Summer 2022

Soil Mesofauna and Microbial Community Response to Mixed Biochar and Compost Application in a Skagit Silt Loam

Jameson Goff
Western Washington University, goffj97@gmail.com

Soil Mesofauna and Microbial Community Response to Mixed Biochar and Compost Application in a Skagit Silt Loam

by

Jameson Goff

Accepted in Partial Completion
of the Requirements for the Degree
Master of Arts

ADVISORY COMMITTEE

Dr. Andy Bach, Chair

Dr. Rebecca Bunn

Dr. Deirdre Griffin

GRADUATE SCHOOL

David L. Patrick, Dean

Master's Thesis

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Jameson Goff

7/23/2022

Soil Mesofauna and Microbial Community Response to Mixed Biochar and Compost Application in a Skagit Silt Loam

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Arts

by
Jameson Goff
July 2022

Abstract

I sampled Skagit silt loam soils from a field trial at the WSU NWREC where biochar and compost were added to potato crops in late spring pre-planting. Soil mesofauna were sampled at mid and late-summer, while soil microbes were sampled at late summer exclusively. Soil treatments included mixed biochar and compost, compost-only, and an unamended control. Mesofauna were extracted with Berlese funnels and sorted to functional groups. F:B ratios and total microbial C were determined using microbiometer test kits. To test whether biochar and compost induced changes to soil mesofauna communities, I used permutational ANOVA. Differences in F:B ratios and total microbial C were tested using a linear mixed effects model with ANOVA. Mesofauna communities differed markedly between mid and late-summer sampling ($p=0.001$) with a shift from Rhyzoglyphus, and Collembola dominance to a more even mix of functional groups including Formicidae, Psocodea, Symphyla, Apocrita, and Diptera with a diminished overall population. However, neither compost nor biochar treatments had a significant effect on soil mesofauna communities ($p=0.291$) nor did they alter F:B ratios and total microbial C ($p=0.246$ and 0.787 respectively). The microbial community favored bacterial dominance with an average F:B ratio of 0.60"0.21 across treatments and total microbial C was 272.50 µg C/g"115.82. The decrease in mesofauna abundance between mid and late summer likely reflects seasonal changes in mesofauna activity due to differences in soil temperature and moisture levels. The F:B ratio favoring bacterial dominance is indicative of chemical fertilizer use and heavy tillage which favors fast growing bacterial groups.

Acknowledgements

I would like to thank my thesis committee, Andy Bach, Rebecca Bunn, and Deirdre Griffin for their patience and guidance in writing this thesis.

TABLE OF CONTENTS

List of Tables and Figures

Tables

Table 1. Wet and dry application rates of compost for each treatment with the low (7 Mg dry C ha^{-1}) and high (15 Mg dry C ha^{-1}) application rates for biochar. Measurements recorded by Stacey et al. (unpublished).

Rate	Compost		Biochar 40%	
	Low	High	Low	High
Wet Weight (Mg ha^{-1})	53.91	115.51	45.04	96.53
Dry Weight (Mg ha^{-1})	23.18	49.68	16.66	35.71

Table 2. Chemical properties of soil amendments for each category of treatment group following mixing and maturation of compost with biochar. These are the properties of each soil amendment immediately prior to field application. Measurements were recorded by Stacey et al. (unpublished).

Treatment	C:N	pH	NO3-N (ppm)	NH4-N (ppm)
Compost-only	26.8	7.9	1328	268
Biochar 20%	32.6	7.9	1524	29
Biochar 40%	34.8	7.6	1955	41

Table 3. Average community composition of mesofauna functional groups by treatment at mid-summer (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Data shown as average percentage of the whole community.

Functional Group	C	CL	CH	BC40L	BC40H	Total
Bulb Mite	71.93%	81.20%	61.22%	58.50%	76.88%	69.95%
Collembola	18.39%	12.02%	19.81%	23.69%	8.62%	16.51%
Oribatid	1.19%	3.35%	11.53%	8.48%	9.68%	6.85%
Larva	3.77%	1.22%	3.22%	4.49%	1.21%	2.78%
Lice	0.00%	0.47%	1.06%	1.11%	2.48%	1.02%
Predatory Mite	1.35%	0.28%	1.18%	2.80%	0.91%	1.30%
Symphylan	1.54%	1.46%	0.84%	0.00%	0.11%	0.79%
Spider	0.00%	0.00%	0.70%	0.37%	0.00%	0.22%
Fly	0.61%	0.00%	0.28%	0.27%	0.11%	0.26%
Beetle	0.31%	0.00%	0.16%	0.27%	0.00%	0.15%
Thrip	0.93%	0.00%	0.00%	0.00%	0.00%	0.19%
Wasp	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ant	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 4. Average community composition of mesofauna functional groups by treatment at late-summer (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Data shown as average percentage of the whole community.

Functional Group	C	CL	CH	BC40L	BC40H	Total
Bulb Mite	38.13%	22.62%	15.63%	30.52%	32.91%	27.96%
Collembola	15.00%	26.67%	6.25%	10.56%	3.85%	12.46%
Oribatid	10.00%	0.00%	0.00%	0.35%	8.55%	3.78%
Larva	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Lice	5.00%	5.00%	6.25%	8.13%	9.40%	6.76%
Predatory Mite	0.00%	0.00%	7.81%	0.00%	1.92%	1.95%
Symphylan	9.38%	6.67%	4.69%	0.00%	0.00%	4.15%
Spider	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Fly	22.50%	24.29%	37.50%	44.39%	32.05%	32.15%

Beetle	0.00%	0.00%	10.94%	5.70%	7.48%	4.82%
Thrip	0.00%	0.00%	3.13%	0.00%	0.00%	0.63%
Wasp	0.00%	8.33%	0.00%	0.00%	1.92%	2.05%
Ant	0.00%	6.43%	7.81%	0.35%	1.92%	3.30%

Table 5. Average community composition of mesofauna functional groups by treatment for compiled data from both rounds of sampling (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Data shown as average percentage of the whole community.

Functional Group	**C**	**CL**	**CH**	**BC40L**	**BC40H**	**Total**
Bulb Mite	52.61%	51.91%	38.42%	44.51%	54.89%	48.47%
Collembola	16.45%	19.34%	13.03%	17.12%	6.23%	14.44%
Oribatid	6.22%	1.68%	5.77%	4.42%	9.11%	5.44%
Larva	1.61%	0.61%	1.61%	2.25%	0.60%	1.34%
Lice	2.86%	2.73%	3.66%	4.62%	5.94%	3.96%
Predatory Mite	0.58%	0.14%	4.50%	1.40%	1.42%	1.61%
Symphylan	6.02%	4.06%	2.76%	0.00%	0.06%	2.58%
Spider	0.00%	0.00%	0.35%	0.19%	0.00%	0.11%
Fly	13.12%	12.14%	18.89%	22.33%	16.08%	16.51%
Beetle	0.13%	0.00%	5.55%	2.99%	3.74%	2.48%
Thrip	0.40%	0.00%	1.56%	0.00%	0.00%	0.39%
Wasp	0.00%	4.17%	0.00%	0.00%	0.96%	1.03%
Ant	0.00%	3.21%	3.91%	0.18%	0.96%	1.65%

Table 6. Raw data of mesofauna community composition by soil treatment in Round 1 of sampling (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Volume of soil used for each sample was 65 in^3.

Treatment	Plot	BulbMites	Collembola	InsectLarvae	Oribatid	Booklice	PredatoryMites	Symphylans	Spiders	Flies	Beetles	Thrips
C	105	90	28	5	1	0	4	0	0	0	0	0
C	305	95	6	2	0	0	1	2	0	2	1	0
C	201	21	10	2	1	0	0	1	0	0	0	1
CL	101	37	8	0	1	0	0	1	0	0	0	0
CL	403	127	4	4	4	2	0	2	0	0	0	0
CL	306	89	19	1	6	0	1	1	0	0	0	0
CH	302	123	17	5	8	0	3	0	0	0	1	0
CH	405	107	28	2	15	4	0	0	0	0	0	0
CH	107	57	56	9	40	3	2	5	5	1	0	0
CH	202	118	34	6	16	0	3	1	0	1	0	0
BC40L	102	81	39	5	3	3	1	0	2	0	0	0
BC40L	407	3	7	1	0	0	1	0	0	0	0	0
BC40L	304	181	8	3	4	0	2	0	0	0	0	0
BC40L	206	52	3	4	27	2	1	0	0	1	1	0
BC40H	207	50	1	2	9	3	0	0	0	0	0	0
BC40H	404	85	3	0	10	1	1	0	0	0	0	0
BC40H	301	24	0	0	1	1	0	0	0	0	0	0
BC40H	106	121	68	4	25	1	6	1	0	1	0	0

Table 7. Raw data of mesofauna community composition by soil treatment in Round 2 of sampling (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Volume of soil used for each sample was 32.5 in^3.

Treatment	Plot	BulbMites	Collembola	Oribatid	Booklice	PredatoryMites	Symphylans	Flies	Beetles	Thrips	Wasps	Ants
C	305	1	0	0	0	0	3	4	0	0	0	0
C	402	1	1	2	1	0	0	0	0	0	0	0
C	105	1	2	0	0	0	0	2	0	0	0	0
C	201	1	0	0	0	0	0	0	0	0	0	0
CL	306	1	1	0	0	0	1	1	0	0	1	0
CL	403	3	0	0	0	0	0	3	0	0	0	1
CL	204	1	12	0	3	0	0	2	0	0	1	1
CH	202	1	0	0	0	0	0	2	1	0	0	0
CH	405	2	2	0	4	1	1	4	1	0	0	1
CH	107	1	0	0	0	0	0	2	0	0	0	1
CH	302	0	1	0	0	2	1	2	1	1	0	0
BC40L	304	3	2	0	1	0	0	3	0	0	0	0
BC40L	102	0	1	0	1	0	0	2	1	0	0	0
BC40L	206	0	0	0	0	0	0	1	0	0	0	0
BC40L	407	63	0	1	1	0	0	3	2	0	0	1
BC40H	404	5	1	3	1	0	0	1	0	0	1	1
BC40H	301	2	1	0	1	1	0	7	1	0	0	0
BC40H	106	3	0	0	2	0	0	3	1	0	0	0
BC40H	207	4	0	1	0	0	0	3	1	0	0	0

Table 8. Results of permutational ANOVA on mesofauna data compiled from both rounds of sampling. * signifies a statistically significant difference in mesofauna community composition. Analysis conducted using the adonis2 function in R. Model: *adonis2(formula = ArthropodFull2 ~ Treatment + Round, method = "bray", by = "margin")*

	DF	SS	R2	Pr(>F)
Treatment	4	0.7901	0.07897	0.283
Round	1	3.9748	0.39727	0.001*
Residual	31	5.2213	0.52186	
Total	36	10.0052	1.0000	

Table 9. Results of ANOVA on F:B ratio data sampled in late summer. Analysis conducted using the anova function on a model generated using the lme function in r. Model: *lme(Fungi ~ Treatment, random=~1|Block, data=FB).*

	DF	F-value	p-value
(Intercept)	1	467.8141	<.0001
Treatment	4	1.5512	0.2498
Residuals	12		

Table 10. Results of ANOVA on microbial biomass data sampled in late summer. Analysis conducted using the anova function on a model generated using the lme function in r. Model: *lme(ugC.g ~ Treatment, random=~1|Block, data=FB).*

	DF	F-value	p-value
(Intercept)	1	61.12041	<.0001
Treatment	4	0.42664	0.7867
Residuals	12		

Figures

Figure 1. Randomized block design of biochar field trial with color coded treatments and dimension of plots.

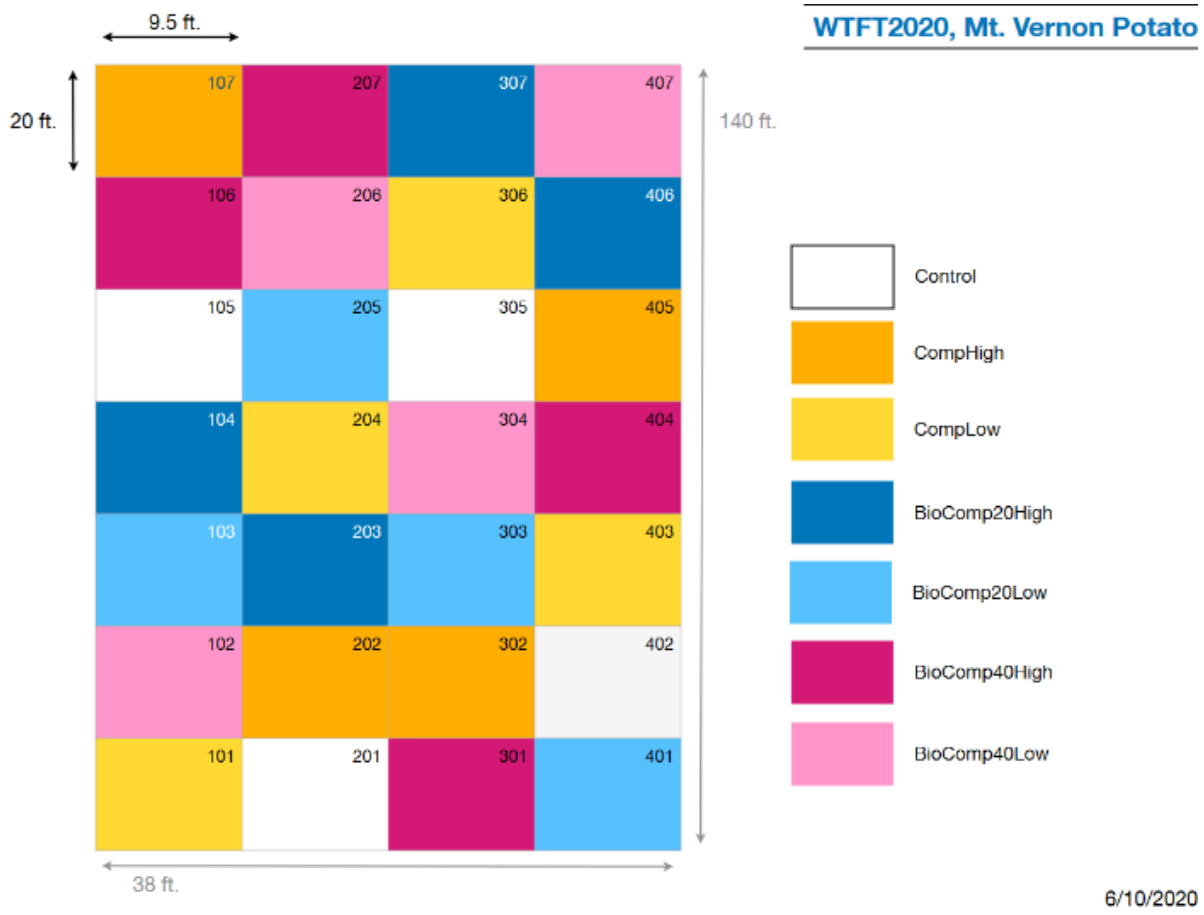


Figure 2. Average number of total mesofauna of all functional groups counted per treatment during the mid-summer round of sampling (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Samples size was 65 in^3. Standard error is shown by error bars below and each treatment had 4 replicates excluding C and CL which had 3.

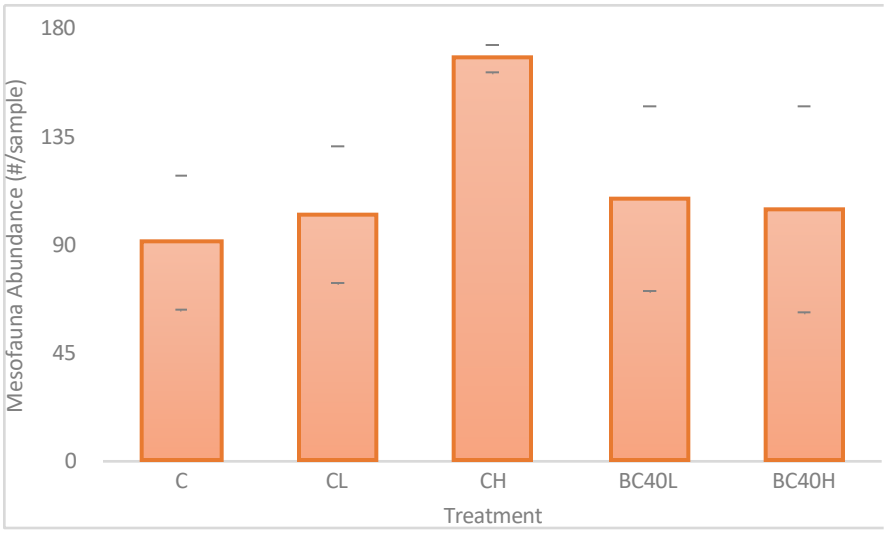


Figure 3. Average number of total mesofauna of all functional groups counted per treatment during the late-summer round of sampling (standardized to match 65 in^3 sample size). (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Samples size was 32.5 in^3. Standard error is shown by error bars below and each treatment had 4 replicates.

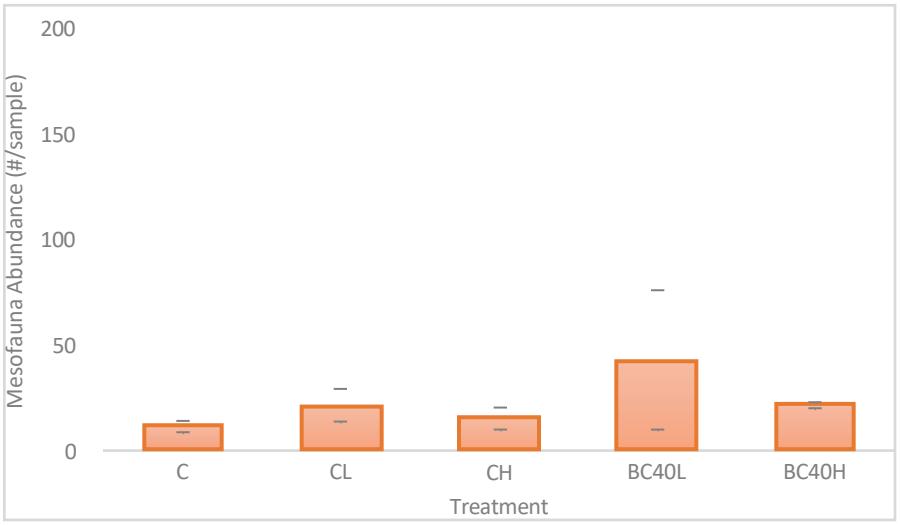


Figure 4. Average number of total mesofauna of all functional groups counted per treatment using compiled data from both sampling rounds. (standardized to match 65 in^3 sample size). (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Standard error is shown by error bars below.

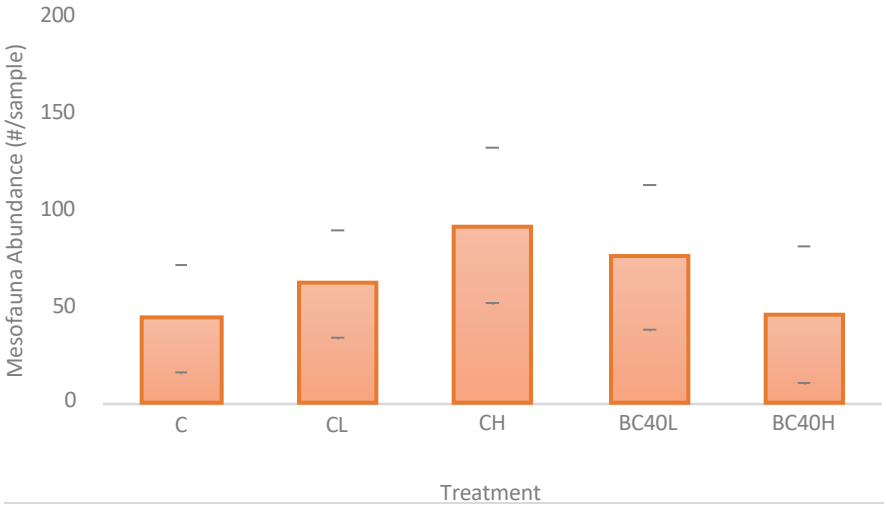


Figure 5. Average community composition of mesofauna functional groups by treatment at midsummer. Data shown as average percentage of the whole community (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Samples size was 65 in^3. Each treatment had 4 replicates excluding C and CL which had 3.

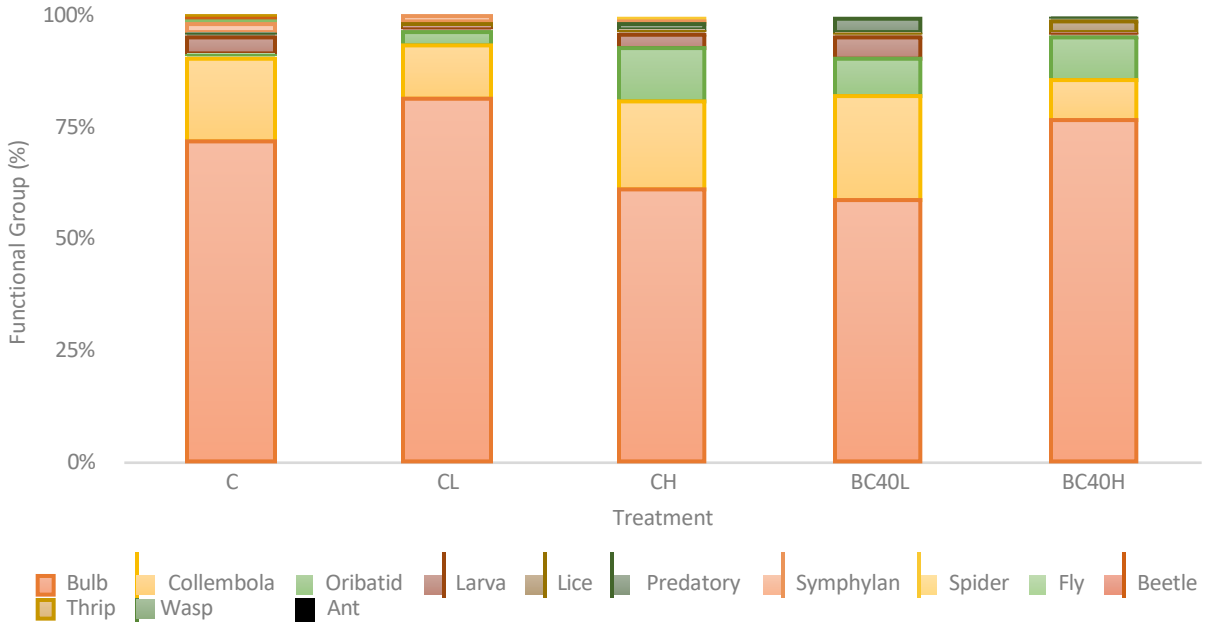


Figure 6. Average community composition of mesofauna functional groups by treatment at late-summer. Data shown as average percentage of the whole community (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Samples size was 32.5 in^3. Each treatment had 4 replicates.

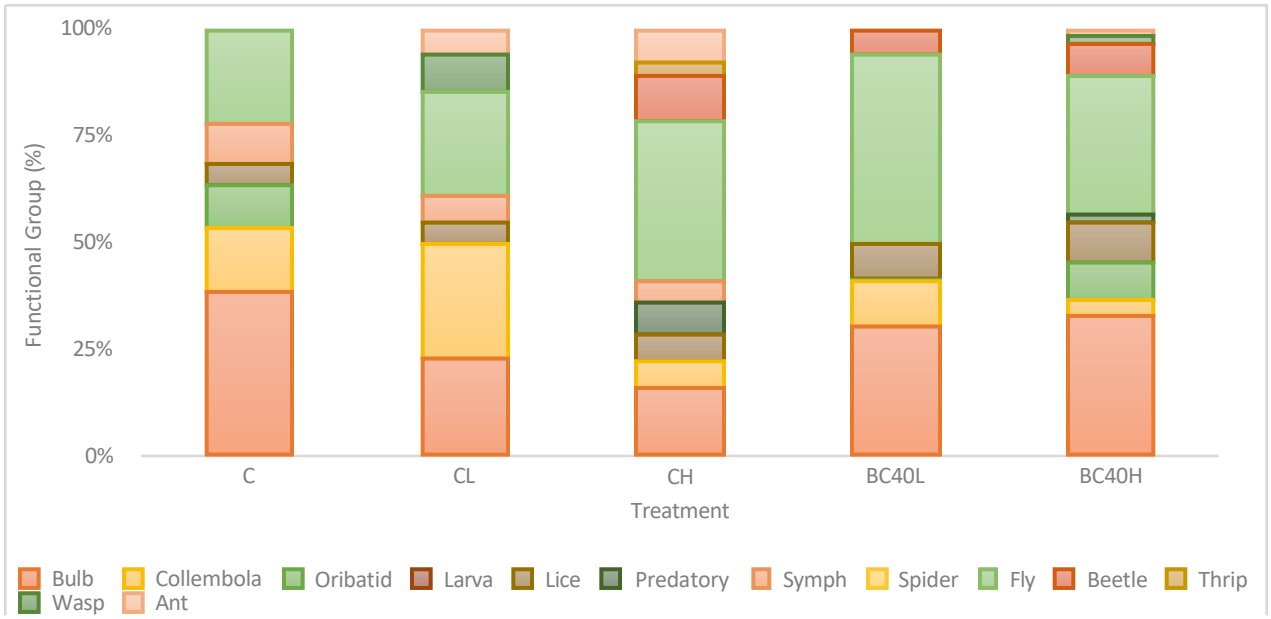


Figure 7. Average community composition of mesofauna functional groups by treatment of both rounds of sampling compiled. Data shown as average percentage of the whole community. (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high).

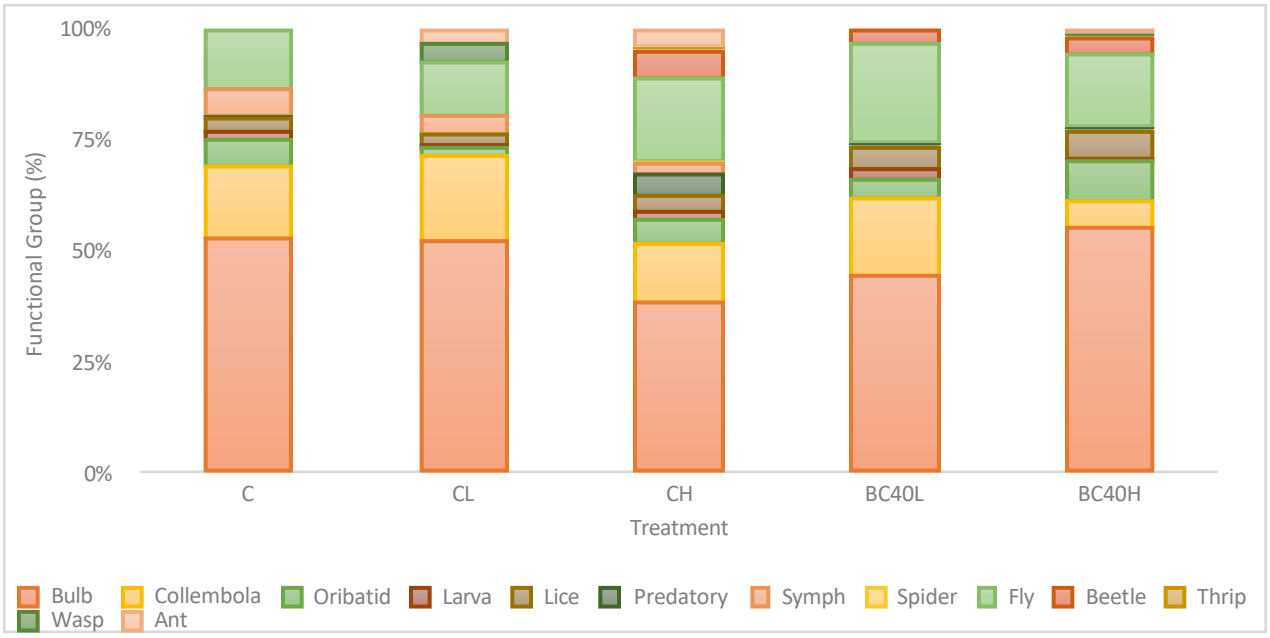


Figure 8. Average F:B ratio per treatment observed after sampling during late-summer in a Skagit silt-loam (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Each treatment had 4 replicates and standard error is shown by error bars below.

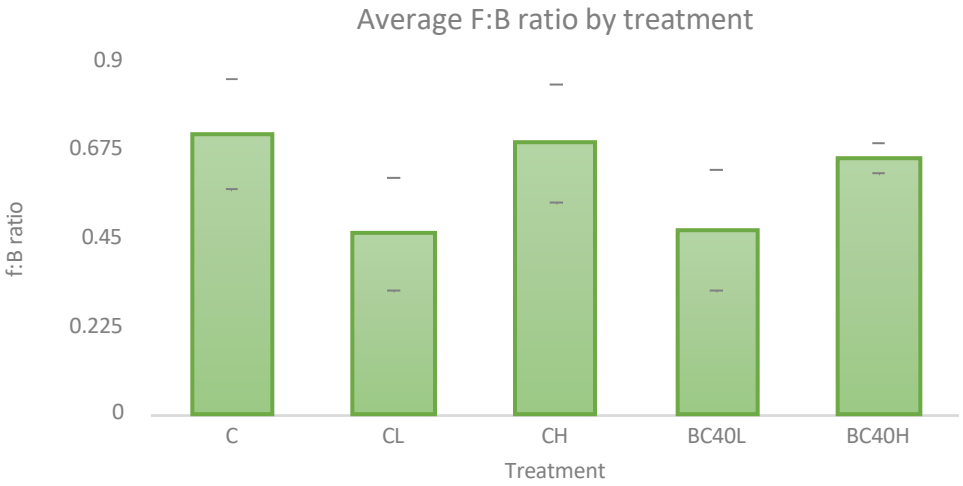
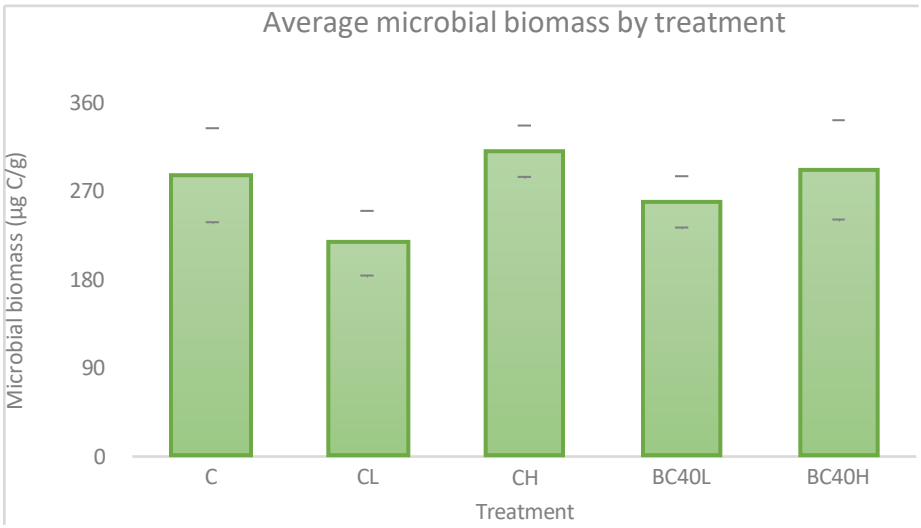


Figure 9. Average microbial biomass per treatment observed after sampling during late-summer in a Skagit silt-loam (C = Control, CL = Compost low, CH = Compost high, BC40L = Biochar + compost low, and BC40H = Biochar + compost high). Each treatment had 4 replicates and standard error is shown by error bars below.



1.0 Introduction

Biochar soil amendments have recently emerged as a sustainable and economically feasible alternative to conventional fertilization strategies in agricultural ecosystems. Addition of biochars to fields often leads to an increase in crop productivity and biochars generally have a positive impact on crop productivity in agricultural ecosystems (Jeffery et al., 2015; Ye et al., 2020; Gao et al., 2016). The effects of biochar on soil biota are less well understood (Warnock et al., 2007, McCormack et al., 2013). To maintain soil health and fertility in agroecosystems long term it is important to promote a diverse community of soil organisms to mediate the renewal of soil nutrients and maintain soil structure (Kennedy, 1999; Altieri, 1999). The goal of my research is to determine how soil biota, including both mesofauna and microbial communities, respond to mixed compost produced from chicken manure and wood shavings and biochar generated from forestry residuals at 871° C in a Skagit silt-loam agricultural soil in a potato crop system.

I will begin by defining biochar and discussing common physiochemical properties that remain consistent across various types (section 2.0). Next will be a literature review justifying the importance of biota in maintaining healthy soils and why measuring their response to soil amendment is relevant (section 3.0), then I will characterize how the practices of potato cropping may alter their abundance and therefore their role in soil ecosystems (section 4.0). Following will be the methodology of my study, and an outline of the variables measured and analyzed (section 6.0). I will conclude with the results (section 7.0) and a discussion (section 8.0) of how treatments affected F:B ratios and mesofauna community composition and what implications this has for farming in the Skagit Valley.

2.0 Properties of Biochar

2.1 Defining Biochar

Though the properties of biochar are highly dependent on its source feedstock and the conditions of pyrolysis there are some that remain relatively consistent among the various types of biochar. This section will provide a working definition of biochar and discuss common physiochemical properties associated with biochars in general.

Biochars are the product of heating organic matter under anoxic conditions to a temperature of 250 ° C or more (Lehman and Joseph, 2015). This process is called pyrolysis and it fundamentally alters the carbon (C) structure of the original organic matter proportionately to its temperature and duration. A defining feature of biochars are the formation of aromatic C rings, which aid in adsorption and mineralization of nutrients during pyrolysis. Pyrogenic C (PyC) in general can be highly recalcitrant in soils varying in mean residence time from 6 to over 5000 years (Lehman et al., 2015b), though research documenting these long residence times are conducted on historic applications of PyC.

The degree of aromaticity and the relative recalcitrance of C structures in biochars is determined in part by the pyrolyzation temperature (Downie et al., 2012). Biochars produced at lower temperatures typically exhibit higher stability in soils and more effective soil C storage, whereas at higher temperatures structure begins to break down into less stable, graphitic sheets (Lehman et al., 2012). Since biochar feedstock is organic matter, the whole process is net carbon negative as some C is stored back in the soil, potentially for millennia in the case of historic precursors to biochar in the form of PyC, rather than decomposing and being released into the atmosphere (Glaser et al., 2015; Lehman et al., 2015b). Both Gerlach et al. (2006) and Gehrt et al.

(2002) have found PyC remains in Germany dating from the Mesolithic and late Neolithic eras, and in the Amazon basin PyC has been found with a mean residence time of over 1000 years (Steiner et al., 2004).

Both nutrient transformations and volatilization occur during pyrolysis resulting in a modest amount of biologically available nutrients in biochars relative to typical soil nutrient pools, but a larger amount of nutrient salts introduced by biochar may constitute a significant increase in the soil environment (DeLuca et al., 2015). Again, nutrient availability from biochar depends largely on the temperature of pyrolysis and source feedstock of biochars through differential volatilization temperatures of nutients. The property of biochars affecting nutrient content that appears most consistent across various parameters is its relatively high pH compared to the source feedstock; this is due to accumulation of ash and alkaline carbonates during pyrolysis (Enders et al., 2012; Yuan et al., 2014).

The capacity of a biochar to inhibit nutrient leaching in agricultural soils is dependent on its pH, the relative quantity of micropores, and its cation exchange capacity (CEC). Nutrients are retained in biochar through sorption to negatively charged particles and through reduced infiltration of water through micropores supplied by biochar. Again these properties are all highly determined by the source feedstock, but can also be manipulated through temperature of pyrolysis. Higher temperatures consistently produce higher surface area with greater CEC and more micropores (Major et al., 2012). Higher heating rates have been observed to produce lower surface areas with more macroporosity (Chia et al., 2015). Though these factors are relatively predictable by the parameters of pyrolysis, not all source feedstocks can be pyrolyzed under the same conditions and each source feedstock introduces its own variability. Moreover, the effect they will

have on the soil environment may be dependent on the physical and chemical properties of the soil its applied to. Though parameters such as heating rate, temperature, and feedstock influence the degree of *relative* surface area to other biochars, most biochars tend to have high surface area (Chia et al., 2015).

These defining properties of biochar illustrate the importance of this research to the scientific community and for agricultural scientists working on soil fertility and crop productivity specifically. Biochar amendment is potentially an effective tool for remediating low pH soils, reducing nutrient runoff, introducing nutrients, and sequestering C; yet despite these promising properties of biochar, its effects on soil biota are less well understood and warrant further research.

3.0 Soil Biota

The chemical and physical properties of soil and biochar discussed above are important for evaluating the productivity of agroecosystems, but the soil biotic community also plays an important role in maintaining soil health and ensuring that productivity in the long term. There are many field studies on the effects of biochar on physical and chemical properties of agricultural soils, but there is a relative lack of research on its effects on the soil biotic community. This section will demonstrate the importance of soil biota to the health of soil environments and to plant nutrition justifying research on their potential response to biochar. For the purposes of this review and my thesis project, in which I collect data on the microbial community in the form of F:B ratios and total microbial C, I will focus on bacteria and fungi broadly. Then I will discuss

the importance of mesofauna occupying the detritivore-microbivore niche as they pertain to soil health.

3.1 Soil Microbes

Bacteria

Bacteria are an integral part of the soil environment; at the bottom of the soil food web, bacteria contribute to nutrient cycling and support the dietary needs of higher organisms. Most bacteria will reach a maximum size of no more than a few microns in length (Prosser and Killham, 2015). Despite the scale in which individuals sequester nutrients being miniscule, the sheer size of their population in soils entails that large quantities of nutrients and C may cycled through the bacterial population.

Bacteria play a very important role in the cycling of nitrogen (N) and phosphorous (P) in soils via organic and mineral inputs. P solubilizing bacteria facilitate the availability of P to plants by releasing it from organic and inorganic sources via production of organic acids (Khan et al., 2009). Various N transforming reactions are also carried out by bacteria in soils; this includes the fixation of atmospheric N into ammonium and subsequent nitrification into nitrates (Kuypers et al., 2018). These transformations alter the bio-availability of N to plants in the soil environment.

Bacteria are not only integral to nutrient cycling in the soil environment, but they can also promote plant growth through symbiotic relationships in the rhizosphere. Plant growth promoting bacteria may stimulate crop productivity through both production of growth promoting hormones and as a biocontrol agent of plant pathogens (Olanrewaju et al., 2017). These functions of

bacteria are synonymous with healthy soils; they contribute to the continuous nutritional needs of plants in their ecosystem by cycling nutrients from decomposing organic matter and mineral sources, while facilitating resistance to disease and promoting plant growth.

Fungi

Fungi have body structures made up of filamentous appendages called hyphae that explore the soil environment by growing radially in various directions (Schaetzl and Thompson, 2005). Their hyphal growth structure allows them to explore the soil in search of nutrients and sources of C whereas bacteria are at the whim of their microhabitat.

The utility of fungi in the soil environment is most apparent in the activity of mycorrhizal fungi. These fungi form mutualistic relationships with plant hosts receiving C in exchange for nutrients (Douds Jr. and Millner, 1999). They have also been documented facilitating increased access to soil water (Davies et al., 1993) and increased pest and disease resistance (Hooker et al., 1994). Their hyphae can act as an extension of the plant roots that they have formed a relationship with. It can allow them to obtain and exchange nutrients with plants from sources in both the mineral soil and the surface layer of organic matter contributing to the process of litter decomposition (Voříšková and Baldrian, 2013; Clark and Zeto, 2008).

In addition to this direct effect on plant growth and nutrition, fungi also play a role in maintaining healthy soil structure through the production of glomalin. Glomalin is a protein excreted by fungi which helps build the structure of soil aggregates and is highly correlated with aggregate water stability (Rillig, 2004). Increased aggregate water stability allows soil to resist the force of rainfall and surface water runoff, and helps keep nutrients and C in the surface layer

of soil from being lost to erosion. Building soil structure, exchanging nutrients between plants, and contributing to litter decomposition are just a few of the myriad processes fungi facilitate to help maintain healthy soils.

Fungal to Bacterial Ratios and Total Microbial C

Above I have shown the importance of bacteria and fungi in maintaining healthy soils; here I will demonstrate how the ratio of fungal to bacterial biomass (F:B ratio) may alter chemical processes in soil as well. Changes in F:B ratios can have implications for long term C storage and cycling of bio-available N in agricultural soils (Waring et al., 2013; Malik et al., 2016). These are manifested through inherent differences in physiology between bacteria and fungi. There is a body of literature suggesting N immobilization tends to increase with higher F:B ratios in soils, yet higher immobilization of N does not imply reduced access for crops since fungi can become the mediators of N uptake resulting in higher N use efficiency (De Vries et al., 2011; Bardgett and McAlister, 1999; De Vries et al., 2007). In agricultural ecosystems higher N use efficiency and less leaching could result in lower reliance on chemical fertilization to maintain crop productivity.

F:B ratios may also be used as potential bioindicators for soil processes necessary for crop production. Both Malik et al. (2016) and Nakamoto et al. (2011) have documented increased soil organic C storage and increased aggregate stability in agroecosystems concomitant with higher F:B ratios. Thus higher F:B ratios may be connected with better soil structure in agroecosystems. Moreover, higher F:B ratios promote dominance of fungal feeders higher up in the soil food web. Smaller biomass and lower turnover rates of fungivores relative to bacteri-

avores allows for immobilization of C and nutrients resulting in less leaching and more efficient use of resources (Didden et al., 1994; Zwart et al., 1994). Though high F:B ratios are indicative of a healthier agricultural soils conventional farming practices which utilize high amounts of chemical fertilizer and have relatively low inputs of organic material typically have relatively low F:B ratios (Chavarria et al., 2018).

The total amount of microbial biomass in soils, comprising both fungi and bacteria among other microbes, is also important for mediating nutrient dynamics and soil structure as described above; however, total microbial biomass may be correlated with other useful soil processes as well. Microbes sequester nutrients in the soil environment through storage as biomass and they contribute to the recalcitrance of nutrients in the soil environment since some microbial components break down much more slowly in the soil environment than plant residues (Kindler et al., 2009; Miltner et al., 2009). Moreover, larger populations of microbes in soil are associated with greater suppression of soil pathogens; it has been posited that this is due to increased competition for resources (Weller et al., 2002).

Both bacteria and fungi play important roles in nutrient cycling, litter decomposition, and forming relationships with crop plants, but the physiology of fungi has implications for more efficient use of N in soils, and for beneficial soil structure. The experiments discussed above have demonstrated that F:B ratios can have an impact on nutrient resources in agroecosystems; this implication warrants further research on the effects of biochar on the microbial community. If we intend to understand how biochar may promote healthier soils with higher N retention and aggregate stability, then measuring F:B ratios is pertinent.

3.2 Soil Mesofauna

Mesofauna are a step above microbes in the soil food web. As with bacteria and fungi above, I will describe the general morphology and niche of detritivore-microbivore mesofauna since these were most abundant in my experiment, then explain their importance within the soil environment and why research should be devoted to their population dynamics. Many soil mesofauna are responsible for processing organic matter in the soil environment. They occupy a similar niche to larger soil invertebrates like earthworms. Though they process a relatively small amount of organic matter by comparison, they are important players in the processing of key nutrients in the soil environment and distributing these nutrients as well (Wierzbicka et al., 2019; Norton and Pelletier, 1990).

Though this functional group is defined by its role in processing fresh organic matter, it also fulfills a host of other duties within the soil environment. Most Acariformes (mites) and Collembola also rely heavily on microbes as a source of food. Fungivore mites often stimulate further growth of mycorrhizal hyphae after a period of grazing (Visser, 1985; Moore et al., 1988). Through digestion and deposition of fecal pellets, mesofauna also contribute to the structure of soils and deposit microbial spores in new habitats for colonization (Walter and Proctor, 2013). These interactions with soil microbes influence decomposition and nutrient cycling through stimulation of microbial growth and facilitation of access to new sources of organic matter.

Grazing on microbes by soil mites and collembola can also influence mineralization rates of nutrients bound in organic form (Filser, 2002). These mesofauna stimulate microbe activity through grazing, but also act as regulators of the microbial population ensuring that rapid bloom-

ing of microbes does not occur (Reichle, 1977). Sudden spikes in the microbial population can result in spikes in nutrient mineralization, rather than storage and release when needed. Thus, detritivore-microbivore mesofauna ensure a slow and continuous release of mineral nutrients into the soil environment, which allows for efficient use of nutrients sourced from organic matter.

The key factors regulating populations of these mesofauna are the availability of microbial biomass for grazing and the volume and structure of surface litter (Ilieva-Makulec et al., 2006; Cao et al., 2011). Mesofauna populations can be sensitive to physiochemical changes and disturbances to the soil environment (Socarrás, 2013), yet in most field experiments measuring abundance, mesofauna populations are responding to trophic cascades rather than directly to physiochemical changes (Cao et al., 2011; Ilieva-Makulec et al., 2006; Xu et al., 2009; Minor and Norton, 2004; Graczyk and Seniczak, 2008). This relationship is one of the strongest predictors of relative abundance of detritivore-microbivore soil mesofauna.

Measuring microbial biomass and F:B ratios is important for understanding the health of our agricultural soils, but understanding and utilizing microbes as an agroecosystem service can only be done within the greater context of the soil food web. This includes an understanding of mesofauna population dynamics, which may regulate and stimulate their activity in a manner providing optimum use of resources. Without mesofauna microbes can mineralize nutrients too quickly or may not have access to resources without their vectoring capacity (Reichle, 1977; Walter and Proctor, 2013). Without microbes as a food source mesofauna abundance declines and so does the ecosystem services they provide. Furthermore, contemporary research on the effects of biochars on mesofauna is scant. If we wish to understand how we can rely on soil biotic

communities to provide productive nutrient cycling and soil structure in agroecosystems, then research on the effects of biochar on mesofauna population dynamics is important.

4.0 Agricultural Soils and the Biotic Community

Agricultural practices are highly varied depending on the methods of individual farmers in question; thus soil biotic communities in agroecosystems can be highly varied as well. The role of microbes and mesofauna in agroecosystems encompasses the processes discussed in sections 2.0 and 3.0 above including facilitating OM decomposition, nutrient cycling, and structural maintenance. Since my research took place on a potato field, here I focus on soil biota in potato systems. Practices common in potato farming have the potential to reduce their abundance and diversity, which may reduce their ecosystem services in the soil environment.

High yield potato varieties have high nutrient requirements and require precise timing of nutrient application during development to maximize tuber development (Zareabyaneh and Bayatvarkashi, 2015; Davenport et al., 2005; Kumar et al., 2017; Alva et al., 2011). To satisfy the high nutrient requirements and precise timing of application needed by potato crops, farmers often use chemical fertilizers (Kumar et al., 2017; Alva et al., 2011) presumably due to better cost effectiveness relative to commercialy produced organic fertilizers (Fang et al., 2021; Alimi et al., 2017). High chemical fertilizer use and low OM input reduces the diversity and abundance of soil biotic communities in agroecosystems resulting in a reduced role in nutrient dynamics and soil structure maintenance (Oehl et al. 2004; Douds et al. 1997).

Potato crops also remain highly susceptible to pathogens despite modern efforts at protection. Global estimates of potato losses vary between 15 and 25% by region even with the use of

protective measures such as crop rotation, mechanical pest traps, and pesticides. In places like Central Africa where protection can be limited exclusively to manual weeding, losses may be as high as 50% (Oerke, 2006; Mills et al., 2004). Due to the potential for high losses in yield, pesticide use in potato systems is common (Cray et al., 2016). However, the effects of pesticides on non-target members of the soil biotic community can further reduce the disease suppressive capacity of soils (Weller et al., 2002; Cook, 2014). General suppressiveness of soils to crop pathogens is directly related to the total microbial biomass since higher populations of microbes act as competition against pathogen agents (Schlatter et al., 2017; Weller et al., 2002). Yet in systems where pesticide use is common, such as potato crops, these services provided by the microbial community are reduced due to reductions in microbial biomass following consistent pesticide application (Ghorbani et al., 2009).

Potatoes and other root crops are sensitive to soil compaction, inadequate aeration, and poor drainage; to accommodate these needs, potato farmers often engage in extensive tilling and ridging of the soil surface in addition to soil disturbance during harvest (Howeler et al., 1993; Carter et al., 2009). While these practices provide for the specific needs of the potato crop, physical disturbance of the soil environment can reduce soil C and soil aggregation. Moreover, disturbance of the soil surface can be harmful to soil biota since this is where much of the biological community resides (Kabir, 2003; Frey et al., 1993; Rieff et al., 2020). Tillage can shred mycelial networks and reduce the abundance of fungal spores (Kabir, 2003), and total microbial biomass has been observed to decrease with higher levels of tillage from no-till, to conservation tillage, to conventional tillage systems (Frey et al., 1993; Carter et al., 2009; Kabir, 2003). Mesofauna communities typically suffer similar consequences with reductions in abundance and lowered

resilience to insecticides under conventional tillage systems (Rodriguez et al., 2006; Rieff et al., 2020). Not only is soil biota abundance reduced, but the community structure of microbes in soils altered by tillage and heavy chemical fertilization typically shifts toward lower F:B ratios (Luo et al., 2019; Chavarria et al., 2018; Bailey et al., 2002). This could have ramifications for soil structure mediated by glomalin production and less efficient use of nutrients through lower immobilization as discussed in section 3.0.

Common potato farming practices such as intensive tillage, pesticide use, and high inputs of chemical fertilizer cause reductions in abundance of soil biota. This can reduce the positive effects of the soil biotic community on the soil environment as discussed in sections 2.0 and 3.0. Therefore, it is important to measure the soil biotic community in potato systems and determine if changes in management practice or soil amendment may help them recover from deleterious practices.

4.1 Biochar in Agroecosystems

Biochars have the potential to alter agricultural soils and soil biotic communities through a variety of physical and chemical mechanisms. This section examines the scant literature on the effects of biochars on soil mesofauna, then delves into the impact of biochars on soil pH and substrate quality respectively, two of the driving factors behind soil biotic community dynamics. I will demonstrate how biochar induces change via these mechanisms and how they might affect soil health indirectly through the biotic community.

Biochar and Soil Mesofauna

Some of the few studies conducted on the effects of biochars on mesofauna examine their relative palatability compared to the natural diets of mesofauna. Mesofauna abundance is highly correlated with the volume and structure of surface organic matter, so the palatability of biochar particles could be a strong predictor of mesofauna response (Ilieva-Makulec et al., 2006; Cao et al., 2011).

In a review of the effects of biochar on soil biota, Lehman (2011) observed mesofauna fecal pellets in charcoal layers of forest soils; he suggests this is evidence for their capacity to ingest carbonized material. Since then, the only study directly measuring the tendency for soil mesofauna to ingest any form of carbonized material comes from Salem et al. (2013) researching hydrochars. Collembola were able to complete their life cycles on a hydrochar particle diet, though fitness was reduced when compared to an all-yeast diet. Fitness was also negatively correlated with higher C:N ratios of hydrochar particles, suggesting that collembola are deriving nutrients from hydrochar rather than ingestion being incidental. In addition to this study, Maaß et al. (2019) observed biochar particles in the gut of collembolans while measuring the ability of mesofauna to laterally transport biochar in soils. Using three different feedstocks, they found that collembola preferentially distributed pine over spelt husk-based biochar particles, corroborating Salem et al.'s (2013) assertion that ingestion is not purely coincidental. The only other study documenting mesofauna response to biochars comes from Gruss et al. (2019) who used avoidance tests to observe a short-term toxicity risk of biochars to collembola.

Though this research suggests that mesofauna may be able to derive nutrients from pyrolyzed materials, it is not indicative of biochar particles being a higher quality food source than their natural diet. These studies are indicative of a neutral response to biochar by mesofauna at

best, and lowered fitness and avoidance at worst. However, most of these studies isolated mesofauna from the greater biotic community in order to understand their response to biochars exclusively. I suggest the mechanism by which biochar amendment will induce changes in mesofauna abundance will be through indirect effects on other organisms in the soil food web as discussed in section 3.2. Changes in the chemical environment of soils typically induce an *indirect* shift in mesofauna abundance operating through trophic interactions (Vilkamaa and Huhta, 1986; Cassagne et al., 2003; Hågvar and Abrahamsen, 1980; Ilieva-Makulec et al., 2006; Cao et al., 2011; Xu et al., 2009; Minor and Norton, 2004; Graczyk and Seniczak, 2008). Mesofauna abundance is primarily dependent on fresh organic matter inputs and trophic interactions. The former factor is weakly related to the chemical properties of biochar; therefore, the following discussion of the effects of biochars on soil biota in agroecosystems will primarily be conducted through the lens of the microbial community.

Biochar Impacts on Substrate Quality and pH

Substrate quality and pH are two of the primary drivers of both biotic community composition and agricultural productivity. Substrate quality is defined by the relative recalcitrance of a substrate, its source material (organic or inorganic) and the ratios of C and nutrients it contains; these factors influence the palatability of substrate to soil mesofauna and the nature of plant-microbe interactions in the rhizosphere (De Vriest et al., 2006; Rousk et al., 2010). PH refers to the hydrogen ion concentration in soils and its capacity to adsorb certain nutrients.

Substrate quality, and the ratio of soil C to N (C:N) in particular, is important to microbial community structure; research suggests that C:N ratios greater than 20 in soil amendments gen-

erally stimulate increased fungal activity over bacterial (Vinten et al., 2002). The surface of biochars is enriched with some bio-available nutrients that mineralized during pyrolysis, as discussed in section 2.0. Through differential temperatures of volatilization and mineralization of nutrients substrate quality is altered during pyrolysis and biochars become a source of organic matter with different nutrient ratios from the source feedstock (Lehman and Joseph, 2015; DeLuca et al., 2015). Moreover, the change in C structure that occurs during pyrolysis provides more recalcitrant forms of C. There are many examples of biochars in agroecosystems changing the substrate quality as documented by Gao et al. (2019) in a meta-analysis. Using data from 124 studies applying various types of biochar Gao et al., demonstrates that mineral N concentrations consistently decline following amendment of soil with biochars; however, when applied in conjunction with organic fertilizers such as compost the opposite effect is observed.

These changes in nutrient ratios can have profound effects on the soil biotic community beginning with microbes and cascading into mesofauna via trophic interactions. Predicting the direction of these effects on microbial biomass and therefore mesofauna is less reliable due to differences in pre-existing soil conditions and differences in the microbial species composition of soils (Lehmann et al., 2011). Moreover, the magnitude of change in substrate quality will vary widely with different types of biochar. In spite of the variable nature of soils and biochars one commonly observed trend in biochar field studies across various types is an increase in root colonization by mycorrhizal fungi following biochar amendment (Blackwell et al., 2010; Treseder and Allen, 2002); however, the degree of mycorrhizal colonization will also depend on the potential for individual crops to form symbioses. Changes in overall microbial biomass have also been observed in both directions in conjunction with changes to substrate quality from biochars (Jin,

2010; Graber et al., 2010). The predictability of the effects of novel substrate quality introduced by biochars is uncertain, but the potential for change in the biotic community is clear.

The connection between microbial community structure and pH is also well documented (Rousk et al., 2010; Rousk et al., 2009). Generally, fungi tend to be more dominant in low pH soils as opposed to bacteria; in alkaline soils, bacteria have a competitive advantage over fungi, rather than fungi being intolerant of higher pH (Rousk et al., 2009; Fierer and Jackson, 2006; Lauber et al., 2009). Biochars can ameliorate low pH soils by introducing alkaline carbonates on its surface and through an increase in ashe content, causing further changes in nutrient availability (Atkinson et al., 2010; Gul et al., 2015). The magnitude of this effect is dependent on the pH of the biochar used as amendment, which can be partially manipulated through conditions of pyrolysis (Tomczyk et al., 2020), and the pH of the soil being amended. There are copious examples of the liming effect of biochars on low pH soils (Teutscherova et al., 2017; Luo et al., 2011; Rees and Morel, 2013); however, slight acidification has also been documented on alkaline soils following biochar amendment (Liu and Zhang, 2012). Regardless of the direction of effect the capacity of biochars to alter pH in agricultural soils has implications for the soil biotic community, and the variable nature of these effects warrants continuous research.

These changes in pH following biochar amendment have been documented as mechanisms of change in the biotic community in field trials, albeit with little consistency in response. Both Teutscherova et al. (2017) and Xiao et al. (2019) have observed increasing abundance of ammonium oxidizing bacteria in conjunction with biochar amelioration of acid soils. In a study on alkaline soils bacterial abundance increased following a drop in pH with biochar amendment (Liu et al., 2021). Li et al. (2019) observed that the microbial community structure shifted toward

fungi in an acidic soil limed by biochar. The variable nature of these responses is likely precipitated by inconsistencies in other properties of biochar, since it is difficult to control for every physical and chemical aspect of it, but in each experiment significant changes in pH following biochar amendment occurred in conjunction with changes to microbial abundance or community structure.

Biochars have the potential to alter the soil biotic community in agricultural soil via the mechanisms discussed above. Changes to the biotic community can have implications for soil health in agroecosystems through changes in organic matter decomposition, nutrient dynamics following decomposition, and soil structure. The unpredictability of biotic response to biochars entails the need for a catalog of studies documenting its various types and their effects on the biotic community.

5.0 Research Objectives

The objective of this experiment was to observe the response of 1) soil mesofauna communities as defined by functional group, and 2) F:B ratios and total microbial biomass. This is important for understanding how biochar may affect long term soil health via litter decomposition, nutrient dynamics, and soil structure in agroecosystems as demonstrated in my literature review above. In my research I applied compost produced from chicken manure and wood shavings and biochar generated from pine forest residuals at 871 ° C to a potato field in a Skagit silt loam soil in order to document their effect on the biotic community.

6.0 Methods and Materials

6.1 Research Site and Experimental Design

My experiment took place on a biochar field trial located at Washington State University's Northwestern Washington Research and Extension Center (WSU NWREC, mtvernon.wsu.edu). We applied compost and seven different biochar-compost mixtures, described below, to potato crops in a Skagit silt loam soil (fine-silty, mixed, superactive, nonacid, mesic Fluvaquentic Endoaquepts; Stacey et al., unpublished). The upper soil layers are composed of alluvium transported from higher up in the Skagit River basin. It has mixed lithology reflecting its fluvial origins; the mountainous eastern portion of the river basin is composed of metamorphic and granitic rock, and volcanic deposits from nearby Glacier Peak and Mount Baker (Pacific International Engineering, 2008; Pringle and Scott, 2001). However, most of the lower substrate layers are composed of volcanic sands and laharic deposits (Pacific International Engineering, 2008). Texture is characterized as fine-silty with high CEC. Mesic soil temperature regime and neutral pH with high CEC provides productive agricultural soil (Stacey et al., unpublished). The water table is seasonally present in the soil profile and there is no drainage or water management system in place, but this does not inhibit agricultural production during the summer dry season (Stacey et al., unpublished data). During the two seasons prior to our biochar field trial the site was planted with legumes and ryegrass as a cover crop, then tilled at the end of each season. Prior to treatment application, the field was fertilized on May 5th, 2020 with monoammonium phosphate (163 kg ha^{-1}), langbeinite (172.6 kg ha^{-1}), potassium chloride (168.1 kg ha^{-1}), ammonium sulfate (103.1 kg ha^{-1}), and urea (196.1 kg ha^{-1}).

Seven treatments with four replications each were applied to the un-planted field on June 15th, 2020 in a randomized block design, though I used only five for my experiment. Treatments included a complete control (C), low and high rates of compost application (CL and CH) and low and high rates of two different mixtures of biochar and compost (BC40L and BC40H). The CL treatment was applied at a rate of 23.18 Mg ha^{-1} of compost and CH was 49.68 Mg ha^{-1}. The BC40L was 16.66 Mg ha^{-1} compost and 7 Mg ha^{-1} biochar, while the BC40H was 35.71 Mg ha^{-1} compost and 15 Mg ha^{-1} biochar. The biochar and compost 20% and 40% treatments were produced by adding and homogenizing biochar to the composting process and allowing this mixture to mature for 33 to 34 days. The exact rates of biochar and compost application in each treatment are recorded in Table 1, and a map of the experimental design is depicted in Figure 1. Biochar and compost were applied to the soil surface by hand, then tilled to a depth of 6 inches using a tractor-pulled rototiller. Each treatment plot was 2.9 by 6.1 meters. Three rows of Chieftan potatoes (*Solanum tuberosum*) were planted at a depth of 15 cm with a two-row planter on June 18th, 2020 in each block.

The biochar used in this project was purchased from Oregon Biochar Solutions in White City, Oregon (chardirect.com). Its source feedstock was forestry residuals from various pine species and Douglas Fir (*Pinus spp*. and *Psuedotsuga menzeissi*). Pyrolysis temperature was 871° C under low oxygen conditions, and resulting particles were screened to between 1 and 4 mm. The compost is a mixture of two source feedstocks: chicken manure and wood shavings. The chemical properties of the original biochar were changed during the maturation process with compost and these are listed below in table 2 along with the properties of the compost alone.

6.2 Sampling Methods

My first round of sampling for mesofauna abundance was taken on the 22nd of July, 37 days after application of biochar and compost. The second was on 29th of September, 105 days after application. I sampled from five of the seven treatments including BC40H, BC40L, CH, CL, and C. From each plot I took 3 subsamples to a depth of 7 inches and combined them into one for a total of twenty samples: one sample per plot and four replicates per treatment. For my first round of samples, I took 65 in^3 of soil and transferred it to the funnels for extraction of mesofauna; for the second round I used 32.5 in^3 as entomologists at WSU NWREC recommended a smaller sample size. Samples for microbial biomass were taken on October 5th from the same treatments as above with two subsamples per replicate sample. Again, samples were taken to a depth of 7 inches. I processed these samples later that day using the microbiometer kits.

To determine the effects of biochar and compost on mesofauna abundance, I used the Berlese funnel method. A bottle filled with 70% ethanol was placed beneath a funnel with a coarse sieve and cheesecloth suspending soil samples over its mouth. Each funnel had a 52-watt light suspended over it kept on for one week following sampling. The light source slowly dries the soil surface causing mesofauna to move downward into the funnel away from the heat. Fauna move through the sieve and are funneled into the bottle to be counted and identified later. Fauna were counted under a stereo microscope at magnifications between 30 and 50x and measurements recorded as abundance of species per sample.

Microbial biomass was assessed using microbiometer test kits (Microbiometer, Montgomery, NY). These kits extract microbes from soil samples and determine the biomass ratio of

fungi and bacteria colorometrically using the microbiometer app (Microbiometer, Montgomery, NY). I sifted 0.5 ml of soil from each sample using the provided microbiometer sifter and combined it with an extraction fluid of Sodium Chloride, Calcium Chloride and water. This was mixed in a vial for thirty seconds by an electric, metal whisker and allowed to settle for five minutes. After five minutes each vial was gently tapped to encourage deposition of soil particles then allowed to sit for another fifteen minutes. The fluid was then pipetted onto a test card and analyzed. Results are reported as a ratio of fungal to bacterial biomass and as total microbial biomass in micrograms of microbial C per gram of soil.

6.3 Statistical Analysis

All statistical analyses for this project were conducted using R software (R Core Team, 2021). To test whether mesofauna communities could be predicted by soil treatments, I used permutational ANOVA (PERMANOVA) with a Bray-Curtis dissimilarity measure (Anderson, 2001). I used round and treatment as fixed factors. I used the adonis2 function from the vegan package (Oksanen et al., 2022) for my PERMANOVA analysis. To determine whether average F:B ratios and total microbial C differed between treatments, I used ANOVA tests. This was done with the ANOVA function on a linear mixed effects models generated with the lme function from the nlme package (Pinheiro and Bates, 2022). My model used treatment as a fixed factor and fungal biomass percentage and micrograms of microbial biomass as response variables respec- tively with block as a random effect. I tested for normality and equal variance using the shapiro.wilk test function ($p > 0.05$) and leven's test ($p > 0.05$) from the same package (Chambers et al., 1992).

7.0 Results

7.1 Mesofauna Data

The soil mesofauna community could not be differentiated by soil treatment ($p = 0.291$), though PERMANOVA could predict the community matrix of mesofauna by round ($p = 0.001$), since late-summer had both a small fraction of the total mesofauna abundance and a different community composition relative to mid-summer. By late-summer only 20% of the total number of mesofauna were observed relative to mid-summer sampling.

The average total mesofauna population per replicate sample at mid-summer in the control group was 91, whereas the CL and CH treatments were 102 and 167, respectively. The BC40L and BC40H treatments were 109 and 105 respectively. By late-summer, the average total mesofauna population - normalized by volume for comparison to mid-summer - in the C group was 12, whereas the CL and CH treatments were 21 and 16, respectively. The BC40L and BC40H treatments were 43 and 22 respectively. Summary charts of mesofauna abundance and community composition in both rounds are shown in Figures 2-7. The variance of overall mesofauna abundance between treatments was very inconsistent, though this was not analysed statistically. Treatments that included both biochar and compost, including BC40L and BC40H, had a much higher average standard deviation in total mesofauna observed than compost or control treatments. The average standard deviation in total mesofauna abundance in BC40L and BC40H was 72.19 and 45.85 respectively, while C, CL, and CH were 27.16, 32.97, and 18.37. Both the highest and lowest populations of mesofauna tended to be found in sample plots from the biochar treatments.

Across all samples, bulb mites accounted for 69% of the mesofauna population at mid-summer; that number dropped to 28% by the end of the summer. Excluding one sample at late-summer, which held an uncharacteristically large amount of bulb mites, this number would be closer to 24% of the total population. Collembolans and oribatid mites were 17 and 7% of the total population at mid-summer; yet they dropped to 11 and 4%, respectively, by late-summer. Collectively, these three functional groups were 93% of the population at mid-summer and dropped to 39% later in the season. Insect larvae also declined from about 3 to 0% by the end of the summer.

Not only did collembolans, bulb, and oribatid mites dominate the mid-summer round of sampling, but they also tended to co-occur within samples. Despite being a relatively small proportion of the population, predatory mites also tended to co-occur with the above functional groups suggesting predatory mites may favor them as prey. The drop in population of these dominant groups accounted for most of the difference in community composition between mid and late-summer, but there was also a slight uptick in functional groups that had a negligible presence at mid-summer. As these dominant functional groups dropped in relative abundance the overall community composition of the site became more evenly distributed.

A number of marginalized functional groups grew in relative abundance such as booklice rising from 1 to 7%, symphylans from less than 1 to 4%, and flies rising from less than 1% of the total population at mid-summer to 32% by late-summer. Similarly, beetles were less than 1%, but grew to 5% by late-summer. Wasps and ants were not observed at the mid-summer sampling at all, but accounted for 2 and 3% of the total population by late-summer. I also observed a very slight increase in relative abundance of predatory mites from mid to late summer though this

primarily manifested from an anomalously large number of them being recorded in one sample during the late-summer round. By the end of the summer the total mesofauna population was a small fraction of the mid-summer population, but the community appeared to be a more even mix of functional groups rather than being dominated exclusively by bulb mites and collembola. Averages of functional group relative abundance by treatment in each sampling round and the compiled data are recorded below in tables 3, 4, and 5.

7.2 Fungal to Bacterial Ratios and Microbial C

Consistent with the mesofauna data, neither average F:B ratios nor total microbail C differed between treatments ($p = 0.246$, $p = 0.7867$, respectively), depicted in Figures 8 and 9, respectively. The average F:B ratio for the control treatment was 0.71, while all other treatments are slightly lower. For CL, CH, BC40L, and BC40H treatments the average was 0.46, 0.69, 0.47, and 0.65, respectively. The standard deviation for the control was 0.28, while the CL, CH, BC40L, and BC40H were 0.08, 0.20, 0.07, and 0.29. As the application rate of amendment used between treatments grew, the average F:B ratio became closer to the control as demonstrated by the CL treatment which had an average much lower than the control, but as more compost is added in the CH treatment the average becomes closer to the control. The same is true for the BC40L and BC40H treatment. Across all samples in the research site, the total average F:B ratio was 0.6, favoring bacterial dominance. Only two samples recorded a greater biomass of fungi than bacteria, and both were barely above an F:B ratio of 1.

Measurements of total microbial C did not differ between treatments either. The average for the control was 286 µg C/g, while CL, CH, BC40L, and BC40H treatments were 286, 216.75,

310, 258.25, and 291.5 respectively. The standard deviation for the control was 98, while the CL, CH, BC40L, and BC40H were 104.6, 160, 50.2, and 183.7. Treatments that had higher average F:B ratios tended to have higher average microbial C, and, similar to the F:B data, treatments with higher levels of amendment tended to have closer average microbial C to the control; these trends are shown below in Figures 8 and 9 which document average F:B ratio and microbial C by treatment. Average microbial C across all samples was 272.5 µg C/g. The lack of significance in statistical testing and lack of any observable trends in data suggest that treatments had no effect on F:B ratio or microbial C.

8.0 Discussion

Across both rounds of sampling neither compost nor biochar-compost mixtures significantly altered mesofauna or microbial communities relative to the control. The results of my statistical testing indicate that pine forest residual biochar pyrolized at 871° C and compost produced from chicken manure and wood shavings have no effect on average abundance and diversity of soil mesofauna and microbes in Skagit agricultural soils on a potato field. Though I observed no difference between treatments in *average* abundance and community structure, the large variance of mesofauna abundance in biochar treatments relative to compost-only and control suggests that biochar may have the capacity to induce increased heterogeneity in the soil environment and spatial variability in the mesofauna community.

I interpret these results with the caveat that my research had a relatively small sample size reducing its statistical power. Moreover, seasonal differences in mesofauna abundance may have provided a stronger mechanism of change than treatment effects, potentially confounding the

results of my experiment. Lastly, the short timescale of my experiment (approximately 3 months) may not have been sufficient time for changes to manifest in microbial biomass and therefore cascade through trophic interactions into the mesofauna community (Xu et al., 2009; Minor and Norton, 2004; Graczyk and Seniczak, 2008). Trophic interactions were the primary mechanism by which I had predicted change in the mesofauna community, thus a lack of change in the microbial community may have precluded any indirect effects of biochar on mesofauna abundance and community structure.

Though changes in the chemical properties of soils following compost/biochar amendment offers another mechanism of change in the biotic community, I did not measure these parameters in the soil following amendment and cannot speak to any differences in soil chemistry that may have manifested from biochar/compost. However, since the pH of soils at my site were not highly acidic (pH 6.5) and the biochar and compost used in this experiment was not particularly alkaline (pH 7.9 for compost treatments and pH 7.6 for biochar) it is unlikely that major changes in soil pH occurred. Most literature on biochars ameliorating the pH of soils are documenting changes on highly acid soils with much more alkaline biochars in contrast with the conditions of this research site (Horák, 2015, Chintala, 2014). Moreover, any potential additions of substrate with different C:N ratios from the soil will likely manifest over a longer timescale than my experiment as mentioned above (Minor and Norton, 2004; Graczyk and Seniczak, 2008).

I suggest that future researchers investigating the effects of biochars on soil biotic communities use longer timescales - a year or more rather than a season - to compensate for seasonal changes in abundance and to allow enough time for changes to manifest in the microbial community. I did not observe changes in the mesofauna or microbial community following biochar

and compost amendment, though I did document potentially useful information on mesofauna population dynamics, and the microbial community in Skagit Valley agricultural soils. Baseline numbers of microbial biomass and documentation of change in seasonal abundance of mesofauna could be useful as a reference for other agroecosystems in the area, or agroecosystems with similar climate patterns and cropping systems as the Skagit Valley.

Mesofauna Population Dynamics

I found evidence of a difference in mesofauna communities between mid and late-summer suggesting that climatic factors may have induced change between those times since all other soil parameters were the same between sampling rounds. This assertion is also founded on a body of literature supporting the notion that soil mesofauna populations are highly variable depending on the season in question. Factors such as soil moisture and temperature, which are seasonally regulated, are linked with mesofauna abundance. In ecosystems that share a similar climate pattern with the Skagit Valley (ie: winter wet/summer dry season) mesofauna abundance tends to bottom out at the end of the summer and peaks in early summer/late spring when soil conditions are both warm and wet (Gkisakis et al., 2016; Shakir and Ahmed, 2016; Wiwatwitaya and Takeda, 2005; Perdue and Crossley Jr., 1989). Though I could not find references documenting the changes in population of some specific functional groups relevant to my research, the transition from mites, collembola, and larvae to flies, beetles, and other larger, more motile mesofauna is reasonable considering their greater capacity to move between favorable soil microsites. I suggest that size and mobility of organisms in soils may imply a greater capacity to endure unfavorable conditions such as late-season drying and cooling. My research indicates that

by late-September in the Skagit Valley mesofauna communities drop to a fraction of their mid-summer numbers and transitioned from mite and collembola dominance to a more even mix of larger and more mobile functional groups.

Information on population dynamics of mites, collembola, and other detritivore-microbivore mesofauna may be useful in understanding seasonal changes in organic matter decomposition rate and subsequent nutrient release to crops. In agricultural systems that do not rely on chemical fertilizers soil mesofauna have a relatively higher impact on nutrient cycling and therefor an indirect impact on crop productivity (Oehl et al. 2004; Douds et al. 1997). Knowledge of their population cycles may help agronomers manage nutrient inputs to minimize inefficiency and nutrient leaching.

Microbial Biomass and F:B ratios

Though I observed no difference in microbial community composition or biomass following soil amendment I did collect baseline data on microbial communities in the Skagit Valley. The average F:B ratio for the whole site was 0.60, and the average total microbial biomass was 272.5 µg C/g, which is within the realm of other conventional agricultural soils (Thangasamy et al., 2017; Bailey et al., 2002; Reilley et al., 2013; De Vries et al., 2006). These numbers may be indicative of the legacy of conventional agriculture in the Skagit Valley, though taking more samples throughout the growing season is necessary since F:B ratios and microbial biomass vary seasonally as well. Once enough data is collected to establish a reliable measurement of F:B ratios in the Skagit Valley this can be used as a reference for the relative health and sustainability of agricultural soils here. Implementing practices that increase F:B ratios and increase total mi-

crobial biomass may provide a healthier and more sustainable agroecosystem with better soil structure and more efficient use of nutrients (De Vries et al., 2006).

9.0 Conclusions

Pine forest residual biochar and compost applied to a Skagit silt-loam agricultural soil did not result in any significant change in mesofauna functional group communities, nor did it alter F:B ratios and total microbial C over a timeframe of approximately 3 months during the summer. Future researchers of biochar's effects on soil biota may consider longer timescales to allow for changes to manifest in the microbial community. Though biochar did not induce changes to the biotic community, I observed a shift in mesofauna community composition and abundance between mid and late-summer sampling rounds from mite and collembola dominant to a more even mix of larger and more motile soil mesofauna. The microbial community at this site is bacterially dominant, which is consistent with literature on conventional agricultural soils, though more sampling is recommended to establish a reliable baseline. Once established, F:B ratios and total microbial biomass may be used as a reference for soil health and sustainable practice in the Skagit Valley.

Works Cited

Alimi, T., Ajewole, O. C., OLubode-Awosola, O. O., & Idowu, E. O. (2007). Organic and inorganic fertilizer for vegetable production under tropical conditions.

Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystems. In *Invertebrate biodiversity as bioindicators of sustainable landscapes* (pp. 19-31). Elsevier.

Alva, A., Fan, M., Qing, C., Rosen, C., & Ren, H. (2011). Improving nutrient-use efficiency in Chinese potato production: experiences from the United States. *Journal of Crop Improvement*, *25*(1), 46-85.

Ananyeva, N. D., Castaldi, S., Stolnikova, E. V., Kudeyarov, V. N., & Valentini, R. (2015). Fungi-to-bacteria ratio in soils of European Russia. *Archives of Agronomy and Soil Science*, *61*(4), 427-446.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26: 32–46.

Andrés, P., Rosell-Melé, A., Colomer-Ventura, F., Denef, K., Cotrufo, M. F., Riba, M., & Alcañiz, J. M. (2019). Belowground biota responses to maize biochar addition to the soil of a Mediterranean vineyard. *Science of the total environment*, *660*, 1522-1532.

Atkinson, C. J., Fitzgerald, J. D., & Hipps, N. A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant and Soil*, *337*(1–2), 1–18.

Bagyaraj, D. J., Nethravathi, C. J., & Nitin, K. S. (2016). Soil biodiversity and arthropods: Role in soil fertility. In *Economic and ecological significance of arthropods in diversified ecosystems* (pp. 17-51). Springer, Singapore.

Bailey, V. L., Smith, J. L., & Bolton Jr, H. (2002). Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biology and Biochemistry*, *34*(7), 997-1007.

Bharadwaj, D. P., Lundquist, P. O., & Alström, S. (2008). Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. *Soil Biology and Biochemistry*, *40*(10), 2494-2501.

Cao, Z., Han, X., Hu, C., Chen, J., Zhang, D., & Steinberger, Y. (2011). Changes in the abundance and structure of a soil mite (Acari) community under long-term organic and chemical fertilizer treatments. *Applied Soil Ecology*, *49*, 131–138.

Carter, M. R., Holmstrom, D., Sanderson, J. B., Ivany, J., & DeHaan, R. (2005). Comparison of conservation with conventional tillage for potato production in Atlantic Canada: crop productivity, soil physical properties and weed control. *Canadian Journal of Soil Science*, *85*(3), 453-461.

Carter, M. R., Noronha, C., Peters, R. D., & Kimpinski, J. (2009). Influence of conservation tillage and crop rotation on the resilience of an intensive long-term potato cropping system: Restoration of soil biological properties after the potato phase. *Agriculture, ecosystems & environment*, *133*(1-2), 32-39.

Cassagne, N., Gers, C., & Gauquelin, T. (2003). Relationships between Collembola, soil chemistry and humus types in forest stands (France). *Biology and Fertility of Soils*, *37*(6), 355–361.

Chavarria, D. N., Pérez-Brandan, C., Serri, D. L., Meriles, J. M., Restovich, S. B., Andriulo, A. E., ... & Vargas-Gil, S. (2018). Response of soil microbial communities to agroecological versus conventional systems of extensive agriculture. *Agriculture, Ecosystems & Environment*, *264*, 1-8.

Chambers, J. M., Freeny, A and Heiberger, R. M. (1992) *Analysis of variance; designed experiments.* Chapter 5 of *Statistical Models in S* eds.

Chia, C. H., Downie, A., & Munroe, P. (2015). Characteristics of biochar: physical and structural properties. In Biochar for environmental management (pp. 121-142). Routledge.

Chintala, R., Mollinedo, J., Schumacher, T.E., Malo, D.D., and Julson, J.L., (2014) Effect of biochar on chemical properties of acidic soil, Archives of Agronomy and Soil Science, 60:3, 393-404, DOI: 10.1080/03650340.2013.789870

Clark, R. B. (1997). Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. Plant and soil, 192(1), 15-22.

Clark, R. Á., & Zeto, S. K. (2000). Mineral acquisition by arbuscular mycorrhizal plants. *Journal of plant Nutrition*, *23*(7), 867-902.

Cray, J. A., Connor, M. C., Stevenson, A., Houghton, J. D., Rangel, D. E., Cooke, L. R., & Hallsworth, J. E. (2016). Biocontrol agents promote growth of potato pathogens, depending on environmental conditions. *Microbial biotechnology*, *9*(3), 330-354.

Crossley, D. A., Mueller, B. R., & Perdue, J. C. (1992). Biodiversity of microarthropods in agricultural soils: Relations to processes. *Agriculture, Ecosystems & Environment*, 40(1–4), 37–46.

Davenport, J. R., Milburn, P. H., Rosen, C. J., & Thornton, R. E. (2005). Environmental impacts of potato nutrient management. *American journal of potato research*, *82*(4), 321-328.

DeLuca, T. H., Gundale, M. J., MacKenzie, M. D., & Jones, D. L. (2015). Biochar effects on soil nutrient transformations. In Biochar for environmental management (pp. 453-486). Routledge.

De Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., & Bloem, J. (2006). Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry*, *38*(8), 2092–2103.

De Vries, F. T., Van Groenigen, J. W., Hoffland, E., & Bloem, J. (2011). Nitrogen losses from two grassland soils with different fungal biomass. *Soil Biology and Biochemistry*, *43*(5), 997-1005.

De Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., Hallin, S., Kaisermann, A., Keith, A. M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N., Prosser, J. I., Thion, C., Thomson, B., & Bardgett, R. D. (2018). Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications*, *9*(1), 3033.

Dhaliwal, S. S., Naresh, R. K., Mandal, A., Singh, R., & Dhaliwal, M. K. (2019). Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. *Environmental and Sustainability Indicators*, *1*, 100007.

Douds Jr, D. D., & Millner, P. D. (1999). Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture, ecosystems & environment*, *74*(1-3), 77-93.

Downie, A., Crosky, A., & Munroe, P. (2012). Physical properties of biochar. In *Biochar for environmental management* (pp. 45-64). Routledge

Fang, P., Abler, D., Lin, G., Sher, A., & Quan, Q. (2021). Substituting organic fertilizer for chemical fertilizer: Evidence from apple growers in China. *Land*, *10*(8), 858.

Farrell, M., Kuhn, T. K., Macdonald, L. M., Maddern, T. M., Murphy, D. V., Hall, P. A., Singh, B. P., Baumann, K., Krull, E. S., & Baldock, J. A. (2013). Microbial utilization of biochar-derived carbon. *Science of The Total Environment*, *465*, 288–297.

Fierer, N. & Jackson, R. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 626–631.

Franche, C., Lindström, K. & Elmerich, C. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant Soil* 321**,** 35–59 (2009). https://doi.org/10.1007/s11104-008-9833-8.

Frey, S. D., Elliott, E. T., & Paustian, K. (1999). Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biology and Biochemistry*, *31*(4), 573-585.

Gao, Y., Wei, Y., & Fan, M. (2011). Nutrient requirements for producing potato tubers. *Chinese Potato Journal*, *25*(3), 182-187.

Gao, S., & DeLuca, T. H. (2016). Influence of biochar on soil nutrient transformations, nutrient leaching, and crop yield. *Adv. Plants Agric. Res*, *4*(5), 1-16.

Ghorbani, R., Wilcockson, S., Koocheki, A., & Leifert, C. (2009). Soil management for sustainable crop disease control: a review. *Organic farming, pest control and remediation of soil pollutants*, 177-201.

Graczyk, R., Seniczak, S., & Wasińska-Graczyk, B. E. A. T. A. (2008). The effect of cattle liquid manure fertilization on the soil mites (Acari) of permanent meadow in Poland. *Journal of Central European Agriculture*, 9(4), 651-658

Gruss, I., Twardowski, J. P., Latawiec, A., Medyńska-Juraszek, A., & Królczyk, J. (2019). Risk assessment of low-temperature biochar used as soil amendment on soil mesofauna. *Environmental Science and Pollution Research*, *26*(18), 18230–18239.

Gkisakis, V., Volakakis, N., Kollaros, D., Bàrberi, P., & Kabourakis, E. M. (2016). Soil arthropod community in the olive agroecosystem: Determined by environment and farming practices in different management systems and agroecological zones. *Agriculture, Ecosystems & Environment*, *218*, 178-189.

Gul, S., Whalen, J. K., Thomas, B. W., Sachdeva, V., & Deng, H. (2015). Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. *Agriculture, Ecosystems & Environment*, *206*, 46-59.

Hågvar, S., Abrahamsen, G. (1980). Colonisation by Enchytraeidae, Collembola and Acari in sterile soil samples with adjusted pH levels. *Oikos*, 245-258.

Hayat, R., Ali, S., Amara, U. *et al.* Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol* 60**,** 579–598 (2010). https://doi.org/10.1007/s13213-010-0117-1

House, G. J., & Brust, G. E. (1989). Ecology of low-input, no-tillage agroecosystems. *Agriculture, ecosystems & environment*, *27*(1-4), 331-345.

Horák, J. (2015). Testing biochar as a possible way to ameliorate slightly acidic soil at the research field located in the Danubian lowland. *Acta Horticulturae et Regiotecturae*, *18*(1), 20-24.

Howeler, R. H., Ezumah, H. C., & Midmore, D. J. (1993). Tillage systems for root and tuber crops in the tropics. *Soil and Tillage Research*, *27*(1-4), 211-240.

Ilieva-Makulec, K., Olejniczak, I., & Szanser, M. (2006). Response of soil micro- and mesofauna to diversity and quality of plant litter. *European Journal of Soil Biology*, *42*, S244–S249.

Jeffery, S., Abalos, D., Spokus, K., & Verheijen, F. (2015). Biochar effects on crop yield. In *Biochar for environmental management* (pp. 301–325). Routledge.

Kabir, Z. (2005). Tillage or no-tillage: impact on mycorrhizae. *Canadian Journal of Plant Science*, *85*(1), 23-29.

Kalayu, G. (2019). Phosphate solubilizing microorganisms: promising approach as biofertilizers. *International Journal of Agronomy*, *2019*.

Kennedy, A. C. (1999). Bacterial diversity in agroecosystems. *Invertebrate biodiversity as bioindicators of sustainable landscapes*, 65-76.

Killham, K., & Prosser, J. I. (2015). The Bacteria and Archaea. In *Soil Microbiology, Ecology and Biochemistry* (pp. 41–76). Elsevier.

Kindler, R., Miltner, A., Thullner, M., Richnow, H., Kastner, M., 2009. Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. Organic Geochememistry 40, 29e37.

Kumar, P., Dua, V. K., Sharma, J., Byju, G., Minhas, J. S., & Chakrabarti, S. K. (2018). Site-specific nutrient requirements of NPK for potato (Solanum tuberosum L.) in Western Indo-gangetic plains of India based on QUEFTS. *Journal of Plant Nutrition*, *41*(15), 1988-2000.

Kuypers, M., Marchant, H. & Kartal, B. The microbial nitrogen-cycling network. *Nat Rev Microbiol* 16**,** 263–276 (2018). https://doi.org/10.1038/nrmicro.2018.9

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. *Applied and Environmental Microbiology*, *75*(15), 5111–5120.

Lehmann, J. and Rondon, M. (2006) Biochar soil management on highly weathered soils in the humid tropics, in N. T. Uphoff, A. S. Ball and E. Fernandes (eds) *Biological Approaches to Sustainable Soil Systems*, Boca Raton, FL:Taylor and Francis, pp 517–530

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota – A review. *Soil Biology and Biochemistry*, *43*(9), 1812–1836.

Lehmann, J., Czimczik, C., Laird, D., & Sohi, S. (2012). Stability of biochar in soil. In *Biochar for environmental management* (pp. 215-238). Routledge.

Lehmann, J., & Joseph, S. (2015). *Biochar for Environmental Management: Science, Technology and Implementation*. Routledge.

Li, M., Wang, Y., Liu, M., Liu, Q., Xie, Z., Li, Z., ... & Chen, Y. (2019). Three Year Field Observation of Biochar Mediated Changes in Soil Organic Carbon and Microbial Activity. *Journal of environmental quality*, *48*(3), 717-726.

Liu, X. H., & Zhang, X. C. (2012). Effect of Biochar on pH of Alkaline Soils in the Loess Plateau: Results from Incubation Experiments. *International Journal of Agriculture & Biology*, *14*(5).

Liu, X., Shi, Y., Zhang, Q. *et al.* Effects of biochar on nitrification and denitrification-mediated N_2O emissions and the associated microbial community in an agricultural soil. *Environ Sci Pollut Res* 28**,** 6649–6663 (2021). https://doi.org/10.1007/s11356-020-10928-4

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., & Brookes, P. C. (2011). Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry*, *43*(11), 2304-2314.

Luo, S., Wang, S., Yao, P. *et al.* Soil microbial communities under film mulching and N fertilization in semiarid farmland. *Nutr Cycl Agroecosyst* 114**,** 157–170 (2019). https://doi.org/10.1007/s10705-019-09998-9

Maaß, S., Hückelheim, R., & Rillig, M. C. (2019). Collembola laterally move biochar particles. *PLOS ONE*, *14*(11), e0224179.

Major, J., Steiner, C., Downie, A., Lehmann, J., & Joseph, S. (20122). Biochar effects on nutrient leaching. *Biochar for environmental management: Science and technology*, *271*.

Malik, A. A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P. G., ... & Gleixner, G. (2016). Soil fungal: bacterial ratios are linked to altered carbon cycling. *Frontiers in Microbiology*, *7*, 1247

McCormack, S. A., Ostle, N., Bardgett, R. D., Hopkins, D. W., & Vanbergen, A. J. (2013). Biochar in bioenergy cropping systems: Impacts on soil faunal communities and linked ecosystem processes. *GCB Bioenergy*, *5*(2), 81–95.

McLauchlan, K. The Nature and Longevity of Agricultural Impacts on Soil Carbon and Nutrients: A Review. *Ecosystems* **9,** 1364–1382 (2006). https://doi.org/10.1007/s10021-005-0135-1.

Meilin, A., & Rubiana, R. (2018, February). Effect of rice husk biochar application to soil insect diversity on potato cultivation. In *IOP Conference Series: Earth and Environmental Science* (Vol. 122, No. 1, p. 012046). IOP Publishing.

Mills, A. A. S., Platt, H. W., & Hurta, R. A. (2004). Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. *Postharvest Biology and Technology*, *34*(3), 341-350.

Miltner, A., Kindler, R., Knicker, H., Richnow, H., Kastner, M., 2009. Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter. Organic Geochemistry 40, 978e985.

Minor, M. A., & Norton, R. A. (2004). Effects of soil amendments on assemblages of soil mites (Acari: Oribatida, Mesostigmata) in short-rotation willow plantings in central New York. *Canadian Journal of Forest Research*, *34*(7), 1417–1425.

Moore, J. C., Walter, D. E., & Hunt, H. W. (1988). Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. *Annual Review of Entomology*, 33, 419–439.

Mundy, C., Creamer, N. G., Wilson, L. G., Crozier, C. R., & Morse, R. D. (1999). Soil physical properties and potato yield in no-till, subsurface-till, and conventional-till systems. *HortTechnology*, *9*(2), 240-247.

Nakamoto, T., Komatsuzaki, M., Hirata, T., & Araki, H. (2012). Effects of tillage and winter cover cropping on microbial substrate-induced respiration and soil aggregation in two Japanese fields. *Soil science and plant nutrition*, *58*(1), 70-82

Norton, R. A., & Behan-Pelletier, V. M. (1991). Calcium carbonate and calcium oxalate as cuticular hardening agents in oribatid mites (Acari: Oribatida). *Canadian Journal of Zoology*, *69*(6), 1504–1511.

Oerke, E. (2006). Crop losses to pests. *The Journal of Agricultural Science*, *144*(1), 31-43. doi:10.1017/S0021859605005708

Olanrewaju, O.S., Glick, B.R. & Babalola, O.O. Mechanisms of action of plant growth promoting bacteria. *World J Microbiol Biotechnol* 33**,** 197 (2017). https://doi.org/10.1007/s11274-017-2364-9

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., Otihara, R. B., ... & Wagner, H. (2013). Community ecology package. *R package version*, *2*(0).

Pacific International Engineering, 2008. Skagit River Basin Hydrology Report Existing Conditions. Edmonds, Washington.

Pagliai, M., Vignozzi, N., & Pellegrini, S. (2004). Soil structure and the effect of management practices. *Soil and Tillage Research*, *79*(2), 131-143.

Palansooriya, K. N., Wong, J. T. F., Hashimoto, Y., Huang, L., Rinklebe, J., Chang, S. X., ... & Ok, Y. S. (2019). Response of microbial communities to biochar-amended soils: a critical review. *Biochar*, *1*(1), 3-22.

Perdue, J. C., & Crossley Jr, D. A. (1989). Seasonal abundance of soil mites (Acari) in experimental agroecosystems: effects of drought in no-tillage and conventional tillage. *Soil and Tillage Research*, *15*(1-2), 117-124.

Pinheiro J, Bates D, R Core Team (2022). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-157, https://CRAN.R-project.org/package=nlme.

Pringle, P.T., and Scott, K.M., 2001. Postglacial influence of volcanism on the landscape and environmental history of the Puget lowland, Washington—A review of geologic literature and recent discoveries, with emphasis on the landscape disturbances associated with lahars, lahar runouts, and associated flooding, in Puget Sound Research 2001, Proceedings: Olympia, WA, Washington State Puget Sound Water Quality Action Team, 23 p.

Reilly, K., Cullen, E., Lola-Luz, T., Stone, D., Valverde, J., Gaffney, M., ... & Griffiths, B. S. (2013). Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop. *Journal of the Science of Food and Agriculture*, *93*(15), 3700-3709.

Rieff, G. G., Natal-da-Luz, T., Sousa, J. P., Wallau, M. O., Hahn, L., & Saccol de Sa, E. L. (2016). Collembolans and Mites Communities as a Tool for Assessing Soil Quality: Effect of Eucalyptus Plantations on Soil Mesofauna Biodiversity. *Current Science*, *110*(4), 713.

Rieff, G. G., Natal-da-Luz, T., Renaud, M., Azevedo-Pereira, H. M., Chichorro, F., Schmelz, R. M., ... & Sousa, J. P. (2020). Impact of no-tillage versus conventional maize plantation on soil mesofauna with and without the use of a lambda-cyhalothrin based insecticide: A terrestrial model ecosystem experiment. *Applied Soil Ecology*, *147*, 103381.

Rillig, M. C. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, *84*(4), 355-363.

Rodríguez, E., Fernández-Anero, F. J., Ruiz, P., & Campos, M. (2006). Soil arthropod abundance under conventional and no tillage in a Mediterranean climate. *Soil and Tillage Research*, *85*(1-2), 229-233.

Roger-Estrade, J., Anger, C., Bertrand, M., & Richard, G. (2010). Tillage and soil ecology: Partners for sustainable agriculture. *Soil and Tillage Research*, *111*(1), 33–40.

Rousk, J., Brookes, P.C. & B˚a˚ath, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralization. *Applied & Environmental Microbiology*,75, 1589 – 1596.

Rousk J, Brookes PC & B˚a˚ath E (2010) Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry*, 42: 926–934

Saha, S. (2010). Soil functions and diversity in organic and conventional farming. In *Sociology, organic farming, climate change and soil science* (pp. 275-301). Springer, Dordrecht.

Salem, M., Kohler, J., & Rillig, M. C. (2013). Palatability of carbonized materials to Collembola. *Applied Soil Ecology*, *64*, 63–69.

Schaetzl, Randall, and Michael Thompson. *Soils Genesis and Geomorphology*. Cambridge University Press, 2015.

Shakir, M. M., & Ahmed, S. (2015). Seasonal abundance of soil arthropods in relation to meteorological and edaphic factors in the agroecosystems of Faisalabad, Punjab, Pakistan. *International Journal of Biometeorology*, *59*(5), 605–616.

Shepherd, M. A., Harrison, R., & Webb, J. (2002). Managing soil organic matter–implications for soil structure on organic farms. *Soil use and Management*, *18*, 284-292.

Singh, P.K., Singh, M. & Tripathi, B.N. Glomalin: an arbuscular mycorrhizal fungal soil protein. *Protoplasma* 250**,** 663–669 (2013). https://doi.org/10.1007/s00709-012-0453-z

Socarrás, A. (2013). *Soil mesofauna: Biological indicator of soil quality*. *36*(1), 8.

Steiner, C., Teixeira, W. G., Lehmann, J., & Zech, W. (2004). Microbial response to charcoal amendments of highly weathered soils and Amazonian Dark Earths in Central Amazonia—preliminary results. In *Amazonian Dark Earths: Explorations in space and time* (pp. 195-212). Springer, Berlin, Heidelberg

Tadesse, Y., Almekinders, C. J., Schulte, R. P., & Struik, P. C. (2017). Understanding farmers! potato production practices and use of improved varieties in Chencha, Ethiopia. *Journal of crop improvement*, *31*(5), 673-688.

Thangasamy, A., Gorrepati, K., Ahammed, T. S., Savalekar, R. K., Banerjee, K., & Chavan, M. K. (2018). Comparison of organic and conventional farming for onion yield, biochemical quality,

soil organic carbon, and microbial population. *Archives of Agronomy and Soil Science*, *64*(2), 219-230.

Tomczyk, A., Sokołowska, Z. & Boguta, P. Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Rev Environ Sci Biotechnol* **19,** 191–215 (2020). https://doi.org/10.1007/s11157-020-09523-3

Treseder, K. K., & Allen, M. F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: A model and field test. *New Phytologist*, *155*(3), 507–515.

Teutscherova, N., Vazquez, E., Masaguer, A. *et al.* Comparison of lime- and biochar-mediated pH changes in nitrification and ammonia oxidizers in degraded acid soil. *Biol Fertil Soils* **53,** 811–821 (2017). https://doi.org/10.1007/s00374-017-1222-0

Vilkamaa, P., & Huhta, V. (1986, January). Effects of fertilization and pH on communities of Collembola in pine forest soil. In *Annales Zoologici Fennici* (pp. 167-174). Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo

Vinten, A. J., Whitmore, A., Bloem, J., Howard, R., & Wright, F. (2002). Factors affecting N immobilization/mineralization kinetics for cellulose-, glucose- and straw-amended sandy soils. *Biology and Fertility of Soils*, *36*(3), 190–199.

Visser, S. (1985). Role of the soil invertebrates in determining the composition of soil microbial communities. In A. H. Fitter, D. Atkinson, D. J. Read, & M. B. Usher (Eds.), *Ecological interactions in soil,* (pp. 297–317). Oxford: Blackwell Scientific Publications.

Voříšková, J., Baldrian, P. Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME J* **7,** 477–486 (2013). https://doi.org/10.1038/ismej.2012.116

Walter, D., and Proctor, H., &Mites in Soil and Litter Systems." *Mites: Ecology, Evolution & Behaviour Life at a Microscale*, Springer Netherlands, 2013, pp. 161–228.

Waring, B. G., Averill, C., & Hawkes, C. V. (2013). Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. *Ecology letters*, *16*(7), 887-894.

Warnock, D. D., Lehmann, J., Kuyper, T. W., & Rillig, M. C. (2007). Mycorrhizal responses to biochar in soil – concepts and mechanisms. *Plant and Soil*, *300*(1–2), 9–20.

Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M., & Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual review of phytopathology*, *40*(1), 309-348

Wierzbicka, A., Dyderski, M. K., Kamczyc, J., Rączka, G., & Jagodziński, A. M. (2019). Responses of soil mite communities (Acari: Oribatida, Mesostigmata) to elemental composition of mosses and pine needles and long-term air pollution in Scots pine (Pinus sylvestris L.) stands. *Science of The Total Environment*, *691*, 284-295.

Wiwatwitaya, D., Takeda, H. Seasonal changes in soil arthropod abundance in the dry evergreen forest of north-east Thailand, with special reference to collembolan communities. *Ecol Res* **20,** 59–70 (2005). https://doi.org/10.1007/s11284-004-0013-x

Xiao, Z., Rasmann, S., Yue, L., Lian, F., Zou, H., & Wang, Z. (2019). The effect of biochar amendment on N-cycling genes in soils: a meta-analysis. *Science of the Total Environment*, *696*, 133984.

Xu, G.-L., Schleppi, P., Li, M.-H., & Fu, S.-L. (2009). Negative responses of Collembola in a forest soil (Alptal, Switzerland) under experimentally increased N deposition. *Environmental Pollution*, *157*(7), 2030–2036.

Ye, L., Camps-Arbestain, M., Shen, Q., Lehmann, J., Singh, B., & Sabir, M. (2020). Biochar effects on crop yields with and without fertilizer: A meta-analysis of field studies using separate controls. *Soil Use and Management*, *36*(1), 2-18.

Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., & Filley, T. R. (2012). Soil microbial community dynamics over a maize (Zea mays L.) growing season under conventional-and no-tillage practices in a rainfed agroecosystem. *Soil and Tillage Research*, *124*, 153-160.