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Relationships Between Organic Matter Contents and Bacterial Hydrolytic Enzyme Activities in Soils: Comparisons Between Seasons

Alexis Wilkerson[1] · **Ola A. Olapade**[1,2]

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Abstract

Microbial assemblages in terrestrial environments, such as soils, utilize hydrolytic enzymes to function biologically in various environments including for the degradation of organic carbon compounds and cycling of nutrients that eventually contributes to the ecological and agricultural productivity of such environments. In this study, 3 soil types (i.e., sandy, loamy and clayey) with varying characteristics were collected within the premises of Albion College in Michigan, with the goal of comparing the occurrences of indigenous bacterial populations and their respective hydrolytic enzyme activities in the soils. The soils were examined for their organic matter content (% OM), while bacterial abundance was determined by combinations of viable counts and nucleic acid staining, and enzymatic activities measured using fluorescein diacetate (FDA) analysis. Results from the study showed loamy soil to have a significantly higher % OM at 30% on average as compared to 2.5% and 6.6% recorded in the sandy and clayey soils. Comparatively, bacterial numbers (both viable and total counts) were also significantly higher in loamy soils than the other two soils. The same trend was observed for FDA analysis with higher fluorescein released in the loamy soil relative to the two other soils. Overall, clear differences were observed in the relationships between % OM and bacterial numbers and hydrolytic enzyme activities among the three soil types and between the two seasons examined. The results suggest that % OM strongly influences both bacterial abundance and hydrolytic enzyme activities in loamy soil and less so in both sandy and clayey soils examined in the study. This study in conclusion revealed potential strong relationships between soil organic carbon and indigenous bacterial populations as well as their FDA activities in various soil types.

Introduction

Microbial activity in terrestrial environments, such as soils and sediments, results mostly from the actions of various hydrolytic enzymes frequently produced by complex aggregates of heterotrophic bacterial assemblages for their energy generation through the decomposition of various organic carbon materials and nutrient cycling [e.g., 1]. The ecological importance of several of these enzymes in the hydrolysis of fluorescein diacetate (3′,6′-diacetylfluorescein [FDA]) for estimating total microbial activity in various environments have been previously recognized [2–6]. This is due to the incontrovertible fact that total microbial activity is generally believed to provide an accurate measure of organic matter turnover in natural systems, given that majority of energy in soil passes through microbial decomposition [1].

FDA is widely believed to be widely hydrolyzed by various enzymes, such as proteases, lipases and esterases [e.g., 2, 5] producing fluorescein that can be easily and rapidly quantified using fluorometric or spectrophotometric approaches [5–7]. For instance, a controlled study by [7] reported the development and effective utilization of the simple but sensitive spectrophotometric method to rapidly quantify total microbial activity using FDA in a range of soils in their study.

Therefore, based on a similar approach that has been well utilized [e.g., 5–7], we have examined in this study, total microbial occurrences and activities in three different soil types, that were targeted because of their varying characteristics, especially in their organic matter content and anthropogenic influences. The main objectives of the study were to determine the relationship between the organic matter availability among the soil types and total bacterial occurrences as well as their enzymatic activities. Also, to compare these

✉ Ola A. Olapade
oolapade@albion.edu

[1] Department of Biology, Albion College, 611 East Porter Street, Albion, MI 49224, USA

[2] The Center for Sustainability and the Environment, Albion College, 611 East Porter Street, Albion, MI 49224, USA

factors measured among the three soils between sampling seasons during the study period.

Materials and Methods

Soil Type Description and Collection

The three different types of soil samples (i.e., sandy, loamy and clayey) used for this study to obtain wide ranging differences in soil characteristics were collected within the premises of Albion College, Albion (42.24445°N 84.7434°W) in Michigan, USA. Fresh top soils (~ 1 m deep) were collected in triplicates during the summer (May–June) and fall (October–November) seasons at the same exact locations for the three soil types and quickly stored in clean Ziplock bags before subsamples were immediately used for various laboratory assays. The sandy soils used were tan to brownish in coloration, comprised of coarse particles and were collected from beneath freshly mowed grasses close to the botanical garden on the campus quad. The loamy soils were dark brown and dark, comprising very smooth particle sizes and were collected within the forested premises of the 25 acres Whitehouse Research Nature Center (WHNC) comprising mostly of oak-hickory, flood-plain trees and extensive marsh and swamp lands. Also collected within the forested premises of the WHNC at Albion College, were the clayey soils used with red and dark brown coloration that consisted of interspersed small particle sizes.

Determination of Soil Organic Matter Contents

The loss of weight on ignition (LOI 360°) procedure was used in this study to determine soil organic matter as previously described by [8] and [9]. Briefly, 5 g of each soil samples were weighed into tarred 20 mL beakers and dried in a muffle furnace for 2 h at 105 °C. The weight of the dried soil samples was recorded and placed back into the furnace at 360 °C for another 2 h incubation, then cooled down to less than 105 °C and weighed again in a daft-free environment under the hood. The percent weight loss-on-ignition (LOI) was then calculated based on the differences in recorded weights before estimating the percent organic matter content from the LOI using regression analysis [9].

Determination of Bacterial Abundance

Viable bacterial numbers in the soil samples were determined using standard microbiological approach as previously described [10]. Briefly, soil samples of 0.1 g were weighed and serially diluted in 9 mL sterile dilution blanks before transferring 100 µL of diluent aseptically into sterile nutrient agar plates in triplicates and incubated at 37 °C for 24 h. Distinct bacterial colonies formed were then counted in each plate using a colony counter and total viable bacterial numbers (CFU) calculated with respect to the dilution factor and the volume plated. Also, total bacterial numbers in the soils were determined by measuring 0.1 g of each soils into 1 mL of sterile deionized water, serially diluted, vortexed and then concentrating onto 0.2 µm pore-size black polycarbonate filters (Poretics, Livermore, CA) before staining with 200 µL of 15 µg/µL of DAPI (4′,6 diamidino-2-phenylindole) solution for between 3 and 5 min. Filters were rinsed with sterile water and then mounted onto glass slides with Type FF immersion oil [11]. Between 300 and 500 of the stained bacterial cells in 10 separate fields were then counted under an epifluorescence microscope.

Measurement of Fluorescein Enzyme Activity

Activities of Fluorescein diacetate [3′,6′-diacetylfluorescein (FDA)] in the soil samples were determined using the approach described by [5] and [6]. First, a standard curve was constructed to calculate the concentration of fluorescein released using a standard stock solution 10 mg fluorescein ($C_{20}H_{12}O_5$, Sigma-Aldrich) in 10 mL of reagent-grade acetone and final volume was then adjusted to 50 mL with sodium phosphate buffer (pH 7.6) for a 602 µM solution. This fluorescein stock was then diluted down in triplicate to 0.0 mg/mL, 0.03, 0.1, 0.3, 0.5 and 1.0 mg/L in 50 mL volumetric flasks and brought to volume with sodium phosphate buffer (pH 7.6), before adding 2.5 mL acetone. The optical densities of the standard solutions were then measured at 490 nm using a spectrophotometer.

FDA activity was measured in the samples by weighing out 1 g of previously air-dried soil in a 125 mL flask and adding 50 mL of 60 mM sodium phosphate buffer (pH 7.6) and 0.5 mL of 5 mM FDA substrate solution (previously prepared by adding 20 mg FDA lipase substrate [($C_{24}H_{16}O_7$, Sigma-Aldrich] in 10 mL acetone). Blanks with no soils, but just the reagents and controls with each soil type containing 0.5 mL acetone instead of the FDA lipase substrate solution were also set up in order to measure color not derived from the hydrolysis of FDA. The triplicate flasks were then arranged on a shaking incubator at 24 h. At varying time intervals, i.e., 0, 4, 6, 8 and 24 h, subsamples of 30 mL of soil slurries from each flask were collected in 50 mL centrifuge tubes and spun down at maximum speed (8000 rpm) after adding 3 mL acetone to stop FDA activity for 5 min at 4 °C. The supernatant was then measured for absorbance at a wavelength of 490 nm. Concentrations of fluorescein released were then calculated in reference to the equation ($y = 1.4789x + 0.0058$) obtained from standard curve prepared prior between the diluted fluorescein standard and their respective OD @490 nm ($R^2 = 0.880$).

Table 1 Results of Student's *t* tests on pairwise comparison of organic matter content and bacterial abundance measured among soil types

Pairwise comparison	Summer			Fall		
	% OM	CFU	DAPI	% OM	CFU	DAPI
Loamy versus Sandy	**0.0409**	NS	NS	**0.0002**	**0.0246**	**0.0180**
Loamy versus Clayey	**0.0480**	NS	**0.0183**	**0.0001**	**0.0069**	**0.0183**
Sandy versus Clayey	NS	**0.0234**	NS	NS	**0.0384**	NS

Significant values are given in bold

Significant level set at 0.05

NS Not significant, *% OM* Percent organic matter content, *CFU* colony forming units, *DAPI* DAPI-stained bacterial cells

Table 2 Results of Student's t tests of pairwise comparison of organic matter content and bacterial abundance (Viable and total) measured among soil types between seasons

Pairwise comparison	% OM	CFU	DAPI
Loamy versus Sandy	**0.0400**	**0.2561**	NS
Loamy versus Clayey	**0.0311**	**0.0296**	NS
Sandy versus Clayey	NS	**0.0234**	**0.0253**
Loamy versus Loamy	NS	NS	NS
Clayey versus Clayey	NS	NS	0.0509
Sandy versus Sandy	NS	NS	NS

Significant values are given in bold

Significant level set at 0.05

NS Not significant, *% OM* percent organic matter content, *CFU* Colony forming units, *DAPI* DAPI-stained bacterial cells

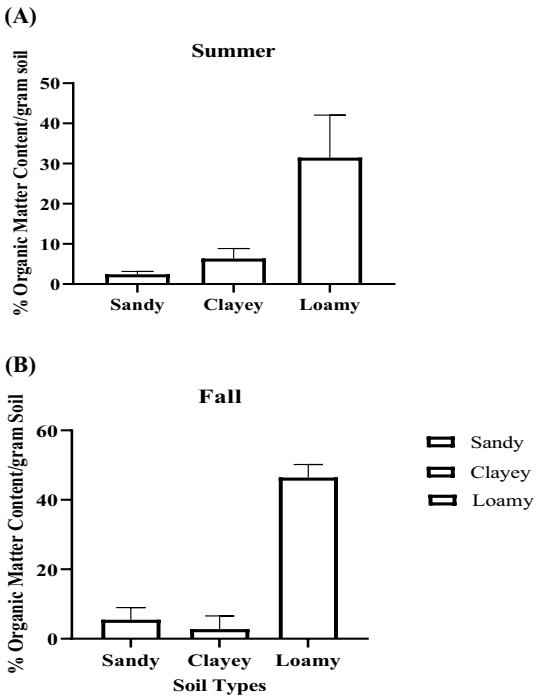

Fig. 1 Percent organic matter content in the soils during the summer (**a**) and fall (**b**) seasons. Mean values ± SD

Statistical Analysis

The Student's t tests and ANOVA analyses were performed to analyze the differences in bacterial abundance and fluorescein diacetate (FDA) hydrolytic activities between different soil types. Post hoc tests were also carried out for pairwise comparison using Bonferroni correction test. Relationships between organic matter contents and bacterial populations a s well as FDA enzyme activity were further examined using linear regression and Pearson Correlation analysis. Statistical significance was set at $p \leq 0.05$.

Results

Soil Characteristics and Organic Matter Content

There were significant differences among the soil types used for the study in their characteristics. Particularly, the organic matter content measured in the loamy soil were found to be much higher than in the two other soil types examined ($p < 0.05$); however, the amounts of % OM were much similar between the clayey and the sandy soil (Table 1). The same trend was observed in % OM when compared among the soils between the two seasons examined (Table 2), with the loamy soil having more significant OM content than measured in the other two soils (Fig. 1).

Bacterial Occurrences

Numbers of viable bacteria enumerated varied significantly in the three soils ($p < 0.05$), with the loamy soil having the highest counts and lowest numbers found in the clayey soil in both the summer and the fall season (Fig. 2). The CFU numbers found among the soil types used were more varied during the fall as compared to the summer (Table 2). Total bacterial numbers based on DAPI-stained

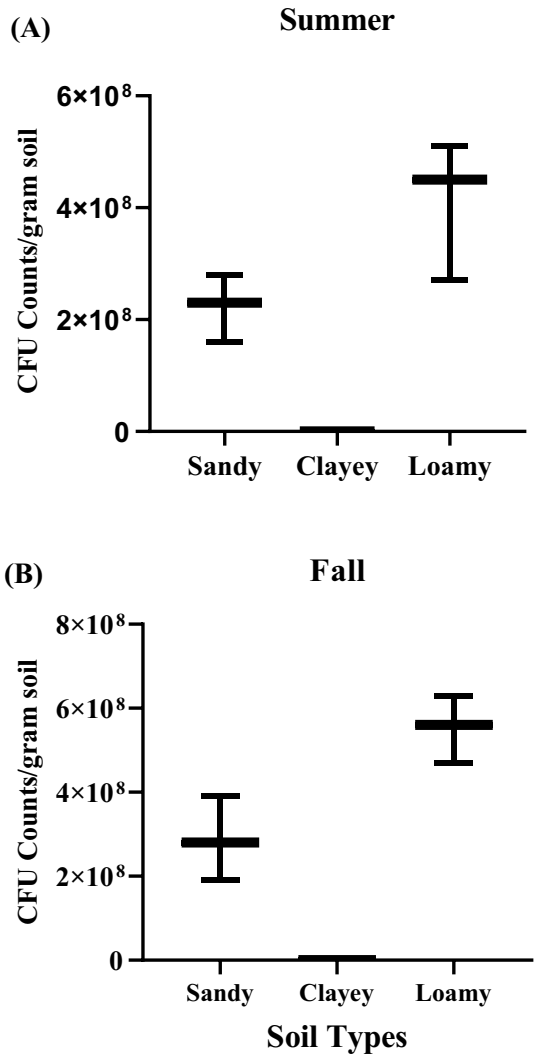

Fig. 2 Numbers of viable bacterial populations in the soils during the summer (**a**) and fall (**b**) seasons. Mean values ± SD

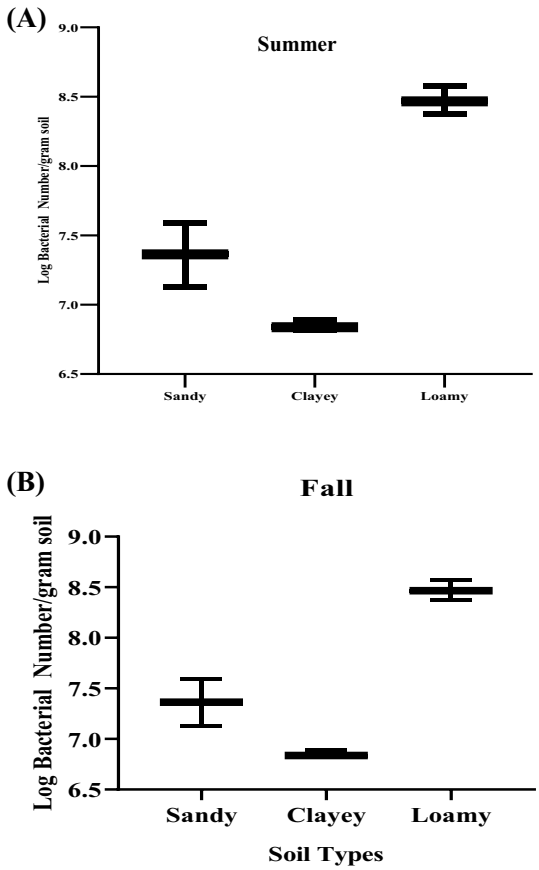

Fig. 3 Total bacterial counts based on DAPI-stained cells in the soils during the summer (**a**) and fall (**b**) seasons. Mean values ± SD

cells followed the same trend as recorded for the viable numbers among the three soils, with the highest numbers found in the loamy soil and the lowest in the clayey soil in both seasons examined (Fig. 3).

Fluorescein Enzyme Activity

The amount of fluorescein released over the 24 h incubation period was significantly higher in the loamy soil and lowest in the clayey soil (Fig. 4). The results of ANOVA showed significant variations among the soils in both the summer ($F = 5.726$, $p < 0.0001$) as well as in the fall ($F = 3.758$, $p < 0.0001$). The results of post hoc tests using Bonferroni correction revealed significant differences between the sampling times among the three soil types examined ($p < 0.05$). Also, significant variations were recorded in fluorescein release after 8 h incubation among the three soils in both summer and fall seasons examined in this study.

Relationships Between OM Content, Bacterial Occurrences and FDA Activity

A strong linear relationship ($R^2 = 0.7285$) was observed between % OM content and viable bacterial counts in the sandy soil, but not such relationship was recorded in both the loamy and sandy soil (Fig. 5a). Similarly, the relationship between % OM content and DAPI-stained bacterial cells in the sandy soil was also strong ($R^2 = 0.6621$), but less so in loamy ($R^2 = 0.1753$) and clayey ($R^2 = 0.484$) soils (Fig. 5b).

When the relationship between % OM content and total bacterial counts were examined among the soils and compared between seasons, the relationship was found in the summer to be stronger ($R^2 = 0.6621$) only in sandy soil (Fig. 6a), but stronger in the fall in both loamy ($R^2 = 0.5517$) and sandy ($R^2 = 0.7167$) soils, while not in clayey soil (Fig. 6b).

The relationships found between the % OM content and fluorescein released in both the loamy and sandy soils were

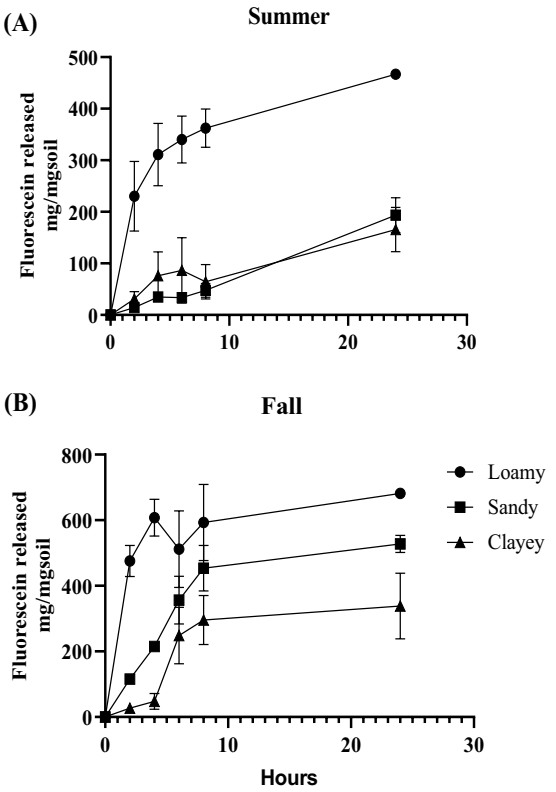

Fig. 4 Concentrations of fluorescein released in the soils during the summer (**a**) and fall (**b**) seasons. Mean values ± SD

very strong in the summer $R^2 = 0.6747$ and 0.7311 (Fig. 7a); as well as in the fall $R^2 = 0.6339$ and 6902 (Fig. 7b), but comparatively weaker in the clayey soil in both seasons. To determine whether total bacterial counts correlated with FDA activity, the relationship between the two were examined in the three soils from both summer and fall, and a very strong linear relationship was found only in the sandy soil in the summer ($R^2 = 0.8946$, Fig. 8a) and fall ($R^2 = 0.710$, Fig. 8), but not in the other two soil types examined during the study.

Discussion

The comparatively high bacterial occurrences as well as FDA activity observed in the loamy soil in this study relative to the other two sources is not at all surprising. Loamy soils are characteristically loaded with complex organic compounds consisting of mostly recalcitrant humic and/or detrital materials [12, 13]. The result obtained from this study corroborates strongly with those observed by Adam and Dunkan [7], who also reported relatively lower FDA activity in both sandy and clayey soils they examined. Contradictory results have been observed in previous studies on FDA activity in different soil types. While many soil enzyme activities have been generally observed to increase with increasing organic matter presence in soils, as typically

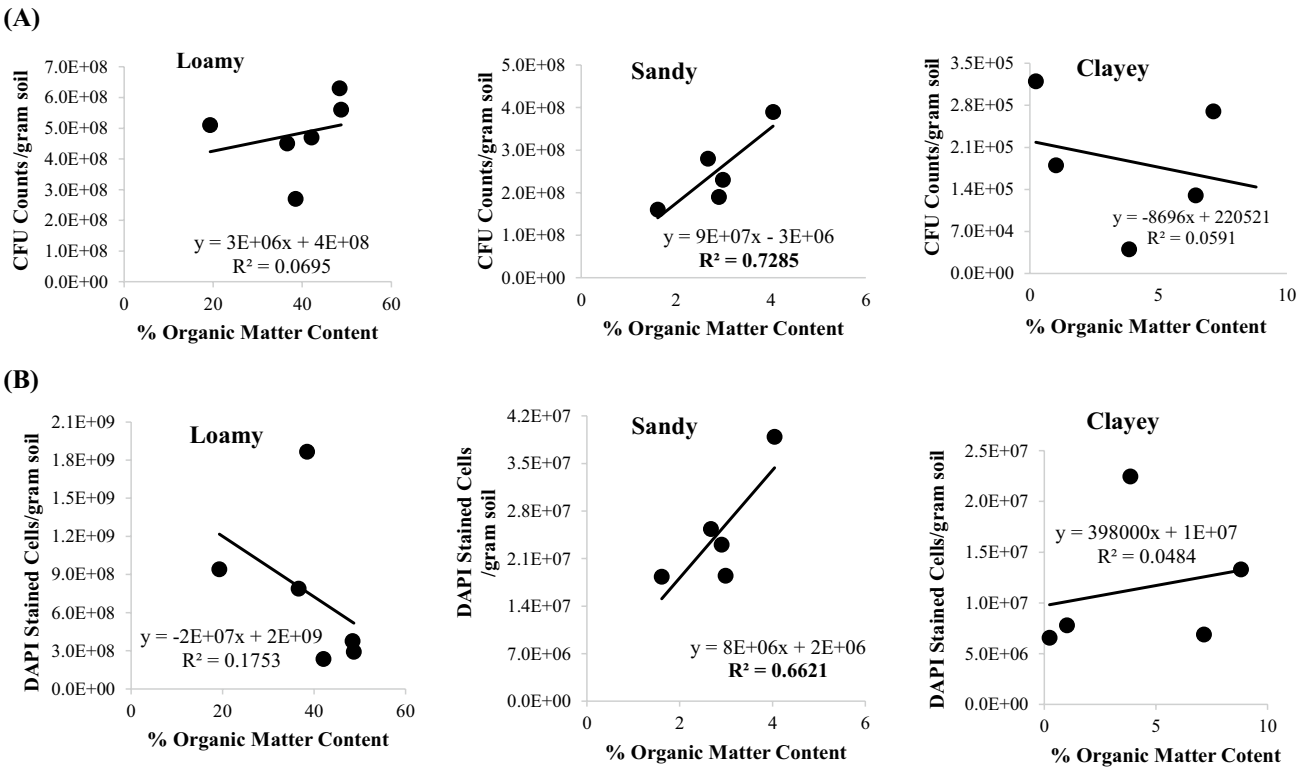

Fig. 5 Relationships between the % OM and viable bacterial populations (**a**) and total bacterial counts (**b**) in the soil types

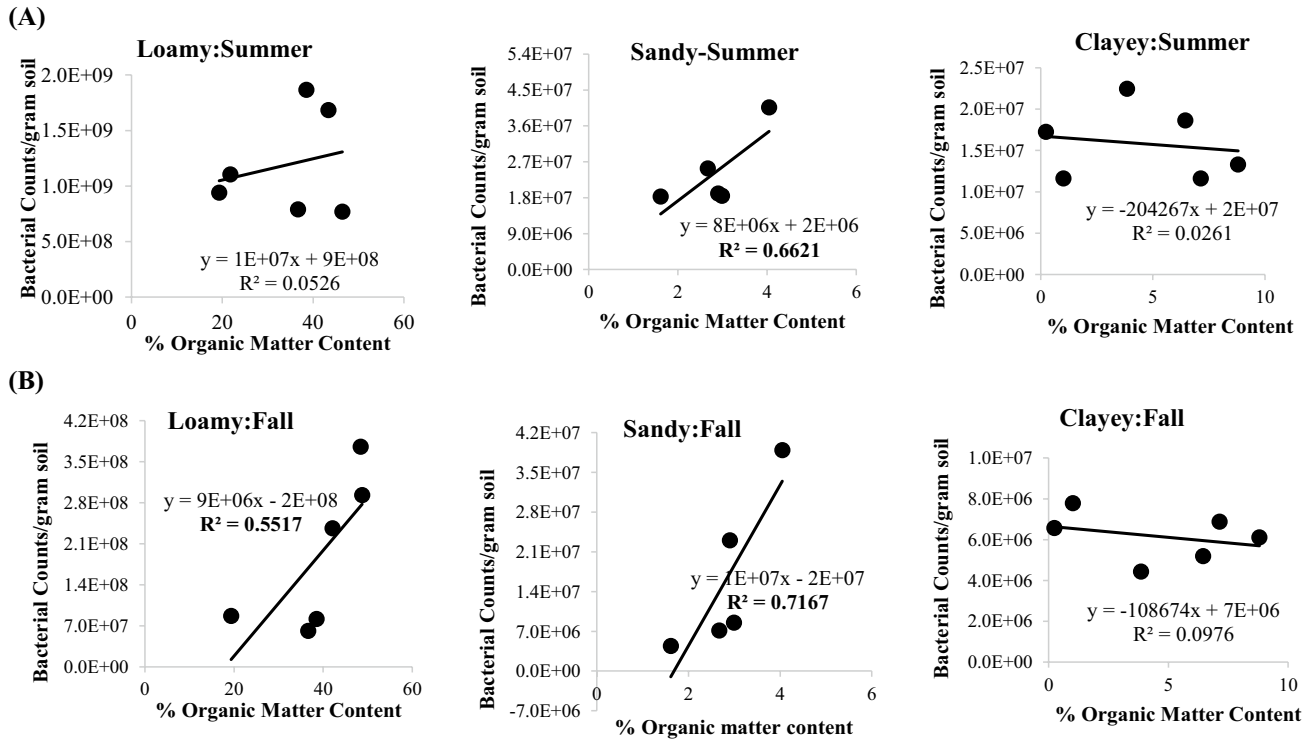

Fig. 6 Relationships between the % OM and total bacterial counts in the soil types during the summer (**a**) and fall (**b**) seasons

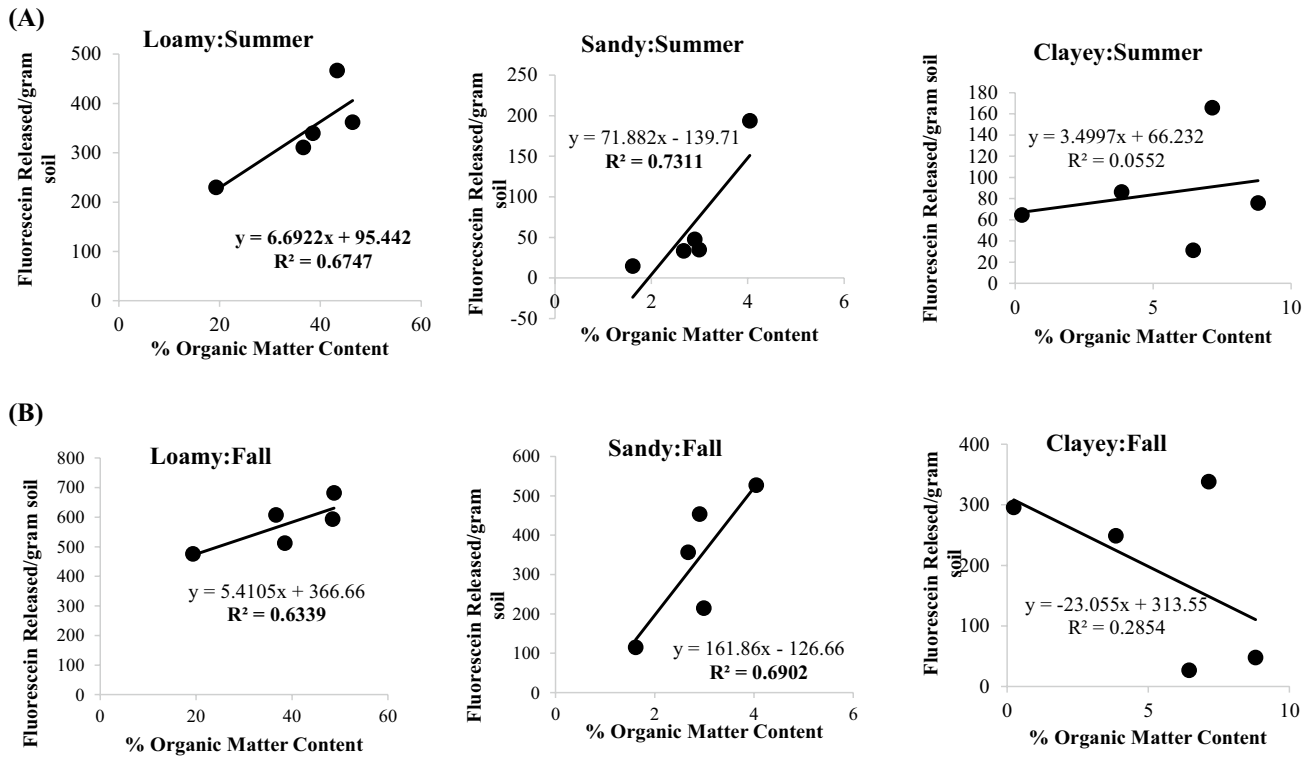

Fig. 7 Relationships between the % OM and fluorescein released in the soil types during the summer (**a**) and fall (**b**) seasons

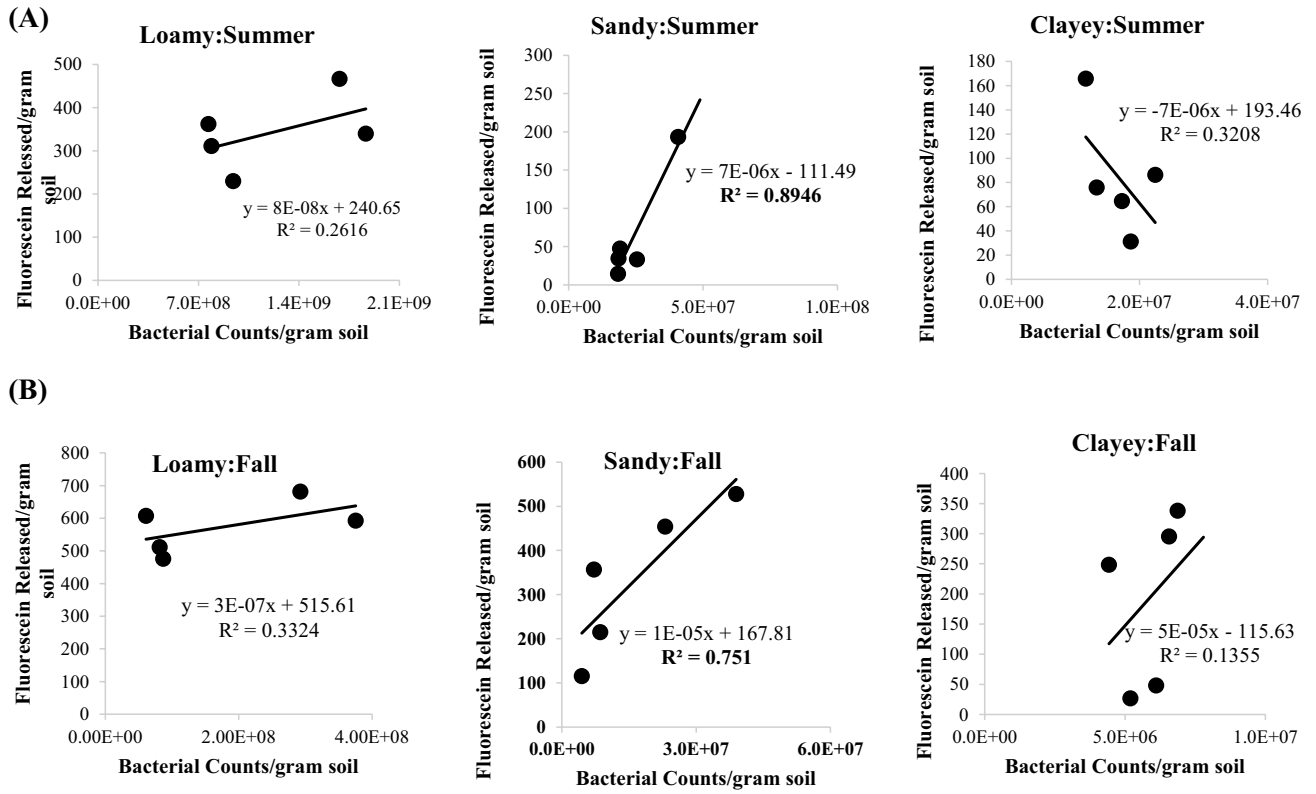

Fig. 8 Relationships between total bacterial counts and fluorescein released in the soil types during the summer (**a**) and fall (**b**) seasons

found in high microbially active loamy soils [e.g., 14, 15], however, a study by Green et al. [5] reported that high clay soils with also high organic matter contents exhibited relatively higher FDA hydrolytic activities among the soils examined in their study.

The strong linear relationships observed in the sandy soil between % OM and microbial occurrences and FDA activity in this study further validates earlier observations that soils that are typically disturbed by human activities such as mowing and fertilization have enzymatic activities per unit soil organic carbon (SOC) higher than those found in forested soils (such as the clayey and loamy soils used in our study [16–18]. This FDA trend was also corroborated by Medeiros et al. [16] in their study that examined enzyme activities per unit SOC in sandy soils with various crop coverage. Similarly, a study by Tokuda and Hayatsu [19] reported higher FDA hydrolytic activity in acidic tea field soils than those in neutral arable soils and also showed a negative relationship between FDA and soil pH, an indication that other characteristics may also be potentially driving microbial activities soils.

FDA hydrolysis is a non-specific, but sensitive test capable of accurately reflecting the hydrolytic activity of soil microbes in a wide range of soil types as generally acceptable measure of total microbial activity [5–7, 20]. FDA is hydrolyzed by all the major enzymes involved in organic carbon decomposition in soils, such as proteases, lipases and esterases [5]. In this study, FDA activity was significantly influenced by % OM in both the loamy and sandy soils, but not clayey, during the two seasons examined. Also, FDA showed strong correlations with bacterial occurrences in sandy soil during the summer (0.89) and fall (0.75) seasons. This strongly indicates that enzyme activities were associated with active microbes in the soils and these microbial cells are the major sources of these soil enzymes. The strong relationship between bacterial numbers and FDA activity as observed in this study further corroborate reported results from earlier similar studies [e.g., 21, 22]. For instance, the study by Gadja et al. [22] also found significant correlation between microbial biomass and enzyme activities with organic carbon content in their study that examined silt and sandy loamy soils. They found all biological activities measured to be generally higher in the silt soil as compared to the sandy loamy soils in their experimental soils.

In this study, we demonstrated that bacterial occurrences and their hydrolytic enzymatic activities in different soil types located within a temperate nature center were affected differently by SOC contents. However, the effects of SOC bacterial populations and FDA activities between the soils were found to be insignificant among the two different

seasons examined. Therefore, a follow-up study on the soil types over a complete seasonal cycle utilizing multivariate analyses may probably reveal differences associated with various environmental factors that may also change temporally among the soils.

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Compliance with Ethical Standards

Conflict of interest The authors declares that there are no conflicts of interests.

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