Meso and Macrofauna Responses to Biochar in Urban Soils

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Abstract

Anthropogenic soils are becoming more prominent across the globe, often seen in urban areas, and ecological soil management presents an opportunity to shift from big business to ecosystem services focusing on local food provision, stormwater management, etc., and resident autonomy, highlighting the need to alleviate continued degradation on urban soils. Among many agricultural soil amendments biochar, a form of charcoal, has numerous favorable qualities including water storing capabilities, plant nutrients retention, and carbon sequestration, as well as potentially positive impacts on soil microbial communities. The particle sizes of biochars vary greatly, and little consensus exists on what size, if any, explains best optimization of biochar functions in soil and what effects this application may have on soil fauna and the processes they carry out. The effect of biochar application on soil invertebrates, specifically arthropods, have not been thoroughly investigated despite increasing recognition that soil fauna, micro-, meso-, and macro- all play key roles in soil health and quality. In this study, we applied 3 distinct particle size distributions of biochar together with compost in treatments across 64 plots. We collected soil invertebrates using pitfall traps to determine faunal responses to biochar application rate and particle size after an initial growing season. We saw significant results in moisture depending on biochar treatment with Mixed showing the highest and Small particles appearing to account for the effects more than Large. Other results suggest no significant difference in faunal responses, specifically relative abundance, diversity, and community composition, to the first season of biochar application. Species richness analysis suggests there are marginally significant trends towards a certain particle size, with Small (sieved; <1mm) treatments collecting the most invertebrates and Mixed (unsieved) treatments suggesting below ground influences, which may also be seen with biomass. This study provides key metrics needed to inform ecological management using biochar, and serves as a model for urban ecology literature, and a model for testing hypotheses about invertebrate community responses to biochar application in urban agricultural settings.

1. Literature Review

Urbanization degrades urban soil health

Since the beginnings of agriculture, the human population has been steadily climbing in number while simultaneously expanding its needs for and uses of soils across centuries and continents (Lal 2007, Brevik et al. 2018). The globe is now filled with portions of land considered undisturbed and others that are highly intensified (Lorenz 2015). Urbanization is an increasing trend in society today. Urban areas are now hosting nearly half of the global population approaching 3.5 billion people (Zipperer and Pickett 2012, Beniston et al. 2016) with two thirds, 5 billion, projected to live in metropolitan areas in 2025, with numbers estimated to reach as high as 6-7 billion by 2050 (Zipperer and Pickett 2012). Rapid urbanization is degrading urban soils. This is evident in several soil models (Effland and Pouyat 1997, Jenny 1994, Jenny 2012) where an additional variable is incorporated to capture anthropogenic ("new soils") soils (Pavao-Zuckerman 2008). Within cities, these "new soils" are likely to have low levels of minerals and nutrients. As urbanization proceeds, urban soils continue to be degraded, following a trajectory of irreparable damage impacting soil properties, chemistry, and functionality.

Urban soils develop by combining somewhat native soils, such as those in grasslands (mollisols), forests (alfisols), or agricultural areas (Pavao-Zuckerman 2008, Kimpe et al. 2000), with soils of differing composition, such as those in gardens, parks, and campgrounds (Morel et al. 2005, Craul 1992, Kimpe et al. 2000), along with non-biological materials often remnants from construction and development projects (Morel et al. 2005). This accounts for the high heterogeneity often associated within urban soils. In addition to mixing natural and unnatural elements, urban soils are also influenced by disrupted soil structure (Lal 2012; Lorenz 2015), compaction (Scharenbroch et al. 2013, Lorenz 2015, Pavao-Zuckerman 2008), pollutant and heavy metal contamination (Scharenbroch et al. 2013, Kumar and Hundal 2016), importing and exporting of materials (Morel et al. 2005), water filtration disruptions (Lorenz 2015). In modern day, it is important to highlight that such soil degradation is connected to urbanization which is deeply tied to socio-, political, and economic factors leading to the commonality of toxic wastes in neighborhoods/areas where non-wealthy, typically non-white, residents reside (Taylor 2014, Schell et al. 2020). Intentional activities that contribute to detrimental and deleterious effects on urban soils continue to modify the land further away from the original qualities (Craul 1992) calling for conservation and restorative soil management practices in urban areas.

Projected city population growth will require additional infrastructure, such as housing and sanitation, alongside opportunities to reduce greenhouse gas emissions, hence the increasing interest in restoring and revitalizing urban soils (Beniston and Lal 2012, Kumar and Hundal 2016). In a report presented at the Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA) 10 conference, Cheng, et al. (2021) acknowledges that soils play an irreplaceable role in key ecological processes and ecosystem services that allows for food production, stormwater management, fostering sustainable cities with green spaces. Urban soils also play a role in other direct benefits like combating heat island effect and improving water and air quality. These essential ecosystem services are dependent on ecological processes and biodiversity (Morel et al. 2015).

An amendment for urban soils: Biochar

Application of biochar to soil is not a new concept and has in fact been used for anywhere between 500-2500 years (Filiberto and Gaunt 2013, Lehmann et al. 2006) mostly gaining attention more recently due to the urgent needs to address a growing food insecurity and environmental concerns, including climate change. Biochar is a form of “black carbon” that remains when vegetation is burned at a certain temperature (pyrolysis) while also devoid of oxygen (Sohi et al. 2010; Lehmann et al. 2011) . The resulting product is a carbon-rich material with characteristics that have been found to both improve soil fertility, and potentially act as a sustainable alternative to current fertilizers and additives that impact soils negatively (Nigh and Diemont 2013, Lehmann and Joseph 2009). Various studies have found biochar to increase both activity and biomass of soil microbes likely due to its ability to provide habitats for microorganisms (Sheng and Zhu, 2018) with Hust and Major (2010) accrediting microbial differences to the high porosity in biochar. Through examining the diversity of microbial communities in Amazonian dark earths or *Terra Preta,* which is known to contain high amounts of black carbon and to be highly fertile, O’Neill et al. (2009) reported a larger populations of bacteria in Anthrosols, a type of soil characterized as being heavily modified by human activity, as compared to the adjacent, nutrient poor soils in the Amazon. Biochar can also retain nutrients (O'Neill et al. 2009), contributing to plant nutrition and reducing the need for fertilizer (Lehmann and Joseph 2009, Marris 2006). This form of black carbon has also been found to increase soil water retention and storing capacities highlighting the potential to reduce irrigation needs (Liang et al. 2006). For example, Glaser et al. (2002) reported higher water retention capacity in Amazonian Dark Earths as compared to adjacent soils with little to no charcoal-like products. Furthermore, biochar can also influence crop production. Lehmann and Rondon (2006) report that plant crop production and biomass increased with the addition of biochar, although the response was highly dependent on the type of crop, the selected feedstock to produce biochar, soils characteristics, and the proportion of biochar to soil that was used (Backer et al. 2016, Li et al. 2017/2016, Lehmann et al. 2006). The authors stated that plant biomass and crop production are likely to respond positively until a certain application rate reaches a maximum or a threshold in which the growth response will then be negative (Lehmann et al. 2006).

Biochar and Soil Fauna

Even with much evidence suggesting that biochar is a beneficial amendment for soils, regarding their ability to improve soil health, there remains much understudied regarding ecosystem processes at work with biochar addition. Although much of the literature on biochar outlines impacts on biota like microorganisms and fungi there are few studies that discuss the impacts of biochar on soil meso and macro arthropods (Lehmann 2011, Ajema 2018). Considering soil animals in the realm of biochar studies is essential given the vital role that soil fauna plays in many soil related ecosystem functions and services (Crossley et al. 1989). Verheof (2004) suggests that soil fauna play key roles at a macro level by developing and shaping soil structure, as well as at a micro level by facilitating mineralization of organic matter. In Ant Ecology, (2010), it was highlighted how disturbed soils, either natural or human influence, can have negative impacts on ant populations, a key group of organisms in soil health as they participate in nutrient cycling and act as indicator species reflecting possible responses of other invertebrates (Philpott et al. 2010). These processes, in turn, influence primary production (Verhoef 2004), energy flow and soil ecosystem's dynamics. Furthermore, soil fauna impact soil structure along with energy flow within soil systems by feeding and fragmenting organic material (Cardoso et al. 2013) in addition to grazing on microbes (Crossley et al. 1989, Domene 2016). This is particularly important with the application of biochar as both of these activities can affect the way activated charcoals interact with soils and how microorganisms respond to the addition. In Palansooriya et. al (2019), for example, the authors reviewed the response of microbial communities to soils amended with biochar (biomass-derived black carbon), highlighting that biochar has the power to alter soil structure and surface area, pore space belowground, soil chemistry (metal content and pH), all of which have been shown to have varying effects on the soil biota. Although there is strong evidence that earthworms ingest biochar (Lehmann et al. 2011, Ajema 2018), there is evidence of other meso and macro fauna species showing both positive and negative effects depending on the species observed, soil characteristics, biochar properties, in addition to biochar application rate and particle size.

In this study, we focus on soil invertebrates response to four biochar treatments: Large (>1 mm) particles, Mixed (unsieved) particles, Small (<1 mm) particles, and a Control (no biochar addition), with Mixed being a combination of the other two particle sizes. We evaluated the response of soil meso- and macro fauna, to the addition of biochar in urban soils. We estimated the diversity, abundance, richness, and community structure of invertebrates under the three biochar treatments. We hypothesize that when biochar, particularly the Large size, mixed with the clay like soils (small particle size) of the urban site where we conducted the study will change the soil structure potentially creating more pore space in between particles. This will allow for increased movement belowground, may it be water or organisms, which can further improve issues urban soils face, like compaction or low water retention. Because of that we further hypothesize that the microbial and fungal communities will be positively impacted, indirectly influencing the invertebrate populations by providing increased food resources (Fig.1).

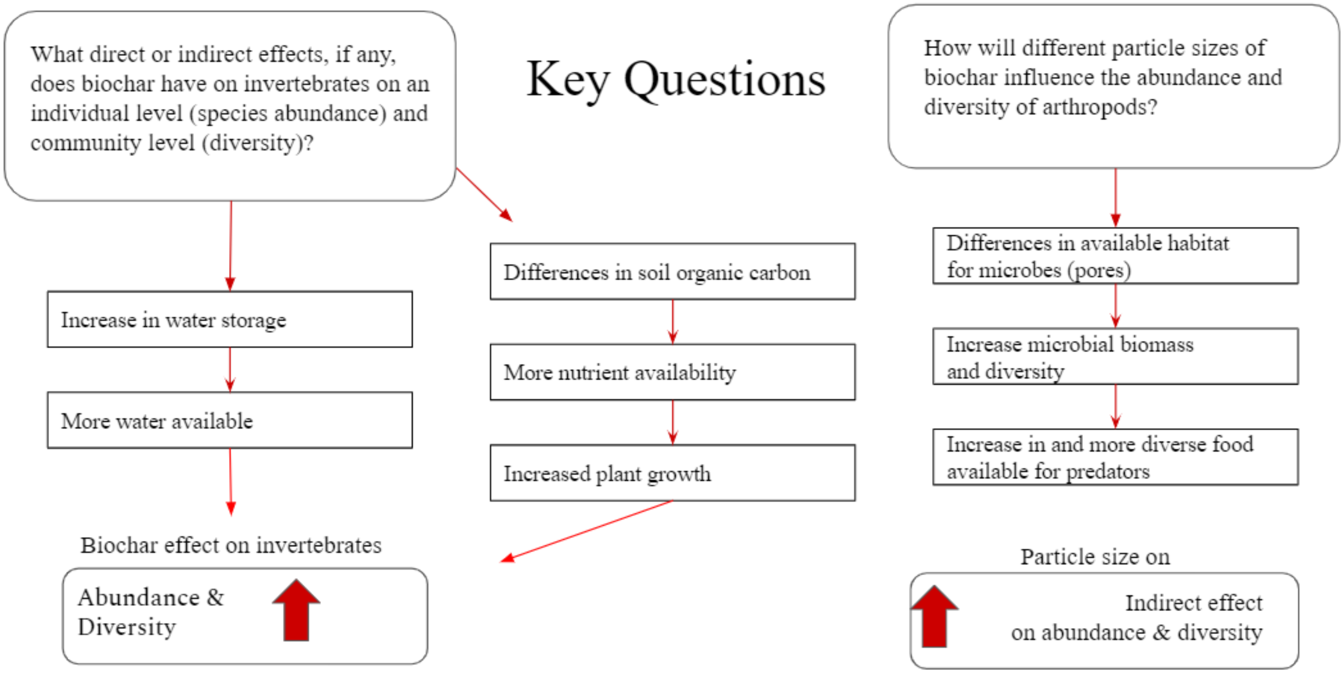


Figure 1 highlights key questions we aim to answer in this study. We are seeking to explore what effects, if any, biochar has on the invertebrate community in an urban environment. We aim to answer those questions looking at abundance and diversity. We anticipate an increase in both given the effects biochar has on water availability, nutrient availability, and influences on carbon and plant production. Further we ask how particle size may influence the repose of invertebrates. We believe with the change in soil structure that biochar provides, specifically in porosity, that will create more micro habitats for microfauna and will therefore indirectly influence the abundance and diversity of soil fauna.

Materials and Methods:

Study site

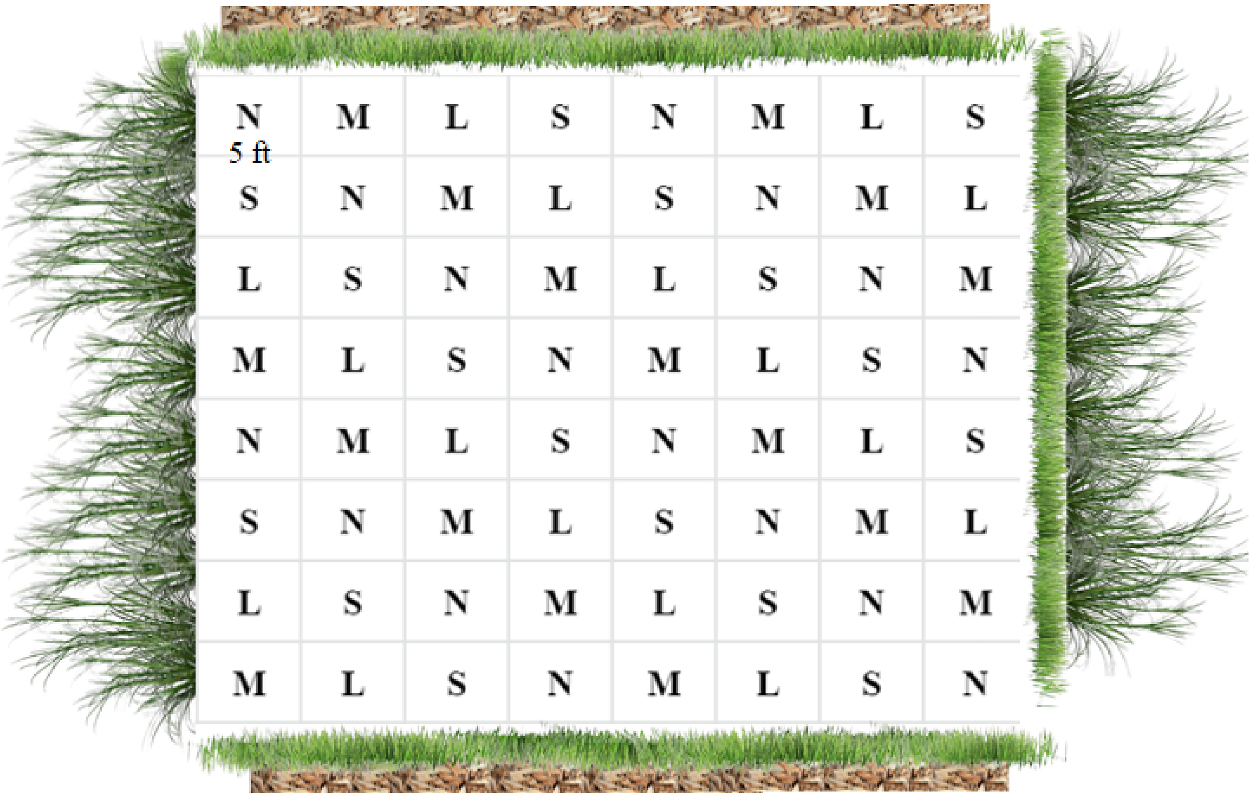
The Detroit Partnership for Food Learning and Innovation is located in the Riverdale neighborhood, near Brightmore, in Detroit of Southeast Michigan, USA. This is Michigan State University's first urban focused research center that hosts a variety of research projects. Its research focus includes "soil sampling and pollution cleanup, pest and crop disease management, forestry, innovative growing systems and community food systems development." Soil characteristics of the research site can be seen in Table 1.

Type	Soil Indicator	Value	Comparison	Issues
Biological	Organic matter (%)	2.8	Very low	Ion exchange Water retention C storage
	Respiration ($mgCO_2d^{-1}$)	0.2	Excellent	
Physical	Aggregate Stability (%)	16.9	Very low	Compaction Infiltration Runoff/Erosion
Chemical	pH		Very high	Plant toxicity Nutrient availability
	Extractable P (ppm)	2.2	Optimal	
	Extractable K (ppm)	107.8	Optimal	

Table 1. Site characteristics based on standard soil testing protocols used by Michigan State University analytical facilities. Soil properties, biological, chemical, and physical were reported.

Experimental design

The research was conducted in a 50 x 50 foot plot (Fig. 2). The plot was cleared of grasses and weeds the previous summer (2019) and covered with a plastic tarp of the same area. The plot is surrounded by short grasses 1-2 feet away from the perimeter. On the right side adjacent to the short grasses are medium to tall grasses that are occasionally trimmed. Two of the sides, front and back, short grasses are disrupted by a row of wood chips. The final side, left, has short grasses, long grasses, and leading to an organic cover crops and vegetable plots including sorghum, buckwheat, maize, and radish. Before outlining the plot, the soil was rototilled at 2 inches and left for 24 hours before biochar application. A grid of 8 rows and 8 columns was established for a total of 64 5 x 5 foot plots (Fig. 2). Roughly 1 foot on all four sides was cleared for pathways around each plot, excluding edge plots. We planted 2 rows of two types of cover crops: Cowpea, *Vigna unguiculata* (from a local organization Keep Growing Detroit distributing seeds from Johnny's Selected Seeds supplier) and Beech wheat *Fagopyrum esculentum* [Johnny's Selected Seeds], 8 individual seeds per row were sown in each 5 x 5 plot. Plants were intercropped as alternating rows. Given the soil type, like whether the soil is classified as clay like or more sand-liem structure and additional resources that may influence the biochar, Cowpea was selected due to its characteristic of aiding nitrogen availability**.** Beech wheat, or buckwheat cover crop is said to attract insects and has the ability to mine soil phosphorus. Black-eyed peas and beech wheat have been recommended to rebuild poor soils, are viable late into Fall (i.e. October), are commonly grown throughout the region, and are annuals, thus they are relatively shallow-rooted.



N 5 ft	**M**	**L**	**S**	**N**	**M**	**L**	**S**
S	**N**	**M**	**L**	**S**	**N**	**M**	**L**
L	**S**	**N**	**M**	**L**	**S**	**N**	**M**
M	**L**	**S**	**N**	**M**	**L**	**S**	**N**
N	**M**	**L**	**S**	**N**	**M**	**L**	**S**
S	**N**	**M**	**L**	**S**	**N**	**M**	**L**
L	**S**	**N**	**M**	**L**	**S**	**N**	**M**
M	**L**	**S**	**N**	**M**	**L**	**S**	**N**

Figure 2 shows the experimental design of the research site along with a photograph of the area after biochar was applied to the soil; L=large particle biochar (>1 mm), M=mixed particle (both large and small), S=small particle biochar (< 1 mm), N=no biochar.

Biochar Application

Biochar was purchased from Wakefield Biochar (Columbia, MO; https://www.wakefieldbiochar.com/). Characteristics are listed in Appendix I. The biochar used is made from pine wood vegetation.

Compost from Tuthill Farm & Composting (South Lyon, MI) was applied to the plot (https://www.tuthillfarms.com/) in May 2020 and remained under the 50 x 50 foot plastic tarp until mid July, 2020. The tarp was removed roughly 1 week prior to application. Biochar was sieved by hand to 1mm resulting in sizes smaller than 1mm and larger than 1mm when separated. Four treatments of biochar - Large, Mixed, Small and None (control with no biochar application) - were applied to the corresponding 5 X 5 foot subplots at proportions of ~1.75% w/w (~5% v/v) 2 days before sowing the seeds. For a second application of the same amount of biochar, the biochar was activated to include nutrients by mixing with compost from Tuthill compost at 1:1 v/v and letting it sit outdoors for 5 days prior to adding it to each subplot one week after the initial sowing of the seeds (2 days after biochar was applied initially) (Sun et al. 2016).

Plot maintenance

From August to September the plot was maintained and watered every 2 days with use of a rotating sprinkler system (Gilmour Sprinkler [circular]). Watering was typically 0.5 - 1 h in the morning or early afternoon. Weeds were removed every few days as well.

Soil moisture

Moisture readings were collected August to September 3 times per week, Monday, Wednesday, Fridays. We used a soil hygrometer (XLUX) with a sensor and a volumetric water content sensor/reader? via small electric potentials with handheld soil moisture meter (Vegetronix). Probes took measurements between 2.5 - 5 centimeters in depth during late morning, 09:00 - 14:30 EST.

Soil Arthropods Collection, Identification and Biomass Estimations

Pitfall traps (16 cm in diameter and depth) were used to collect soil fauna. One pitfall trap was placed near the center of each plot and were left open for 72-96 hours in late August 2020. Seventy percent ethyl alcohol was added to each pitfall trap (Greenslade and Greenslade 1971). Coverings of a wider diameter were hoisted above traps to avoid flooding if it rained, although that did not occur.

All invertebrates, excluding 11 individuals specimens, were identified by a collective effort of 6 researchers using taxonomic keys, invertebrate guides (literature and online formats), and other useful online resources (Dunn 1996; Eisenbeis and Wichard 2012). See Appendix II for details on the specific sources. Size class determination into either meso- (<2 mm) or macro- (>2 mm) was made by Order: with meso- including Collembola, Acari, Ixodida, and Psocoptera; and macro- including all other observed arthropods.

Biomass was recorded for each sample (all arthropods in each pitfall trap) from each plot. Samples were dried for 72 - 96 hours prior to weighing. Each sample was removed from its storage container, placed on a tared weigh boat, placed on a scale (Sartorius) and the mass was recorded.

Statistics

Soil moisture measurements were pooled from 3 sub-measurements per plot to a single average per plot (n = 16 per treatment), and maintained separately by sampling day. All other variables were originally measured at the plot level, so raw values were used for analysis. Shannon diversity (information entropy) was calculated using the ‘diversity’ function in the ‘vegan’ package (v2.5.7) in R (v3.6.1, 2019). All variables were then checked for normality using the Shapiro-Wilks test, as the base ‘shapiro.test’ function in R. All variables were not normally distributed, so Kruskal-Wallis tests were run to study treatment effects, as the ‘kruskal.test’ function in R. Dunnett post-hoc tests were also run for certain variables with pairwise comparisons reported, using the ‘DunnettTest’ function in the ‘DescTools’ package (v3.6.2). Finally, morphospecies relative abundances were ordinated by plot using the NMDS method, which focuses on ranking rather than other more strict (e.g. Euclidean) distance metrics, as the ‘vegdist’ function from the ‘vegan’ package in R, and treatment effects were then studied using a PERMANOVA, as the ‘adonis’ function also from the ‘vegan’ package in R.

RESULTS

Moisture

Overall, soil moisture varied significantly over the season, as well as by treatment. Specifically, moisture of the top 5 cm of soil showed a wide range of values, from 10 to 100% humidity, with apparently regular fluctuations every ~15 days, despite being watered every other day (Fig. 3). In Fig 3c., data points show that on randomly selected days (5, 24, 32, 38) the none plot is consistently the driest with the lowest levels of relative humidity. Soil relative humidity showed significant differences among all biochar treatments compared to controls ($p \leq 0.01$, AIC = 4292). Specifically, days 5, 24, 32, and 38 were representative in showing significant differences from control ($p \leq 0.05$, AIC ≤ 247). Volumetric water content showed a significant difference of only the mixed treatment compared to control ($p = 0.0085$, AIC = 2504).

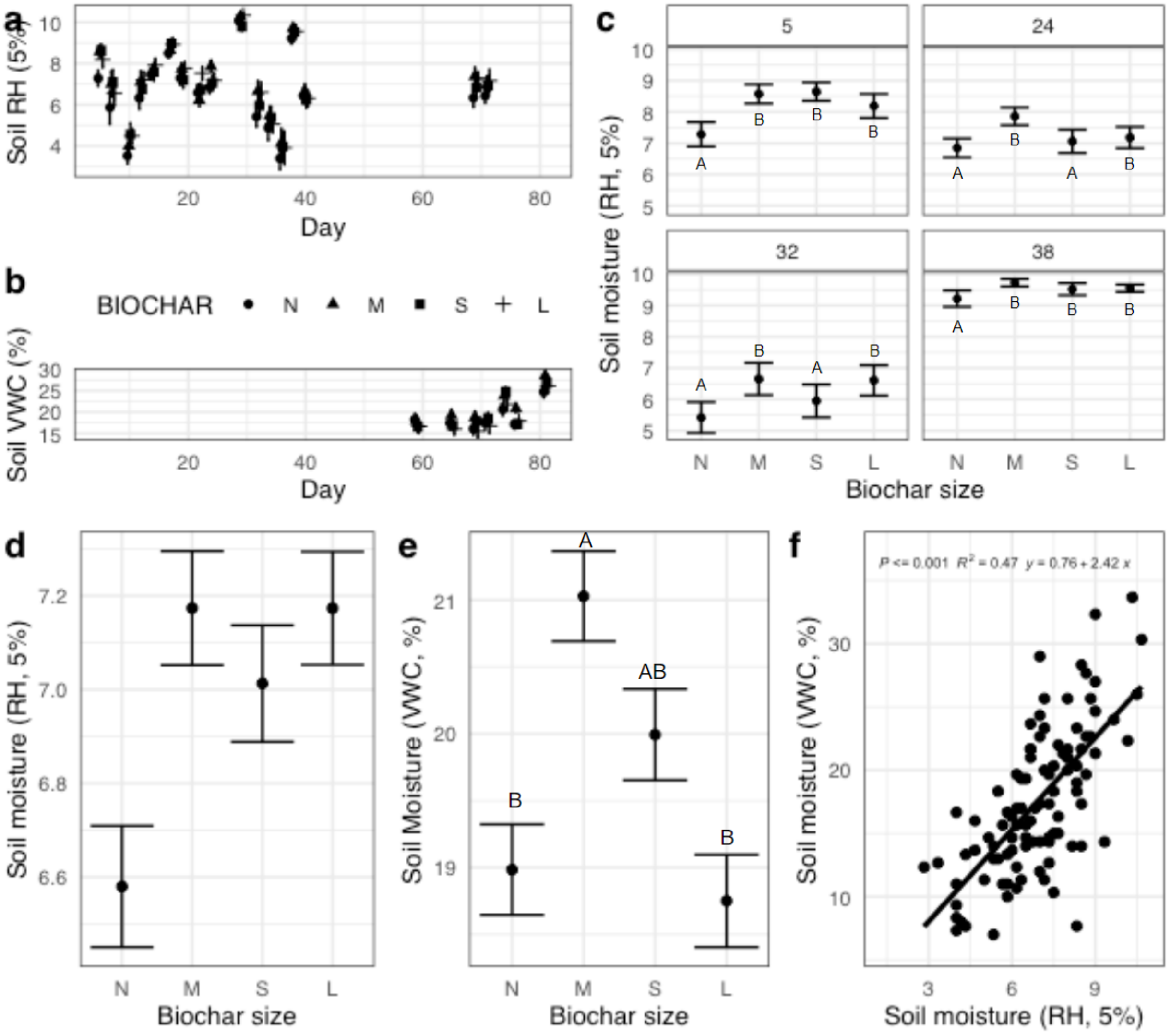


Figure 3. Soil moisture in the top 5 cm, measured as (a) relative humidity (RH) over time and (b) volumetric water content (VWC) over time. (c) RH by biochar treatment for specific days, (d) RH by treatment over all days measured, (e) VWC by treatment over all days measured, and (f) how RH and VWC values correlate among all data. Treatment replication is *n* = 16. Error bars show ±1 SE.

Invertebrates

The most abundant arthropod groups recorded in our samples were Collembola (879), with Hymenoptera (279), Acari (165 total individuals), Araneae (85), and Hemiptera (55) distantly following. Overall, mesofauna (< 2 mm) were more abundant than macrofauna (> 2 mm) across all treatments and the control.

Abundances by Treatment

No consistent pattern for total abundance of individuals was found related to biochar treatments. The least total number of individuals were collected from the Mixed biochar treatment (372), while Small collected the most total individuals (421). We found a total of 11 orders and divided them into two groups, mesofauna and macrofauna. Mesofauna consists of Acari, Collembola, Isopod, Ixodida, Psocoptera. Macrofauna consist of Araneae, Coleoptera, Diptera, Gastropod, Hemiptera, Hymenoptera. 24 individuals remain unknown at this time (Table 2). For Macrofauna, the mixed biochar treatment had the least total number of individuals (105) while the control had the most (139). For Mesofauna, the Large particle biochar treatment had the least total number of individuals (259), while the Small particle biochar had the most (279) (Table 2).

		Treatment				
Size	Order (shorthand)	None	Small	Large	Mixed	*Sum*
Macrofauna						
	Araneae (Area)	21	26	19	19	*85*
	Coleoptera (Cole)	27	12	20	25	*84*
	Diptera (Dipt)	0	3	3	1	*7*
	Gastropod (Gast)	1	0	2	0	*3*
	Hemiptera (Hemi)	7	22	18	8	*55*
	Hymenoptera (Hyme)	83	69	73	54	*279*
Mesofauna						
	Acari (Acar)	42	44	48	31	*165*
	Collembola (Coll)	218	229	206	226	*879*
	Isopod (Isop)	0	0	2	2	*4*
	Ixodida (Ixod)	6	5	3	5	*19*
	Psocoptera (Psoc)	0	1	0	0	*1*
	Unknowns (Unkn)	8	10	5	1	*24*
	Totals	*413*	*421*	*399*	*372*	*1605*
	Macro Fauna total	*139*	*132*	*135*	*105*	*513*
	Meso Fauna total	*266*	*279*	*259*	*264*	*1068*

Table 2. Totals of all invertebrates sampled, separated by order, per treatment (Small = < 1 mm, Mixed = > 1 mm). All calculations represent the total sum of individuals per order and the total of individual specimens per treatment across all 16 replicates. Unknown (Unkn) were excluded from Macro Fauna total and Meso Fauna total.

Abundance by Order

Total abundance did not vary significantly among biochar treatments compared to control ($p > 0.10$, AIC = 1100). Relative abundances of macro and meso fauna varied across biochar treatments but were not statistically significant ($p > 0.10$, AIC = 273) and did not follow any consistent pattern (Fig. 4). Results show significant differences in relative abundances among orders ($p < 0.05$, AIC = 124), driven by “Area”, “Coll”, “Hyme”, and “Ixod” (Fig. 4). Finally, there was a marginally significant effect of invertebrate size class (meso vs. macro) on relative abundances ($p < 0.001$, AIC = 273) and significant effect on absolute abundances ($p < 0.001$, AIC = 1495)

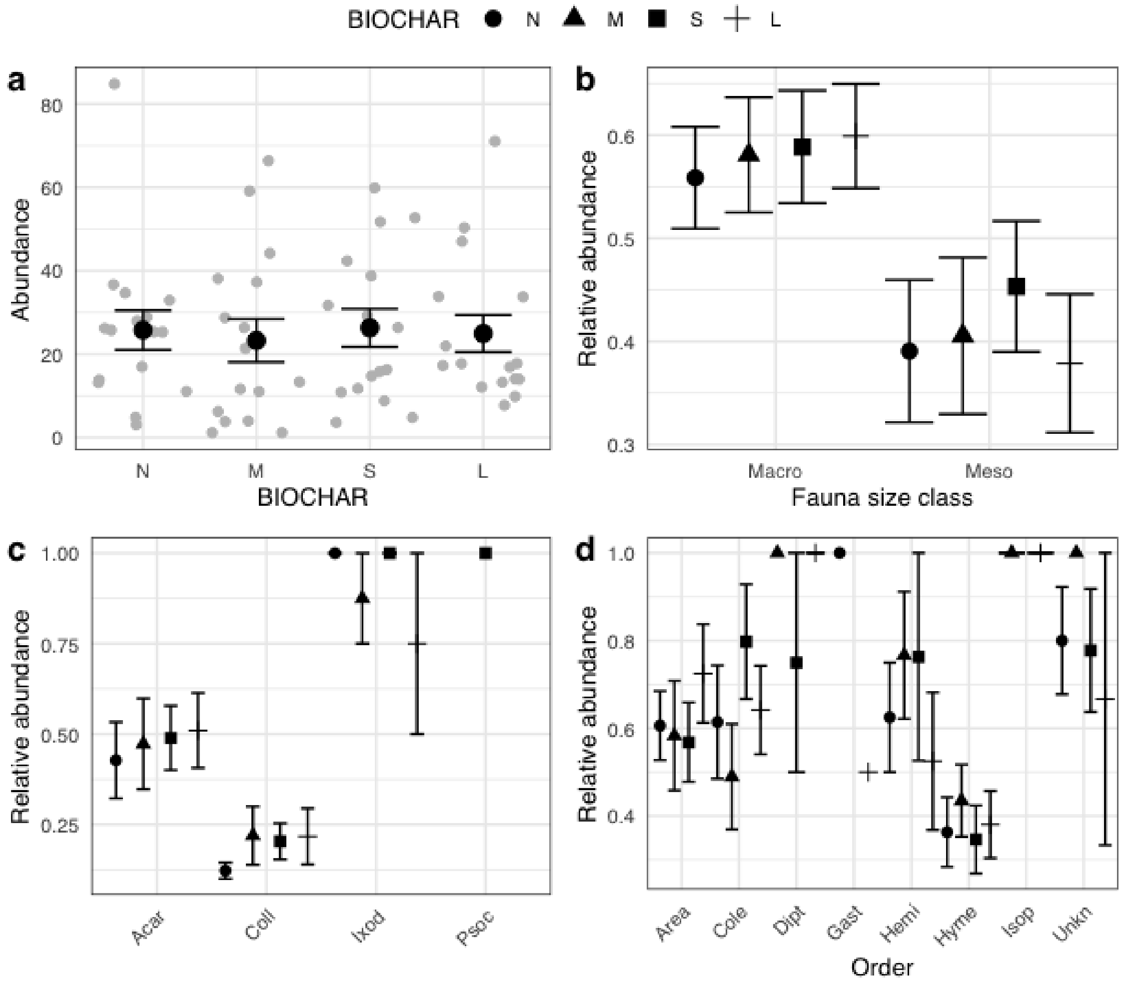


Figure 4. (a) Absolute abundance by treatment, and relative abundance by (b) treatment and invertebrate size class, (c) mesofauna Order, and (d) macrofauna Order. Treatment replication is *n* = 16, and N = None (control), M = Mixed (unsieved), S = Small (<1 mm), L = Large (>1 mm). Error bars show ±1 SE. Invertebrate orders are Acari (Acar), Collembola (Coll), Ixodea (Ixod),

Psoc (Psoc), Areanae (Area), Coleoptera (Cole), Diptera (Dipt), Gastropoda (Gast), Hemiptera (Hemi), Hymenoptera (Hyme), Isoptera (Isop), and unknown (Unkn). Error bars show ±1 SE.

Biomass

Overall, biomass showed slight yet weak differences across treatment, with a marginally significant difference of ~0.25 g between Small plots and control plots (p = 0.07, AIC = -163) (Figure 5).

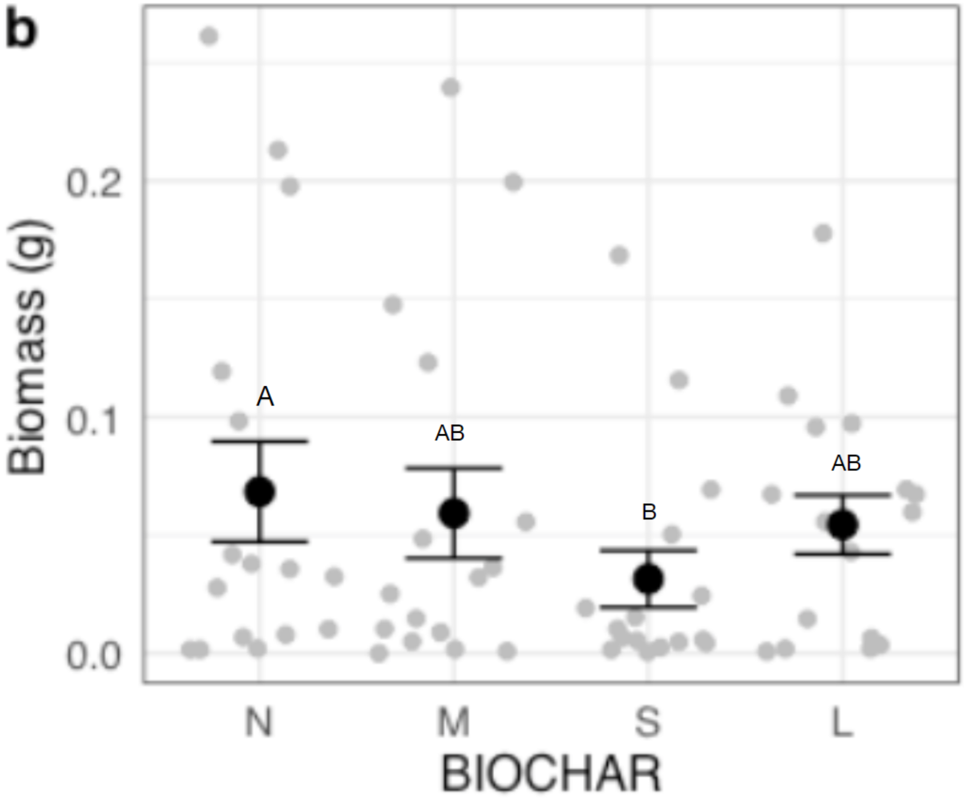


Figure 5. Average biomass of soil invertebrates per plot by biochar treatment of None (control), Mixed (unsieved), Large (> 1 mm), and Small (< 1 mm) particle sizes (n = 16). Error bars show ±1 SE, raw plot values shown in gray and means in black.

Diversity and Richness

There was no significant difference in Shannon diversity among biochar treatments ($p > 0.10$), though there was a marginally significant difference between Mixed and control plots ($p < 0.05$) (Fig. 6). When separated by order, biochar treatments showed no significance differences ($p > 0.10$) in regards to richness, but there was significant difference in richness between body size (meso vs macro) of collected invertebrates in response to biochar ($p < 0.05$).

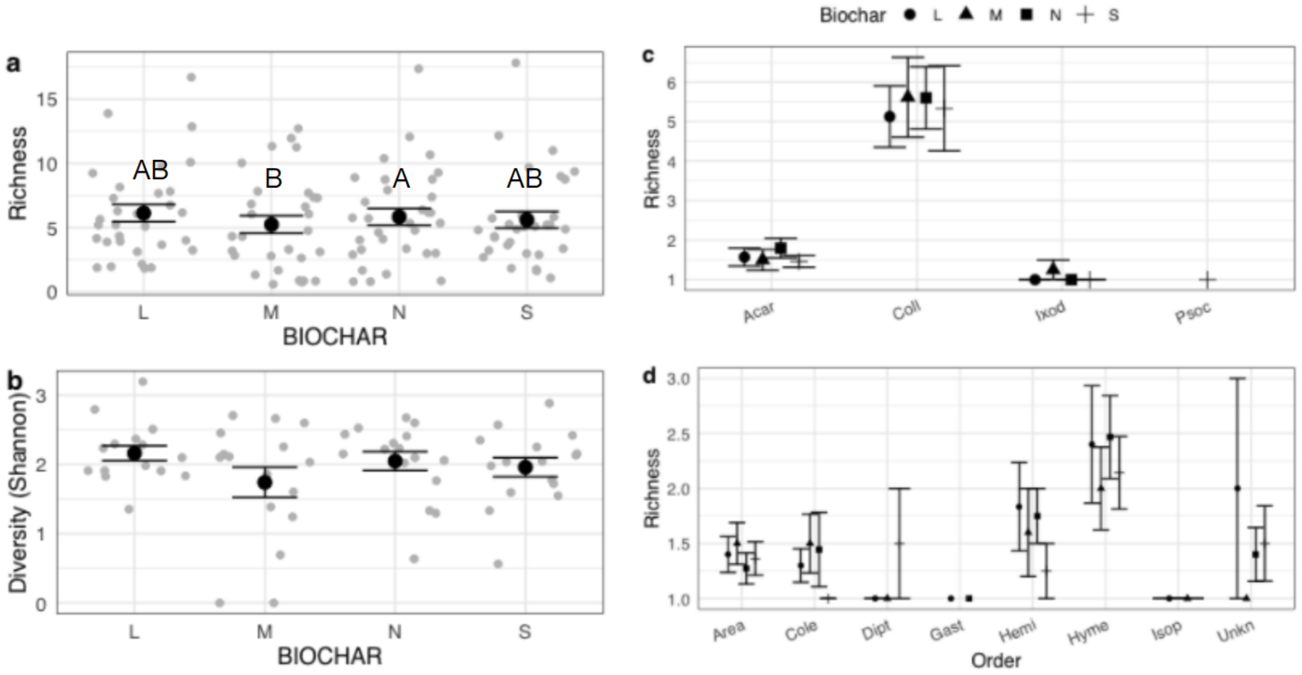


Figure 6. Biochar treatment averages of morphospecies (a) richness and (b) Shannon diversity, along with morphospecies richness of (c) mesofauna and (d) macrofauna orders. Treatments are None (control), Mixed (unsieved), Large (> 1 mm), and Small (< 1 mm). Invertebrate orders are Acari (Acar), Collembola (Coll), Ixodea (Ixod), Psoc (Psoc), Areanae (Area), Coleoptera (Cole), Diptera (Dipt), Gastropoda (Gast), Hemiptera (Hemi), Hymenoptera (Hyme), Isoptera (Isop), and unknown (Unkn). Error bars show ±1 SE.

Community composition

Community composition shows no significant difference by treatment indicated by the overlap of ellipses. The size of the ellipses corresponds with variation among plots. Small particles and no-biochar had the least variation among plots and mixed biochar had the most variation among plots. However, these differences were not statistically significant.

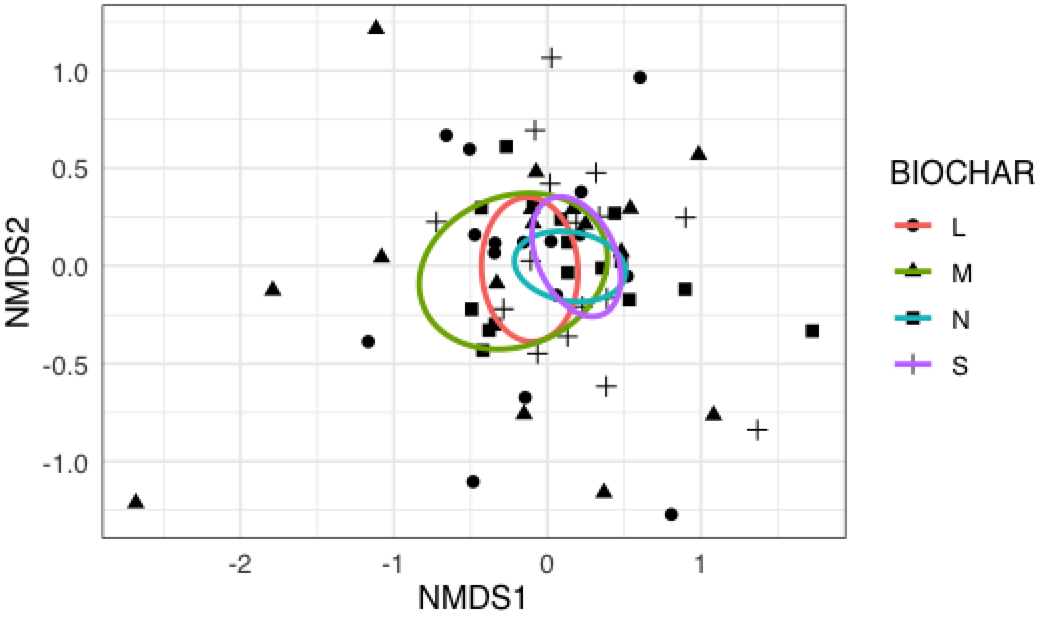


Figure 7. NMDS ordination of invertebrate community composition based on morpho-species relative abundances at the plot level (n = 16). Ellipses show 95% CI around centers by treatment group. Each dot is a plot associated with a biochar treatment: None (control), Mixed (unsieved), Large (> 1 mm), and Small (< 1 mm).

DISCUSSION

This study provides evidence that biochar has the ability to retain moisture in soil (Fig. 3). We found that this varies based on particle size, with Small biochar particles retaining less water than Mixed and Large particles biochar. However, this effect did not have an observable impact on the arthropod community on this particular urban farm. Biochar has been reported to increase microbial biomass and alter the microbial community, such as increasing the fungal:bacterial ration found on soil, improve plant productivity, enhance nutrients, and change soil structure (Maraseni 2010; Garbuz et al. 2020; Andrés et al. 2019; McCormack et al. 2019; Lehmann et al. 2011). We hypothesized that in addition to these benefits, varying biochar particle sizes, particularly the Large particle size with clay soils, made of assorted smaller particle sizes, would mix to create a loam like soil potentially increasing the availability of nutrients and microorganisms which may support higher resources for the soil fauna, specifically meso or macro invertebrates. We predicted that alteration in themicrbial community would be reflected in invertebrate diversity and abundances however no such effects were observed in this study. Instead, arthropod communities were significantly shaped by other factors like size class (meso vs. macro) and taxonomic order (Table 2, Fig. 4, Fig. 6). Overall, the research on biochar and its impacts on meso and macro fauna is largely understudied (Castracani et al. 2015) and therefore, there are few studies that can serve as comparison.

Time Frame

There are a few reasons that may explain the lack of observed effects of biochar on soil invertebrates in this study including the short duration of sampling. Our study lasted a little more than one month from the initial application of the biochar to the collection of the arthropod samples. Some studies of similar timeframe see no effect on any response (Castracani et al. 2015, Marks et al. 2014). However, we think it is possible that invertebrates had the potential to respond to treatment after this short period of time because several studies have reported changes in the microbial activity (Domene et al. 2015) and mesofauna reproduction based on biochar type (Marks et al. 2014) within a shorter time frame (28 days) with others reflecting indirect effects on macroinvertebrates after longer periods of time (up to 30 months) (Andrés et al. 2019; McCormack et al. 2019). Despite this, future studies of net effects of biochar application on the meso and macro faunal community would likely benefit from longer management periods.

Mesofauna responses

A more ecological reason for net neutral invertebrate responses may be opposing effects at the population level on different life stages - namely positive for juveniles and negative for adults. Studies show that microorganisms, both bacterial and fungal, react to biochar application, often positively (McCormack et al. 2019, Warnock et al. 2007, Dangi et al. 2020, Rousk, Dumpster, Jones 2013) in part due to higher soil moisture. This is most likely to support the immature stages of some insects, who require moist environments, and whose molting may even promote microbial growth (Maas et al 2015). For example Domene (2015) assessed mesofauna responses, specifically in collembola (*Folsomia candida*) and enchytraeid (*Enchytraeus crypticus*), to corn stove biochar and found increased microbial biomass and activity as biochar application increased, up until a certain point (day 61). With this, authors observed avoidance by

Collembola, but saw significant preference in the *Enchytraeus* annelid worms, at different application rates (0.2%, 7%), which is interesting since this study also resulted in an increase in enchytraeid reproduction at 0.5 and 2% application rates (Domene et al. 2015). In another study (Marks et al., 2014), it was found that depending on biochar characteristics, the effects on mesofauna (collembola and enchytraeids) could result in either high adult mortality, high reproduction, or no effect at all. The authors attributed this to the high variability in the production of and the application of biochar further supporting that meso and macro faunal responses are often dependent on the type of biochar (pine wood, corn stover, bamboo, etc.), pyrolysis temperature (550°C, 600°C, 800°C ect.) application rate (0.2-14%, or others), and even particle size in some cases (0.5mm, <2mm, <5mm, etc.) (Marks et al. 2014). Another consideration here relates to He et al. (2018) and Proderna et al. (2019) both reference several studies that state different sizes of biochar result in different levels of effectiveness and influences, with fine sizes (75–150 µm) more successful at immobilization and absorption of metals and aqueous solutions with little discussion on larger particle sizes of biochar. Interestingly, it's possible that biochar can offer resources that foster reproduction and potential risks for invertebrates, depending on the size or taxonomic order.

Macrofauna responses

The variable effects of biochar addition on soil fauna is also reflected in several studies focusing on earthworms and biochar, in which results are variable, but many suggest certain biochar additions could be toxic for adult earthworms. For example, Prodana et al. (2019) found biochar, specifically wood chip biochar, to be toxic for earthworms observed through avoidance and decreased body mass. However Garbuz et al. (2020) found that the combination of biochar and earthworms as a treatment revealed significant positive effects on plant production, collembola abundance, and beneficial chemical properties for soils possibly suggesting neutral effects on the earthworm population, even as it was exposed to a 1% application rate of biochar. Our study supports the possibility that different effects on organisms are due to body size, with smaller micro and mesofauna potentially being supported by and benefiting from biochar compared to macrofauna which tend to be negatively impacted. Direct effects of biochar have been more evident in the research, but this study suggests indirect effects can also be a point of insight and observation.

With this in mind, we propose this study be used as a model experiment to explore the effects of biochar application on more taxa defined as mesofauna and macrofauna overall. Mesofauna, like Acari have been reported to play a role in soil fertility, perhaps by way of contributing to the below and above ground food chain (Lehmitz et al. 2012). Assessing the impacts of biochar, in a variety of ways, is crucial to understanding both direct and indirect impacts this amendment can have on soil invertebrates, which are known to be vitally important in soil health (Crossley et al. 1989). In a review by Lehmann et al. (2011), it is clear that biochar can produce a wide range of responses in the microbial and fungal communities, plant production, micro, meso, and macro faunal communities in addition to affecting soil properties and characteristics. Very few studies, if any, explore the impacts of biochar on large macrofauna, such as Coleoptera or Hemiptera making it somewhat difficult to synthesize a consensus from this and other studies. In addition to potential toxicity on macroinvertebrates, as seen with earthworms, dispersal techniques and patterns may contribute to the lower abundances of macrofauna, especially those with flight

(Desender, K., 2000) capabilities, so larger study areas/plots may be a help in invertebrate collection methods. A more comprehensive understanding of biological, chemical, and physical properties of biochar are needed in addition to how specific properties impact varying soil communities. In this approach, studies will also need to apply the soil amendment to various soil types as well, since evidence suggests biochar responses may also be dependent on soil type (Garbuz et al. 2020).

CONCLUSION

As the human population becomes more urbanized, the reliance on urban soils for food production could increase. Research to enhance and detoxify highly degraded urban soils is critical to this effort. Biochar amendments have become increasingly popular as a way to enhance soil quality and sequester carbon (Lehmann, 2011). Although the use of biochar has shown promise for improving soil quality and increasing soil microbial biomass, little is known about how biochar amendments affect the broader soil ecosystem, including the soil meso and macro arthropods. In this study we tested the effect of different types of biochar with respect to particle size (Small, Mixed and Large) and found no significant effects on the arthropod community, including abundance, diversity, richness and species composition.

Regardless of the lack of effect on the arthropod fauna, biochar has the ability to encourage several essential ecosystem services like water retention and carbon sequestration, that can improve urban soil health, and help mitigate the effects of climate change (Cheng et al. 2021). Our study did find a positive effect of biochar on soil moisture retention. As urbanization increases nationally and globally, such locations require healthy soils for ecosystem services, food production, and climate change mitigation and biochar is a solution with great potential. Studies show that biochar has the potential to support several beneficial characteristics and help restore degraded soils. Many studies highlight the influences on soil composition and the microbial and fungal communities in response to biochar but few focus on the impacts it may have on soil fauna and the role particle sizes play. In this study, data does support that biochar has an advantageous impact on soil moisture and there may be possible trends highlighting responses by soil fauna. Although no significance was found in relation to soil fauna’s response to biochar, meso- and macrofauna abundance, richness, and biomass do suggest preferences and avoidance may have occurred in the short study. For those reasons, we encourage further research on biochar particle sizes, particularly course, large sizes, the impacts that can have on the faunal communities, mostly focusing on meso and macrofauna, and pursuing a field study with a longer timeframe with detailed observations regarding both above and belowground, micro and macro observations.

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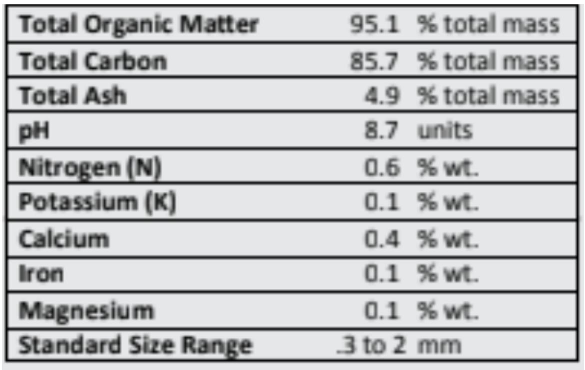
Appendix Supplementary Material

Appendix I: Characteristics of Biochar from Wakefield Biochar



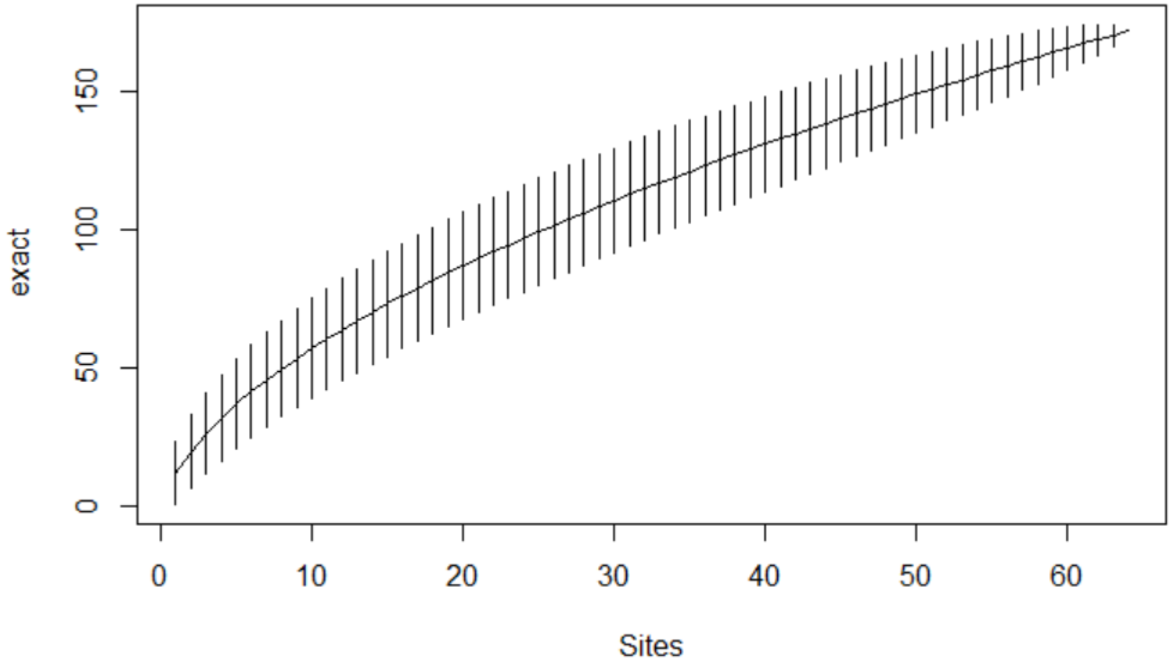
Total Organic Matter	95.1	% total mass
Total Carbon	85.7	% total mass
Total Ash	4.9	% total mass
pH	8.7	units
Nitrogen (N)	0.6	% wt.
Potassium (K)	0.1	% wt.
Calcium	0.4	% wt.
Iron	0.1	% wt.
Magnesium	0.1	% wt.
Standard Size Range	.3 to 2	mm

Appendix II: List of resources used in invertebrates Identification

Books
Insects of the Great Lakes Region / Gary A. Dunn.
Atlas on the Biology of Soil Arthropods
Springtails
Cave Life of Wales
Texas A&M AgriLife Extension
Insect Ecology and Integrated Pest Management
The Michigan Entomologist
Springtails - Families of Springtails
Springtails - Slideshow
Springtails - Familty Determination
Mites
Michigan's 5 Most Common Ticks
Acarid
Major Mite Taxa_Identification Tool
Chaos of Delight - All About Mites Oribatida
Ants
http://antkey.org/en/gallery?page=1

General
Identification Tools/ Keys
Michigan Identification tool
Insect Identification Key
Bug Guide
What's that Bug?
Insect Connect - Identify to Order

Appendix III: Species accumulation curve



Appendix IV: Statistical Analysis

Category	***Variable (per plot)***	***Type***	***Distrib.***	***Indep. Var.***	***Type***	***Test***	***Post-hoc***
Soil matrix	*Volumetric water content*	Contin.	Gamma	*Biochar particle size; Block within Day*	Discrete; Discrete, Count	GLME	EMM
	Relative humidity	Contin. (by 5%)	Gamma	*Biochar particle size; Block within Day*	Discrete; Discrete, Count	GLME	EMM
Inverts.	*Total biomass*	Contin.	Inverse Gaussian	*Biochar particle size; Block*	Discrete	GLME	--
	Total abundance	Count	Poisson	*Biochar particle size; Block*	Discrete	GLME	--
	Relative abundance (order level)	Contin.	Gamma	*Biochar particle size; Block; Invertebrate size class*	Discrete	GLME	--
	Morpho-specie s richness	Count	Poisson	*Biochar particle size; Block*	Discrete	GLME	--
	Morpho-specie s diversity	Contin.	Inverse Gaussian	*Biochar particle size; Block*	Discrete	GLME	EMM