, gram-positive and -negative bacteria, actinomycetes, fungi and AMF were significantly increased following the organic fertilizers. To increase the microbial community size and activity, and fertility of urban agricultural soils, organic fertilization may be a better alternative to inorganic fertilization. However, future studies are needed on nutrient losses, soil contaminations, and soil physical properties for better understanding of the soil quality in organic fertilizer applied urban agricultural soils.

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