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The Impacts of Urbanization on Soil: The Effect on Decomposition Rates and Microbial Communities

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ABSTRACT The anthropogenic impacts of industrialization have left many ecosystems polluted. Soil is essential for ecosystem functions, so understanding how it reacts to anthropogenic change is important to understanding larger ecological change. My study examined soil organic matter decomposition, microbial biomass carbon (MBC) and the fungal to bacteria ratio (F:B) across an urban-suburban-exurban landuse gradient in San Luis Obispo, California. A cotton strip assay as well as microBIOMETER® testing was performed on three geographic locations in and outside of the city. The cotton strip decomposition acts as an indicator for organic matter breakdown as microorganisms break down the cellulose in the strips causing them to lose mass and tensile strength. My null hypothesis is the geographic location of the sites (urban, suburban, exurban) will not have a significant effect on the rate of soil organic matter decomposition, MBC, and F:B carbon. The three sites were sampled over a five-week period and the cotton strip mass loss, MBC, and F:B were measured. A two-way ANOVA as well as Tukey's post-hoc tests were utilized to test for significant findings. The decomposition rates for the Urban, Suburban, and Exurban sites were: 0.0189g/week < 0.0204g/week < 0.0304g/week respectively. One-way ANOVA of the decomposition rates, as well as MBC and F:B among the sites showed no significant differences. ($p > 0.05$ for all). The tests failed to reject the null hypothesis, indicating a lack of significant difference in cotton decomposition rates and microBIOMETER® results between the three sites. This study works to bridge the connection between urbanization and the functions of soil.

INTRODUCTION

The composition and function of soils are an essential part of ecosystems. They provide crops we need to feed the world's population, sustain ecosystems, regulate the climate and more.

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The biological processes of soils, specifically the role of microbial populations within them, are vital to the future of sustainable ecosystem management. Soils serve as a vital reservoir of biodiversity, hosting millions of microorganisms that play crucial roles in nutrient cycling, ecosystem maintenance, water reclamation, and carbon storage.

Microbial communities composed of fungi, bacteria, and other organisms are largely responsible for maintaining soil fertility through decomposition of organic matter. These communities and decomposition rates are often used as an indicator of a soil's ability to perform its functions (Ananyeva et al., 2021; Berg and McClaugherty, 2008). Measurements such as microbial biomass carbon (MBC), and the fungal/bacteria ratio (F:B) used in this study show the fertility of soil. Microbial populations decompose plant and animal residues and soil organic matter to release carbon dioxide and plant available nutrients. F:B ratios affect plant biomass through efficiency of carbon flow, where efficiency increases with higher fungal populations. Thus, a high MBC value means more microbial communities are present. A higher fungal value in the F:B ratio indicates higher fertility. The pairing of these tests gives insight into the health of microbial communities in the soil. Organic matter decomposition via these communities is an important process for nutrient regeneration, maintaining productivity and influencing ecosystem structure (Swift et al., 1979).

There are multiple ways of measuring organic matter decomposition rates (Lin et al., 2013; Martin et al., 2023). Two common ways are through a litter-bag or cotton strip assay. Ideal soil decomposition proxies are replicable, user-friendly, cost efficient, and should accurately measure soil conditions under human impacts. (Tiegs et al., 2013). The cotton strip assay method utilizes strips of cotton to analyze the rate in which soil microbial communities break down the cellulose present in the cotton. The change in mass and tensile strength of the cotton when removed from the soil over a fixed time period provides a reliable rate of organic matter decomposition. Cotton-strip assays are also sensitive to anthropogenic impacts such as urbanization and agriculture (Clapcott et al., 2010), and pH (Hildrew et al., 1984). Furthermore, they are often paired with tests to measure microbial populations (e.g. soluble organic carbon, fluorescein diacetate (FDA) hydrolysis, etc.) (Nachimuthu et al., 2022; Eggins, 1989; Zhang et al., 2020). In this study, MBC and F:B were measured via microBIOMETER® (Prolific Earth Sciences, Montgomery, NY; https://microbiometer.com/) to examine this relationship.

microBIOMETER® provides a cost-effective, simple, and replicable measure of microbial biomass, (Nickens et al., 2024). Based on a review of literature, little to no studies of microbial decomposition of organic matter using cotton strip assays have used microBIOMETER to measure MBC and F:B, likely due to its more recent release in 2018.

This connection between microbial communities and decomposition rates in soils has been widely investigated under an array of factors. One such factor is geographic location of the soil, specifically across sites with increasing levels of urbanization, or an urban gradient (Turnquist et al., 2016). A common and very detrimental problem for soils around the world are anthropogenic impacts, with studies showing that urbanization has significant effects on soil pollutants (Martin et al., 2023; You, 2016). These soil pollutants across urban, suburban, and exurban environments can harm microbial communities, fungi, bacteria, plant growth, etc., leaving the soil unable to perform necessary functions such as decomposition, (Wang et al., 2022). The inability of soils to decompose materials can have devastating impacts on human health as well as agricultural productivity (You, 2016).

Soil microbial decomposition is often examined in relation to ecological species evenness or plant diversity, (Lin et al., 2013; Tresch et al., 2019). However, microbial organic matter decomposition across an urban-to-exurban landuse and pollution gradient is relatively underexplored. Ecosystem functions and services will be affected with climate change (Keiser and

Bradford, 2017); hence, it is imperative we fully understand the stressors we are placing on soils. Understanding how anthropogenic impacts are affecting soils' key functions may better equip us for conserving soils in the uncertain future.

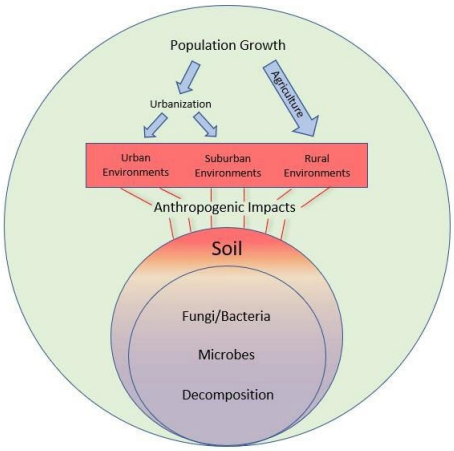

Figure 1. The urban-suburban-exurban gradient effects on the soil sphere visualizes the scope of the study, showing how testing for decomposition rates and microbial properties are reflective of anthropogenic impacts.

The aim of this study is to determine if and how the rate of soil organic matter decomposition, the mass of MBC, and the F:B ratio change along an urban-suburban-exurban landuse gradient in San Luis Obispo, California (Figure 1). Based on the aim of this study, the following null and alternative hypotheses were:

Ho: Geographic location of the sites (urban, suburban, exurban) will not have a significant effect on the rate of soil organic matter decomposition, MBC, and F:B carbon.

Ha: Geographic location of the sites (urban, suburban, exurban) will have a significant effect on the rate of soil organic matter decomposition, MBC, and F:B carbon.

METHODS

2.1 Study Sites

Cotton strips were buried at three sites along an urban-exurban land use gradient across San Luis Obispo, CA. Site 1 was the most urban, located in the heart of the city, characterized by houses with smaller lot sizes and yards, as well as less foliage. This site was residential, located near a highway. The area does not have major industrial activity, with most development being minor renovations. Site 2 was a suburban location characterized by houses with larger yards and more extensive foliage. The area is strictly residential, with this site having no development within the last 20 years. Site 3 was a remote exurban location in a field characterized by scrub and shrub vegetation (Figure 2).

Figure 2. Shows the three sample points used for cotton analysis. The blue point is site one (urban), the red point is site two (suburban) and the green point is site three (exurban). As well as the soil type distribution displayed by Web Soil Survey. The soil type used for this study is represented by 163/162. Labeled a Los Osos-Diablo complex.

These sites best represented the desired urbanization gradient of the city. Furthermore, to limit variability in decomposition rate caused by differences in soil properties, the three sites were located in the same mapped soil - the Los Osos-Diablo complex (Soil Survey Staff, Web Soil Survey, http://websoilsurvey.sc.egov.usda.gov/ (Figure 2).

2.2 Cotton Strip Assay Procedure

45 unprimed, heavyweight cotton strips, 15 per site, (Tiegs et al. 2013) were cut to 2.5cm by 7.5cm. Each strip weighed ~1 g, thus allowing for a controlled initial weight. The strips were labeled via permanent marker with their site, week, and replicate for accurate weight loss analysis after being pulled (e.g. Urban 1a, Suburban 3b, etc.). Each site had three replicate strips per week labeled a, b, or c. At each site,

the strips were placed in a 10-cm deep hole, with a spacing of at least 30 cm between the strips. The holes were then refilled with soil and lightly pressed to ensure soil to cotton contact. Cotton strips were buried in an approximate $4m^2$ square area at each site in a grid pattern.
Three replicate strips were removed each week from each site over the five-week period. The strips were removed, cleaned, dried, and weighed to the nearest hundredth of a gram to measure mass loss. Along with the cotton strips, 48 soil samples were taken across the three sites, 16 samples per location. These samples were scraped directly off the strip upon removal or were collected within 1 cm of the sidewall of the hole. An initial MBC and F:B test was made at each site prior to burying the cotton strips using the microBIOMETER® . Soil microbes are bonded to particles and each other via a substance they secrete. The microBIOMETER® test extracts the microbes which then settle to the bottom of the test tube. The soil-colored microbes remain suspended in the fluid and are sampled via color intensity. Finally, a computerized analysis of the color intensity provides MBC and F:B values. Each week, when cotton strips were pulled, soil samples were tested immediately for MBC and F:B carbon via the microBIOMETER® test.

2.3 Statistical Analysis

The mass loss of each replicate (n=3) was determined and utilized for a two-way ANOVA statistical analysis between the three sites. Additionally, Tukey's post-hoc test was also utilized to look for significant pairwise differences between the three groups. All statistics were performed using R 4.1.1. (R Core Team, 2024). The average decomposition rates were also calculated for the three sites—Urban, Suburban, and Exurban—based on the mass loss over the five weeks. Decomposition rate was determined by dividing the mass loss by time. Weekly decomposition rates were averaged to obtain the overall average for each site. While decomposition rates are directly related to mass loss, a one-way ANOVA was also done on the decomposition rates to test for statistical significance. Similarly, two-way ANOVA tests were conducted for both the MBC and the F:B data along with Tukey's post-hoc test.

RESULTS

Figure 3 and Table 1 show the mass loss recorded for the cotton strips at each site. The absence of strip Suburban 4b is due to a strip not being recovered after likely being dug up by an animal.

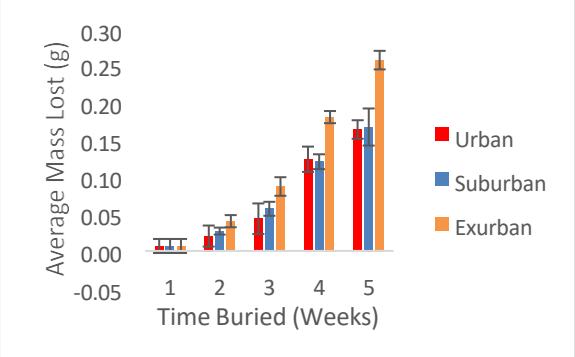

Figure 3. Shows the average mass loss of the cotton strip replicates, where error bars represent the standard deviation of the mean of the replicate values.

The statistical analysis of the mass loss at the three sites revealed no significant differences among the sites. The two-way ANOVA results comparing each site (Urban, Suburban, Exurban) did not show significant variation in mass loss ($p > 0.05$ for all). Additionally, Tukey's post-hoc test, which compared the pairwise differences between the sites, also did not identify any statistically significant differences. However, the two-way ANOVA results did see significant results in mass loss between weeks for the Exurban site (Exurban: $p < 0.05$). Based on both the ANOVA and post-hoc test results, we can conclude that the statistical analysis supports the null hypotheses.

The decomposition rates for the Urban, Suburban, and Exurban sites were: 0.0189g/week < 0.0204g/week < 0.0304g/week respectively. One-way ANOVA of the decomposition rate values for every strip among the sites showed no significant differences. ($p > 0.05$ for all).

Both the two-way ANOVA as well as the Tukey's post-hoc test did not show significant differences in either the MBC or F:B of the three sites ($p > 0.05$ for all). Although the microBIOMETER® tests did not provide any

noticeable trends over the five-week period, across all three sites there was significant increase in both MBC as well as the fungal proportion of the F:B from the initial week to the first week (Tables 2, 3, Figures 4, 5).

Week	Urban Initial Mass(g)	Pull Mass(g)	U Mass Lost(g)	Suburban Initial Mass(g)	Pull Mass(g)	S Mass Lost(g)	ExUrban Initial Mass(g)	Pull Mass(g)	E Mass Lost(g)
	0.93	0.93	0	0.87	0.87	0	0.9	0.9	0
	0.92	0.9	0.02	0.89	0.89	0	0.9	0.9	0
1	0.91	0.91	0	0.88	0.86	0.02	0.92	0.9	0.02
	0.89	0.88	0.01	0.92	0.89	0.03	0.92	0.87	0.05
	0.93	0.89	0.04	0.9	0.87	0.03	0.89	0.86	0.03
2	0.94	0.93	0.01	0.91	0.89	0.02	0.9	0.86	0.04
	0.88	0.86	0.02	0.91	0.86	0.05	0.89	0.82	0.07
	0.92	0.85	0.07	0.88	0.83	0.05	0.9	0.8	0.1
3	0.89	0.85	0.04	0.93	0.86	0.07	0.9	0.81	0.09
	0.92	0.82	0.1	0.91	0.8	0.11	0.92	0.74	0.18
	0.93	0.8	0.13	0.89	N/A		0.87	0.7	0.17
4	0.93	0.79	0.14	0.88	0.75	0.13	0.92	0.73	0.19
	0.93	0.75	0.18	0.91	0.71	0.2	0.9	0.63	0.27
	0.92	0.77	0.15	0.87	0.73	0.14	0.93	0.67	0.26
5	0.9	0.74	0.16	0.9	0.74	0.16	0.88	0.64	0.24

Table 1. Mass loss of cotton strips over the five-week period across the Urban, Suburban, and Exurban sites.

	MBC (ug C/g)		
Weeks	Urban	Suburban	Rural
0	211	193	228
	371	450	323
	300	597	554
1	322	477	539
	348	378	366
	240	442	375
2	295	493	434
	411	569	508
	270	589	398
3	234	643	388
	383	548	306
	351	N/A	488
4	284	461	455
	452	414	568
	431	635	523
5	443	663	634

Table 2. MBC (Microbial biomass carbon) count for each soil sample collected over the five-week period. Where each row is a replicate of the site.

	F:B - Fungal Level		
Weeks	Urban	Suburban	ExUrban
0	0.2	0.2	0.3
	0.8	0.8	0.6
	0.4	1.2	1
1	0.4	0.9	1
	0.7	0.7	0.7
	0.4	0.8	0.7
2	0.4	1	0.8
	0.6	0.9	1
	0.4	1.2	0.7
3	0.3	1.3	0.5
	0.3	1	0.7
	0.4	N/A	0.8
4	0.3	0.8	0.7
	0.9	0.8	1.1
	0.8	1.9	0.8
5	1.3	1.8	0.8

Table 3. Fungal to bacterial ratio in terms of fungal level (e.g. 0.7:1) for soil samples collected over the fiveweek period.

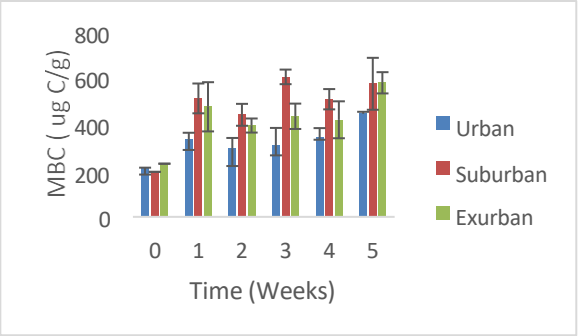

Figure 4. Shows the average MBC of the replicates at the sites in (ug C/g) where standard error bars are the standard deviation of the mean values.

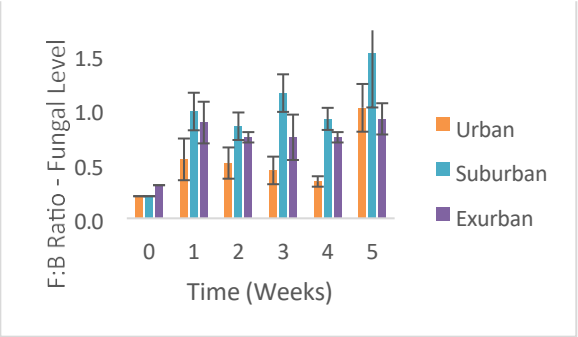

Figure 5. Shows the F:B ratio (via fungal level) where standard error bars are the standard deviation of the mean values.

DISCUSSION

The findings of this study show no significant difference between the Urban, Suburban, and Exurban sites in terms of mass loss, soil decomposition rates, MBC values, or F:B ratios. While these values were not found to be significantly different, they can still provide some insight into the difference in soil quality between the sites. The increased rate of decomposition for the exurban site is interesting as it is not supported by the microBIOMETER® results which saw no consistent trends, with the suburban site even having the highest MBC and F:B values. This could be due to several factors not measured in this study, such as temperature, pH, oxygen levels, humidity, etc. These factors could have an array of unseen effects. Any of these effects could have significantly affected MBC and F:B and therefore the ability of microorganisms to decompose organic materials, (Brady & Weil, 2010).

The introduction of the cotton strips did impact microbial communities in the soil in direct contact

of the cotton strips. The overall increase of both MBC and the F:B ratio after the introduction of the cotton strips into the soil does suggest that the cotton strips are loci of increased microbial activity. This increase in microbial activity has been a noted effect of both the cotton strip assay as well as litter-bag assays. (Tresch et al., 2019; Eggins, 1989). This increase in MBC and higher fungal values in the F:B ratio than initially found are indicators of more active, fertile soil that can perform the functions we rely on them for.

The gaps and shortcomings of this study are quite prevalent and may have impacted the results. First, only one $4m^2$ for each geographic location was chosen to represent an urban "gradient" for the city of San Luis Obispo. This was due to a constrained time frame. Ideally, if replicated, this study should utilize more sample sites within the urban-rural-exurban locations.

Second, the fertility of the soil tested was quite low when compared to ideal ranges provided by microBIOMETER®. This is possibly due to the region's arid climate as well as performing the study during the dry season. A region with more fertile soil likely would have seen more decomposition, and thus, possibly more substantial differences in decomposition rates.

Furthermore, the cotton strip assay is most commonly done with a tensile-strength test as opposed to mass loss and decomposition rates. While tensile strength is directly affected by mass loss, it is still a beneficial, and a more accurate, measurement for analysis of cellulose breakdown in the cotton strips. (Tiegs et al. 2013). Not performing this test was an oversight. Equipment for the test was not readily available less than 48 after extracting the strips which is the recommended time frame (Nachimuthu et al., 2022; Tiegs et al., 2013).

Lastly, doing the study over a longer period will yield a more complete picture of soil organic matter decomposition. While there is no standardized length of time, an analysis of the world data set (Eggins, 1989) concluded a mean time of 191.83 days or about 27 weeks is used for a cotton strip assay, a significantly longer period then was available for this study. This could lead to significantly different results as the strips were not allowed to fully decompose in this study. This is also supported by the significant difference in mass loss for the exurban site between weeks, suggesting that not enough time was given for all three sites to demonstrate the possible effects they have on decomposition rates.

While this study may not have had significant results, it does work to bridge an important gap in soil science, the connection between anthropogenic impacts and the functions of soil. With the scale and reach of anthropogenic impacts growing in our ecosystems, it is imperative we examine its effects on all aspects of them. With soils providing so much for ecosystems, continuing to understand how we are affecting them is crucial for our future.

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