Agriculture **2015**, *5*, 1060-1075; doi:10.3390/agriculture5041060

OPEN ACCESS

agriculture

ISSN 2077-0472

www.mdpi.com/journal/agriculture

Article

Effects of Biochar Blends on Microbial Community Composition in Two Coastal Plain Soils

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Academic Editor: Bin Gao

Received: 31 July 2015 / Accepted: 26 October 2015 / Published: 3 November 2015

Abstract: The amendment of soil with biochar has been demonstrated to have an effect not only on the soil physicochemical properties, but also on soil microbial community composition and activity. Previous reports have demonstrated significant impacts on soil microbial community structure. These impacts are modulated not only by the biochar composition, but also on the soil's physicochemical characteristics. This indicates that soil characteristics must be considered prior to biochar amendment. A significant portion of the soils of the southeastern coastal plain are severely degraded and, therefore, candidates for biochar amendment to strengthen soil fertility. In this study we focused on two common soil series in the southeastern coastal plain, utilizing feedstocks endemic to the area. We chose feedstocks in four ratios (100% pine chip; 80:20 mixture of pine chip to poultry litter; 50:50 mixture of pine chip to poultry litter; 100% poultry litter) prior to pyrolysis and soil amendment as a biochar product. Soil was analyzed for bioavailable nutrients via Mehlich-1 extractions, as well as microbial community composition using phospholipid fatty acid analysis (PLFA). Our results demonstrated significant shifts in microbial community composition in response to biochar amendment, the effects of which were greatest with 100% poultry litter biochar. Strong relationships between PLFAs and several Mehlich-1 extractable nutrients (Al, Cu, Fe, and P) were observed.

Keywords: biochar; soil microbiology; southeastern United States

1. Introduction

When used as a soil amendment, biochar has the potential to improve the health and fertility of degraded soils. These benefits come in the form of increased soil aggregates with concomitantly increased water retention capabilities, improved soil carbon (C) levels, as well as increased plant-available nutrients, and improved plant biomass [1]. Along with the demonstrated physical and chemical improvements associated with biochar soil amendment, the positive effect of biochar addition on soil microbial communities has likewise been extensively documented [2]. However, despite its wide ranging success as a soil amendment, biochar is not without its detractions, with reports demonstrating negative impacts to soil pH and cation exchange capacity [3], reduced soil aggregation [4], decreases in beneficial soil microorganisms [5], and lower crop yields [6].

The disparity between these reports indicates that there may not be a single biochar source suitable for the amendment of all soils; in other words, not all manufactured biochars may be suitable for all soil types. This finding resulted in Novak proposing the idea of "designer biochars", tailored to meet the needs of a particular soil [7]. This proposal was further expounded upon by Spokas *et al.* [8], the results of which have been supported by research by Sigua *et al.* [9] who clearly demonstrated that soil type can influence biochar stability. Herath *et al.* [10] likewise reported that biochar effects on soil physical properties were dependent upon the biochar, the rate of amendment, and soil series. While this has resulted in the ability to adjust amendments so that they provide the proper amount of benefit to soil characteristics, what is still largely unknown is how these designer biochars affect overall soil microbial community composition.

A report by Kolb *et al.* [11] utilizing a biochar derived from a manure mixture feedstock, demonstrated that both biochar application rate and soil type modulated microbial responses. Similarly, Kelly *et al.* [5] utilized a switchgrass biochar to measure microbial activity on a pair of soils. Interestingly, these reports provided two different looks at the effects of biochar amendment, with the Kolb *et al.* [11] report showing positive gains in microbial responses over a period of several months while the Kelly *et al.* [5] report demonstrated negative impacts on plant biomass and an altered microbial community structure, a result supported by Ippolito *et al.* [12] who similarly noticed a negative priming effect when applying a hardwood biochar to an Aridisol. Ippolito *et al.* [12] further demonstrated that this negative priming effect was eliminated when manure was co-applied. Ducey *et al.* [13] reported significant impacts on microbial N-cycling gene abundances when using switchgrass biochar as a soil conditioner, and Ippolito *et al.* [14] demonstrated a relationship between biochar amendment rate and microbial abundance as measured by 16S rRNA gene abundances.

Despite the potential benefits provided by biochar as a soil amendment, adoption of this management practice can often be hampered by fiscal realities. The review by Spokas *et al.* [8] highlights the impact that economics plays on the feasibility of biochar application and stresses that "designer biochars"—designed specifically to ameliorate local soil deficiencies—could improve the economics of the practice. Since pyrolysis feedstock transportation costs (to and from pyrolysis

stations) can be a significant expense, materials relevant to the area should be considered for cost reduction. For the southeastern coastal plain, this means a reliance on pine chip and poultry litter, two readily-available waste materials inherent to the region [15,16].

The objective of this study was to assess the impact of biochar application on soil microbial community structure. To achieve this objective, we looked at two soils (Coxville and Norfolk soil series) endemic to the region. Biochar was created from two-locally acquired feedstocks, pine chip and poultry litter biochars blended in four different ratios (PC:PL), and subsequently added to the soils at the rate of 2% (w/w). The ratios were as follows: 100% pine chip biochar (PC:PL 100:0); an 80:20 mixture (PC:PL 80:20); a 50:50 mixture (PC:PL 50:50); and a 100% poultry litter biochar (PC:PL 0:100). The effects of biochar addition on soil microbial community structure were assessed by phospholipid fatty acid (PLFA) analysis.

2. Results

2.1. Soil Characteristics in Response to Biochar Amendment

Biochar amendment demonstrated significant impacts on measured soil characteristics when analyzed 78 days post-amendment (Table 1). For the highly acidic Coxville soil, amendment with 100% poultry litter biochar (PC:PL 0:100), as well as a 50:50 mixture of pine chip with poultry litter biochar (PC:PL 50:50), significantly increased ($p < 0.05$) soil pH in comparison to the non-amended, control soil. In all instances, the amended soils still remained acidic in nature, with the PC:PL 0:100 biochar-amended soil reaching a pH only as high as 5.7. In comparison, all Norfolk soil samples amended with PC:PL biochar mixtures, with the exception of the 100% pine chip biochar (PC:PL 100:0), caused significant increases ($p < 0.05$) in soil pH over the slightly acidic (pH 6.0), non-amended, control soil. For these soils, their pH's ranged from neutral (pH 7.0 for the PC:PL 100:0) to slightly alkaline (pH 7.9 for the PC:PL 0:100).

Table 1. pH and soil organic carbon (SOC) after 78 day incubation period.

		pH	SOC ($g \cdot kg^{-1}$)
Coxville	Control	5.1 ± 0.1 ^{c,†,‡}	47.8 ± 0.1 ^{c}
	PC:PL 100:0	5.0 ± 0.1 ^{c}	57.8 ± 0.9 ^{a}
	PC:PL 80:20	5.2 ± 0.1 $^{b\,c}$	55.9 ± 0.8 $^{a\,b}$
	PC:PL 50:50	5.5 ± 0.1 $^{a\,b}$	54.1 ± 0.7 ^{b}
	PC:PL 0:100	5.7 ± 0.1 ^{a}	54.6 ± 0.9 ^{b}
Norfolk	Control	6.0 ± 0.1 ^{c}	12.6 ± 0.5 ^{c}
	PC:PL 100:0	6.3 ± 0.1 ^{c}	22.5 ± 0.5 ^{a}
	PC:PL 80:20	7.0 ± 0.1 ^{b}	22.8 ± 0.3 ^{a}
	PC:PL 50:50	7.7 ± 0.1 ^{a}	20.1 ± 0.3 $^{a\,b}$
	PC:PL 0:100	7.9 ± 0.2 ^{a}	17.7 ± 2.5 ^{b}

† Means and standard deviations; ‡ columns statistically grouped according to Duncan's multiple range test based on a $p < 0.05$ level. Those with the same letter are not significantly different. Each soil series grouped individually.

Analysis of soil organic carbon (SOC) demonstrated that amendment with all four PC:PL mixtures resulted in significantly increased SOC levels for both Coxville and Norfolk soils (Table 1). For the

Coxville soil (47.8 $g \cdot kg^{-1}$), SOC was highest with the PC:PL 100:0 biochar amendment (57.8 $g \cdot kg^{-1}$), and decreased as the amount of pine chip biochar in the mixture also decreased (54.6 $g \cdot kg^{-1}$ SOC in the PC:PL 0:100 biochar-amended soil). A similar trend was observed in the Norfolk soil which saw the highest SOC levels when amended with the PC:PL 100:0 and PC:PL 80:20 biochar mixtures (22.5 and 22.8 $g \cdot kg^{-1}$ SOC respectively). Levels of SOC decreased to 17.7 $g \cdot kg^{-1}$ in the PC:PL 0:100 biochar mixture; however, it too was significantly increased over the 12.6 $g \cdot kg^{-1}$ of SOC in the non-amended control soil.

The results of the Mehlich-1 extractions can be found in Table 2. Coxville soil Mehlich-1 extractable Al, Cu, Fe, and Mn nutrients did not significantly change for any of the biochar mixtures. When compared to the control soil, significant increases ($p < 0.05$) in K and P were noted in PC:PL 80:20, PC:PL 50:50, and PC:PL 0:100 biochar-amended soils, while Na, Mg, Ca, and Zn concentrations significantly increased in the PC:PL 50:50 and PC:PL 0:100 biochar-amended soils.

Unlike in the Coxville soils where Al concentrations did not respond significantly to biochar amendment, Norfolk soil amended with PC:PL 0:100 biochar saw a significant increase in Al concentrations when compared to the control soil. For Norfolk soils receiving biochar amendment, Mg, Mn, and P significantly increased in the PC:PL 80:20, PC:PL 50:50, and PC:PL 0:100 biochar-amended soils as compared to the control. Additionally, Ca, K, Na and Zn saw significant increases in the PC:PL 50:50 and PC:PL 0:100 biochar-amended soils, while Fe was only significantly increased in the PC:PL 0:100 biochar-amended soil. More details on of the interactions between these two soil series and the aforementioned biochar amendments are reported by Novak *et al.* [17] and Sigua *et al.* [9].

2.2. Microbial Community Composition in Response to Biochar Amendment

High-throughput PLFA analysis was performed according to Buyer and Sasser [18], with PLFAs assigned to taxonomic microbial groups according to Bartelt-Ryser *et al.* [19]. Total PLFA—expressed as a ratio to the peak area of methyl-hexadecanoate (C16:0)—in Coxville and Norfolk soils revealed differing responses to biochar amendment (Figure 1). In Coxville soils, total PLFA increased concomitantly with the ratio of poultry litter contained in the biochar mixture; and reached significantly increased levels at PC:PL 50:50 (7.03) and PC:PL 0:100 (7.10) as compared to the non-amended control soil (6.62). In Norfolk soils however, total PLFA peaked at PC:PL 80:20 (7.06) before declining to a low of 6.41 in the PC:PL 0:100 biochar amendment. Despite the decrease in PLFA totals in Norfolk soils amended with increasing ratios of poultry litter biochar, microbial stress indicators either decreased significantly (as in the case of saturated *vs.* monosaturated PLFAs (S/M) for all treatments), or remained similar (as in the case of cyclo *vs.* precursor PLFAs (cy/pre) as PC:PL 100:0 and PC:PL 0:100) to control soils (Figure 2). It should be noted that PC:PL 80:20 and PC:PL 50:50 showed a significantly lower cy/pre ratio as compared to control soils (Figure 2).

Table 2. Mehlich-1 extractable nutrients after 78 days incubation.

		Mehlich-1 Extractable Nutrients (mg·kg^{-1})									
		Al	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**	**Zn**
Coxville	Control	650.9 ± 60.2 $^{a\,b,\dagger,\ddagger}$	235.8 ± 16.0 ^{c}	0.7 ± 0.1 ^{a}	48.3 ± 4.2 $^{a\,b}$	42.8 ± 3.8 ^{d}	37.4 ± 1.3 ^{c}	15.4 ± 1.6 ^{a}	7.8 ± 1.2 ^{c}	66.2 ± 7.7 ^{d}	3.1 ± 0.1 ^{c}
	PC:PL 100:0	675.8 ± 102.4 $^{a\,b}$	234.7 ± 34.5 ^{c}	0.6 ± 0.1 ^{a}	53.2 ± 7.7 $^{a\,b}$	45.7 ± 7.6 ^{d}	36.2 ± 5.5 ^{c}	18.4 ± 3.3 ^{a}	7.6 ± 0.2 ^{c}	68.7 ± 11.8 ^{d}	3.1 ± 0.7 ^{c}
	PC:PL 80:20	759.8 ± 27.8 ^{a}	304.1 ± 7.1 $^{b\,c}$	1.0 ± 0.2 ^{a}	60.5 ± 3.9 ^{a}	130.2 ± 3.4 ^{c}	52.9 ± 1.1 ^{c}	19.9 ± 1.3 ^{a}	24.3 ± 2.0 $^{b\,c}$	110.7 ± 7.2 ^{c}	3.9 ± 0.4 $^{b\,c}$
	PC:PL 50:50	637.4 ± 70.9 $^{a\,b}$	381.6 ± 39.8 $^{a\,b}$	0.8 ± 0.1 ^{a}	48.5 ± 6.1 $^{a\,b}$	271.8 ± 33.2 ^{b}	84.0 ± 11.8 ^{b}	17.0 ± 3.1 ^{a}	60.2 ± 18.5 $^{a\,b}$	165.9 ± 25.8 ^{b}	5.4 ± 0.9 $^{a\,b}$
	PC:PL 0:100	587.9 ± 18.9 ^{b}	416.6 ± 37.2 ^{a}	0.9 ± 0.1 ^{a}	44.3 ± 2.2 ^{b}	388.3 ± 30.7 ^{a}	108.4 ± 11.8 ^{a}	17.1 ± 1.7 ^{a}	95.9 ± 23.5 ^{a}	228.6 ± 13.4 ^{a}	6.9 ± 1.0 ^{a}
Norfolk	Control	89.3 ± 6.5 ^{b}	176.5 ± 6.0 ^{c}	ND §	8.4 ± 0.8 $^{b\,c}$	30.6 ± 10.2 ^{c}	22.7 ± 0.7 ^{d}	3.7 ± 0.2 ^{d}	4.5 ± 0.4 ^{c}	11.1 ± 1.5 ^{d}	3.3 ± 0.1 ^{c}
	PC:PL 100:0	86.2 ± 14.0 ^{b}	193.7 ± 4.6 ^{c}	ND	9.1 ± 0.5 $^{a\,b}$	25.3 ± 2.5 ^{c}	23.5 ± 0.8 ^{d}	4.0 ± 0.8 ^{d}	3.3 ± 0.3 ^{c}	11.4 ± 1.6 ^{d}	2.9 ± 0.2 ^{d}
	PC:PL 80:20	87.3 ± 9.5 ^{b}	204.9 ± 13.4 ^{c}	ND	6.7 ± 0.3 ^{d}	64.6 ± 3.7 ^{c}	32.9 ± 2.5 ^{c}	6.4 ± 1.0 ^{c}	11.3 ± 1.0 $^{b\,c}$	28.9 ± 1.7 ^{c}	3.1 ± 0.1 $^{c\,d}$
	PC:PL 50:50	101.0 ± 5.0 $^{a\,b}$	330.5 ± 6.2 ^{b}	ND	7.4 ± 0.3 $^{c\,d}$	148.4 ± 2.8 ^{b}	77.2 ± 2.9 ^{b}	9.3 ± 0.7 ^{b}	31.1 ± 1.9 ^{b}	112.7 ± 2.6 ^{b}	6.5 ± 0.1 ^{b}
	PC:PL 0:100	121.2 ± 3.8 ^{a}	509.9 ± 17.5 ^{a}	ND	9.6 ± 0.1 ^{a}	244.9 ± 39.4 ^{a}	130.0 ± 4.0 ^{a}	13.5 ± 0.1^{a}	61.5 ± 19.3 ^{a}	236.5 ± 7.0 ^{a}	9.8 ± 0.1 ^{a}

† Means and standard deviations; ‡ columns statistically grouped according to Duncan's multiple range test based on a $p < 0.05$ level. Those with the same letter are not significantly different. Each soil series grouped individually; § ND, not detected or below detectable levels.

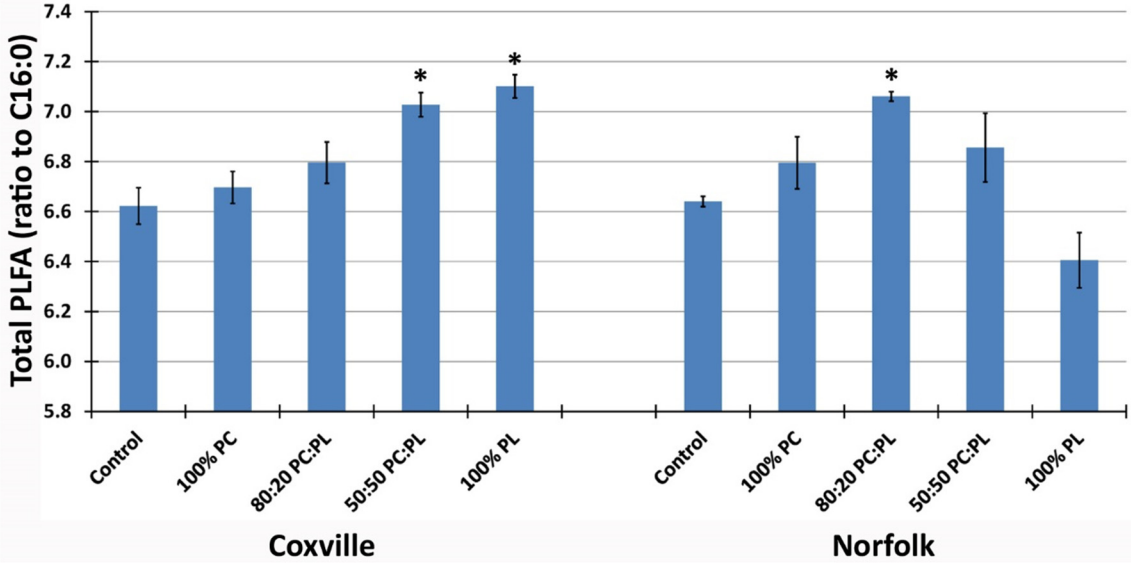

Figure 1. Total microbial biomass, presented as a ratio to C16:0 for Coxville and Norfolk soils amended with PC:PL 0:100, PC:PL 80:20, PC:PL 50:50, or PC:PL 0:100 biochar mixtures added at a 2% w:w volume. * represents statistical significance ($p < 0.01$) as compared to the control.

Comparisons with other microbial groups revealed that Gram-positive to Gram-negative (G−/G+) ratios increased at higher poultry levels for both soil series (Figure 2), a consequence of both increasing G− and decreasing G+ relative abundances (Table 3). Fungal to bacterial (F/B) ratios were lower in Coxville soils as compared to Norfolk soils, but F/B ratios remained comparable to control soils for all biochar treatments across both soil series (Figure 2). Relative abundances of fungal PLFA's remained equable across all treatments for both Coxville and Norfolk soils. Similar results were generally seen for both actinomycetes and eukarya group PLFA's as well (Table 3). The degree of the effect of biochar amendment on each soil type is also evident in Table 3. In the Coxville soils, Gram-positive bacterial relative abundance was reduced from 37.39% in the control soil to 34.99% in the PC:PL 0:100 biochar-amended soil—a decrease of 2.4%. Inversely, Gram-negative bacterial relative abundances increased from 36.04% to 38.44%—an increase of also 2.4%. Meanwhile, in the Norfolk soil, Gram-positive bacterial relative abundance was reduced from 34.80% to 28.63%—a decrease of 6.17%, while Gram-negative bacterial relative abundance increased from 35.24% to 41.00%—an increase of 5.76%.

To assess the differences in microbial community composition in response to biochar amendment, we utilized principle component (PC) analysis of microbial PLFAs, the biplot of which is shown in Figure 3. Total variance explained by the first pair of axes was 96.4% (PC1, 77.2%; PC2, 19.2%). Pearson (r) and Kendall (tau) correlations with the ordination axes are listed in Table 4. Along the first axis, strong relationships ($r < \pm 0.75$) were observed with pH, Al, Cu, Fe, and SOC. A similarly strong relationship along the second axis was observed with P. While the two soil series separated along the first axis (Figure 3; PC1), biochar treatments separated primarily along the second axis (Figure 3, PC2) with 100% poultry litter biochar-amended soils (PC:PL 0:100) being the most distant groups away from their respective controls (Figure 3); the PC:PL 50:50 groups were the second most distant group to their respective controls. An overlay showing specific PLFA markers grouped according to microbial

taxonomic groups revealed findings similar to that found in Table 3. In this table G− abundances increased—and G+ abundances decreased—as poultry litter biochar amendment increased. Overall, both G+ bacteria and actinomycetes were found in higher relative abundances in Coxville soils, while fungal PLFA's were found in higher relative abundance in Norfolk soils.

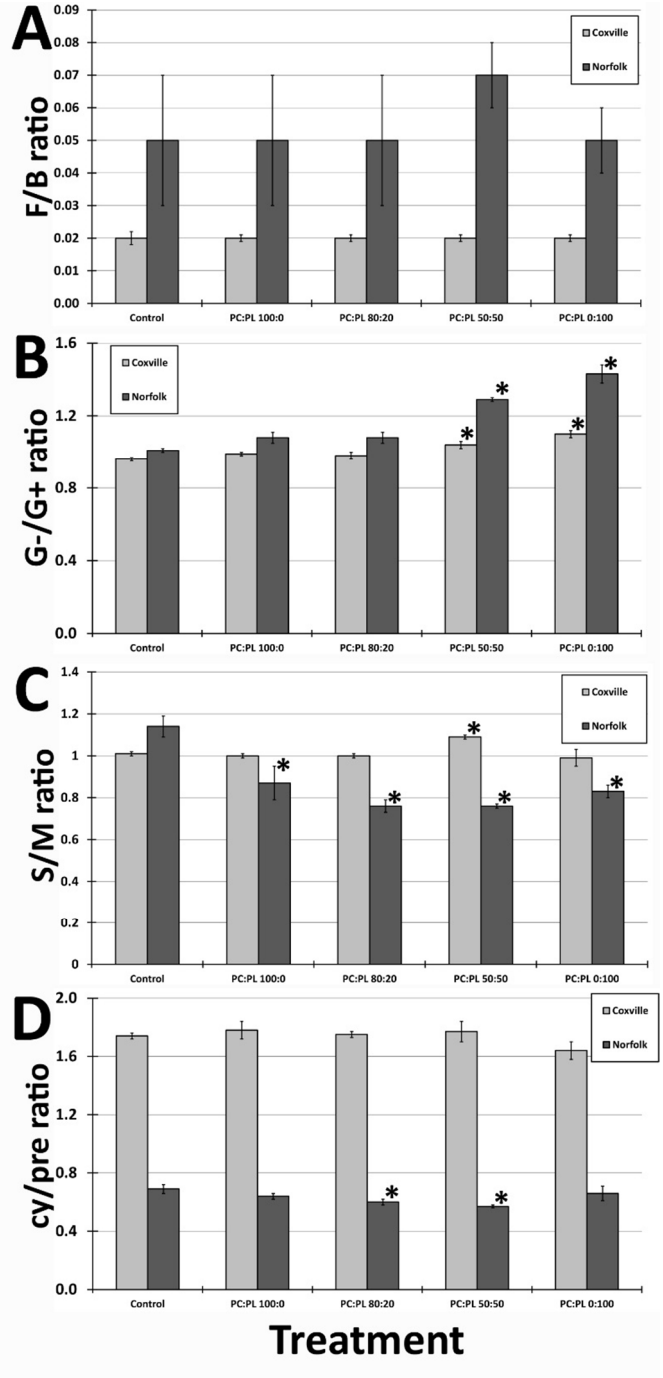

Figure 2. Ratios between PLFA groups in treatment soils. Data are means and standard deviations (n = 3). Panels are as follows: (**A**) fungal to total bacterial PLFAs (F/B); (**B**) Gram-negative to Gram-positive bacterial PLFAs (G−/G+); (**C**) saturated to monosaturated PLFAs (S/M); and (**D**) cyclopropyl to precursor PLFAs (cy/pre). * indicates statistical significance of biochar treatment comparative to the control treatment of the same soil series.

Table 3. Relative abundances (% of total) of PLFA groups in biochar treatments.

Soil	PLFA Group	Control	PC:PL 100:0	PC:PL 80:20	PC:PL 50:50	PC:PL 0:100
Coxville	Gram-positive	37.39 ± 0.18 †	36.82 ± 0.20	37.03 ± 0.88	35.86 ± 0.32 *	34.99 ± 0.20 *
	Gram-negative	36.04 ± 0.03	36.35 ± 0.32	36.17 ± 0.22	37.15 ± 0.34 *	38.44 ± 0.48 *
	Actinomycetes	19.90 ± 0.27	20.10 ± 0.14	19.94 ± 0.35	19.61 ± 0.18	19.49 ± 0.31
	Fungi	1.52 ± 0.18	1.59 ± 0.08	1.62 ± 0.43	1.81 ± 0.04	1.74 ± 0.16
	Eukarya	0.58 ± 0.30	0.42 ± 0.08	0.55 ± 0.32	1.00 ± 0.02	0.96 ± 0.11
Norfolk	Gram-positive	34.80 ± 0.44	33.91 ± 0.93	33.55 ± 1.17	29.93 ± 0.68 *	28.63 ± 0.38 *
	Gram-negative	35.24 ± 0.74	36.58 ± 0.48	36.24 ± 0.89	38.68 ± 0.81 *	41.00 ± 1.09 *
	Actinomycetes	18.25 ± 0.37	17.69 ± 0.93	17.64 ± 0.44	17.22 ± 0.23 *	18.36 ± 1.32
	Fungi	4.61 ± 1.55	4.19 ± 2.00	4.59 ± 1.56	5.61 ± 1.18	4.61 ± 1.18
	Eukarya	0.74 ± 0.02	0.97 ± 0.21	0.82 ± 0.10	1.35 ± 0.86	1.12 ± 0.04 *

† Mean and standard deviations; * indicates statistically significant differences ($p < 0.05$) compared to control sample.

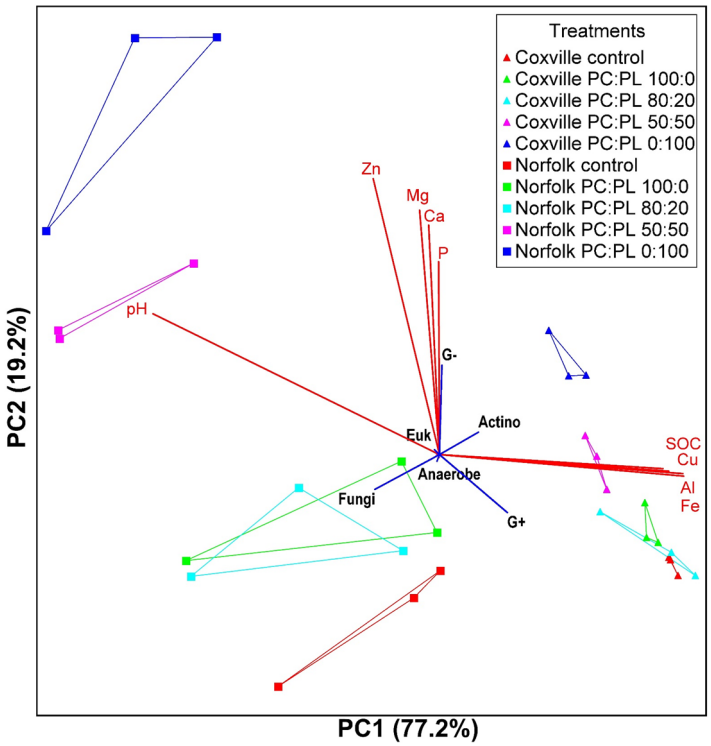

Figure 3. Principle component (PC) analysis ordination plots using PLFA groups indicating changes to the microbial community structure of non-amended Coxville (▲) and Norfolk (■) soils, or the same soils amended with PC:PL 0:100, PC:PL 80:20, PC:PL 50:50, or PC:PL 0:100 biochar mixtures added at a 2% w:w volume. Numbers in parentheses along each axis represents the percent variance explained. Joint plot vectors (red lines) were selected for display based on a combined axis r^2 cutoff of 0.60 (see Table 4). Blue lines represent microbial PLFA groups and their directional trend along the two axis plot.

Table 4. Pearson and Kendall correlations with ordination axes.

	Axis 1			Axis 2		
	r	***r*²**	**tau**	***r***	***r*²**	**tau**
pH	−0.957	0.917	−0.795	0.018	0.000	−0.181
Al	0.795	0.631	0.526	0.377	0.142	0.127
Ca	−0.244	0.059	−0.012	0.734	0.538	−0.031
Cu	0.768	0.582	0.570	0.386	0.149	0.142
Fe	0.797	0.635	0.568	0.352	0.124	0.127
K	−0.126	0.016	−0.054	0.714	0.510	0.034
Mg	−0.358	0.128	−0.045	0.573	0.588	0.000
Mn	0.495	0.245	0.376	0.553	0.305	0.066
Na	−0.152	0.023	−0.020	0.721	0.520	0.050
P	−0.187	0.035	0.041	0.819	0.670	0.066
Zn	−0.506	0.256	−0.149	0.658	0.432	−0.062
SOC	0.727	0.529	0.529	0.415	0.172	0.065

3. Discussion

While the two soils used in this study are both commonly found in the southeastern coastal plain, they have two very different chemical compositions. The Coxville soil series is poorly drained and forms closed depressions in upland landscapes. These areas collect organic matter transported from upslope locations, and the poor drainage results in anoxic conditions that tend to slow decomposition rates. Not only do these soils typically have lower pH values, but the organic matter produces a fine material which traps aluminum silicates, as well as holds Cu, thereby preventing both the aluminosilicates and Cu from being lost through leaching. This is evidenced quite clearly in Table 2, with Coxville soil Al, Fe, and Cu levels all significantly greater ($p < 0.001$) than those of the Norfolk soils. Additionally, the lower pH of the Coxville soil series would favor more soluble Al to remain in soil pore water [20]. The lower organic carbon content of the Norfolk loamy sand resulted in a wider dispersion along the second axis (Figure 3, PC2) as well as a greater effect on the shifts in relative abundances of both Gram-positive and Gram-negative bacteria, when compared to the Coxville loam. This was most likely a result of the lower buffering capability of the Norfolk soils resulting in a larger pH range between the control soil and biochar treatment soils. This, in turn, would also afford microorganisms greater access to nutrients—such as P—supplied by the biochar. In fact, previous work showed that soluble P leached from these biochars, allowing them to be made readily available for uptake and utilization [17].

While an extremely important nutrient for plant growth, P is often found in limiting concentrations in soil. While biochar is often looked to as a C source, when the proper feedstocks are utilized it can also serve as a considerable source of other nutrients—P included. A drawback to this, however, is that when added to soil at levels over those required by crop nutrient demands, residual P can often be lost from the soil by surface runoff [21]. In sandy soils, P can also be lost through leaching into groundwater [22]. For this study, only the PC:PL 80:20 biochar mixtures added at a 2% w:w rate provided P in agriculturally-recommended amounts (15 to 30 $mg \cdot kg^{-1}$) [17]. In this study, extractable P played a significant role in microbial community composition (Figure 3) for both soil series. In addition to

negative water quality impacts, exorbitant P levels can have a deleterious effect on soil fertility, usually due to interactions between P and other micronutrients [23]. For example, phosphorus-induced Zn deficiency has been reported to lower crop yields [24]. Singh *et al.* [24] reported that this induced deficiency occurred around 80 to 160 $mg \cdot kg^{-1}$ of applied P, levels that were reported in this study at the higher poultry litter mixture application rates. Despite this however, the increased P levels found in the 100% poultry litter biochar treatment (PC:PL 0:100) did not appear to stress the microbial communities as determined by S/M and cy/pre ratios.

For Coxville soils receiving biochar amendment, neither S/M (with the sole exception of PC:PL 50:50) or cy/pre ratios fluctuated significantly from the control soil. Stable S/M ratios, and high cy/pre ratios in the Coxville soils, may be indicative of a relatively stable microbial community [25]. Stable F/B ratios, and G−/G+ ratios at all but the highest rates of poultry litter biochar amendment (*i.e.*, PC:PL 50:50 and PC:PL 0:100, treatments which also resulted in significantly higher total PLFA (see Figure 1)) further demonstrate the stability of the soil microbial communities in the Coxville soils. This is visually demonstrated in the PC analysis, where the Coxville control soil clusters with the PC:PL 100:0, and PC:PL 80:20 biochar amendment treatment soils (Figure 3). There is also very little movement along the primary axis of the PC plot for the Coxville soils receiving biochar amendment, potentially indicative of a stabilizing effect on microbial community composition provided by high soil SOC levels, particularly when compared to the Norfolk soils. The greatest change to the microbial community composition of the Coxville soils is reflected in the second axis, correlating primarily with increasing P, and seen more dramatically in the PC:PL 50:50 and PC:PL 0:100 microbial communities, a pattern similar—but diminished in its intensity—to that of Norfolk soils (Figure 3). These shifts in microbial community structure are attributed primarily by significant increases in Gram-negative bacteria, with significant and concomitant decreases in Gram-positive bacteria for both soil series (Table 2). That the change along the second axis is diminished for the microbial communities in the Coxville soils suggests that while the Coxville soil may provide a more stabilizing environment for microorganisms, it cannot completely abate the influences brought about by biochar amendment in these soils.

For the Norfolk soils, F/B ratios and G−/G+ ratios showed a pattern similar to the Coxville soils, though significant differences manifested when comparing S/M and cy/pre ratios. For the Norfolk soils, the cy/pre ratios in the PC:PL 80:20 and PC:PL 50:50 treatments were significantly lower than the control soil, potentially indicating an actively growing microbial community [26], reflected in the total PLFA (Figure 1). Additionally, S/M ratios for Norfolk soil series decreased as poultry litter biochar ratios increased, indicating that microbial communities were less stressed under the increasing P conditions. This is consistent with research that showed P availability is a limiting factor for microbial growth in soils [27,28]. Of additional interest is that excess P levels did not appear to have an effect on fungal PLFA levels. When bioavailable P is found in excess levels, plants no longer require arbuscular mycorrhizal fungi (AMF), which serve a primary role in providing soluble P to their hosts [29]. While AMF are only a small portion of the total fungal population in the soil, we saw no trends in the data to indicate that fungal populations were affected by biochar amendment. It should be noted however that fungal PLFA levels (and therefore F/B ratios) were considerably low in this study, potentially in part because this study did not include microbe:plant interactions—thereby limiting AMF activity. Future studies focusing primarily on the effect designer biochar plays on the composition and abundance of

soil fungal populations—and AMF in particular—in the presence of plants, would provide considerable benefit to the subject.

In addition to providing nutrients to plants and microbes, biochar can also be utilized as a soil conditioner. Ameliorating soil acidity/alkalinity issues, increasing SOC, and improving physical properties such as water holding capacity, with the express goal of increasing soil fertility, have all been objectives of soil biochar amendment. In our study, both Coxville and Norfolk soils responded to biochar amendment, significantly increasing pH and SOC levels. In fact, the feedstocks utilized in this study were chosen precisely for the predicted effects they would impact on the soil, post-addition. Combining nutrient-dense feedstocks—poultry litter high in P, for example—with nutrient-poor feedstocks—such as pine chip—can result in "designer biochars" [17]. These biochar blends can be used to neutralize acidic soils without overloading soils with plant-available P. Traditional fertilizers can then be co-applied to offset any potential negative priming effects in regards to microbial community activity and structure. The benefits of this co-application are demonstrated by Elzobair *et al.* [30] who reported that a combination of biochar and dairy manure led to significant increases in soil organic C, extractable P, and NO_3-N as well as significant increases in microbial community biomass as measured by PLFA.

Biochar amendment to soils has frequently been shown to modulate soil microbial responses, as measured by activity and community composition. Rapid, short-term responses have often been reported upon introduction of biochar to soils as the local microbial communities are stimulated by an influx of nutrients or changes in pH. For example, Rutigliano *et al.* [31] demonstrated an increase in microbial activity up to three months after woody biochar amendment to soils used for wheat cropping, though these responses were undetectable over a year later. As the authors also noted, this study also coincided with increases to wheat and grain yields as compared to control soils [32]. This is in contrast to the report by Kelly *et al.* [5] which demonstrated a negative response in plant biomass upon introduction of a switchgrass biochar. Using a similar feedstock to measure nitrogen cycling gene abundances, Ducey *et al.* [13] reported a rapid, significant increase in several nitrogen cycling genes, levels of which remained significantly higher than the control soil over a period of six months. These reports all demonstrate the capacity of biochar amendment to rapidly affect microbial communities; the impacts of which are controlled by the type of feedstock utilized, production method, and biochar application rate, and the soil to be amended. Of these potential impacts, biochar production and application rate could potentially have the lowest variability, given the ability of manufacturers to produce biochar that meets strict chemical characteristic criteria and the precision of agricultural machinery for land application. Soils mapped as a particular pure series, will still have inclusions of related soil series causing them to be highly variable in morphogenic characteristics (e.g., texture, horizon depths, SOC contents, *etc.*). Local land usage, regional flora and fauna, along with the spatial variability of microbial communities can all influence how the soil will respond to biochar application. Considerable efforts will be needed to tease out how specific soils, preferably at the field scale, respond to biochar designed specifically to ameliorate local shortfalls in soil physical and chemical characteristics.

4. Experimental Section

4.1. Feedstocks and Pyrolysis

Poultry litter (PL; *Gallus domesticus*) was collected in Orangeburg County, SC. Pine chip (PC; *Pinus taeda*) was collected in Berkeley County, SC, USA. The process for pyrolysis of PC and PL has been previously described [9,33]. Briefly, the PC and PL were hammer milled into 6 mm flakes, blended on a per weight basis, and then pelletized through a 6.4 mm die using a PP2000 pellet mill (Pellet Pros, Inc., Davenport, IA, USA). PL alone and PC:PL blends required the addition of deionized water to achieve a 30% moisture content for pelletization, while PC alone required the addition of a 50:50 (w:w) addition of deionized water and soybean oil to achieve a 30% moisture content for proper pelletization. Pelletized feedstocks were then subjected to slow pyrolysis at 350 °C in a retort [33] with a residence time of 2 h. After pyrolysis, pellets were retained by sorting with a 2 mm sieve. For this particular study, a post-pyrolysis step was incorporated to grind all biochar pellets into a dust capable of passing through a 0.42 mm sieve.

4.2. Soils and Biochar Incubation

Coxville and Norfolk soil series Ap (*i.e.*, plowed topsoil) horizons were chosen for this study as this uppermost soil horizon provides a root environment for plants, as well as serving as a habitat for the majority of soil microorganisms [34]. The Ap horizons were collected in 2011 and 2012 respectively, by mechanical removal of the top 15 cm of soil from the Clemson University, Pee Dee Research and Education Center, Darlington, SC. These Ultisols are commonly found in the Southeastern United States coastal plain region. The poorly-drained Coxville (fine, kaolinitic, thermic, Typic Paleaquults) and well-drained Norfolk (fine-loamy, kaolinitic, thermic, Typic Kandiudults) were collected from a field site used for switchgrass production since 2007. Collected soil was air-dried and passed through a 2 mm sieve. For the study, a total of 9 $g \cdot kg^{-1}$ of biochar was mixed into 450 g of air-dried soil (2% addition) and then placed into open-top flower pots with drainage holes. Each treatment was performed in triplicate, and a non-biochar treatment for both the Coxville and Norfolk soils was included as a control. Chemical characteristics of the biochars and soils used for this study can be found in Table 5. Deionized water was mixed into each pot to achieve a 10% soil moisture content (w:w), and then gently packed to a bulk density of 1.3 to 1.4 $g \cdot cm^{-3}$. These pots were then laboratory incubated at 10% moisture content for a period of 78 days. The pots were leached with between 1.2 to 1.3 pore volumes of deionized water on days 30 and 75 as part of a companion study [17]. At the end of the study, on day 78, the pots were destructively sampled.

4.3. Soil and Biochar Chemical Characterization and Phospholipid Fatty Acid (PLFA) Analysis

C and N analysis of biochar feedstocks was conducted by Hazen Research, Inc. (Golden, CO, USA) following American Society for Testing and Materials (ASTM) D3172-13 and D3176-15 standard methods [35,36]. Biochar pH was measured using a 1:2 (v:v) biochar:deionized water mixture after shaking at 200 rpm for 2 h. At the end of the 78 day incubation period, soil samples were collected from each pot for chemical analysis. Samples were allowed to air-dry and then tested for pH and SOC,

as well as Mehlich-1 extractable nutrients [37]. SOC measurements were performed using the loss on ignition method in a muffle furnace set for dry oxidation at 575 °C for 16 h following ATSM E1755-01 [38]. The weight difference pre- and post-oxidation was used to calculate the quantity of SOC ($g \cdot kg^{-1}$). Soil pH was measured as previously described [39]. Mehlich-1 extractable Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn concentrations were measured using an inductively coupled plasma-atomic emission spectrometer (ICP-AES). At the study endpoint (day 78), a total of 15 g of fresh soil from each pot was snap-frozen in liquid nitrogen and shipped on dry ice to MIDI Inc. (Newark, DE, USA) for high-throughput PLFA analysis as described by Buyer and Sasser [18]. PLFAs with retention times lower than C14:0 and greater than C22:0 were removed prior to data analysis.

Table 5. Chemical properties of soil and biochar utilized in study.

Soil Series	pH	SOC † ($g \cdot kg^{-1}$)	TN † ($g \cdot kg^{-1}$)	
Coxville	5.1	26.3	1.8	
Norfolk	5.9	3.9	ND ‡	
Feedstock	**pH**	**C ($g \cdot kg^{-1}$)**	**N ($g \cdot kg^{-1}$)**	**C:N ratio**
Pine chip	5.3	787	3.7	213:1
Poultry litter	9.4	511	56.1	9:1

† SOC, soil organic carbon; TN, total nitrogen; ‡ ND, not detectable or below detectable limits.

4.4. Data Analysis

Duncan's multiple range test, and other statistical analyses were performed using SAS version 9.2 (SAS Inst., Cary, NC, USA). To account for extraction efficiencies of PLFA from biochar-amended soils [40], PLFAs were normalized as a ratio to C16:0 [41]. To assess the effects of the various biochar amendments on the two soils used in this study, multivariate analysis ordination as well as Pearson and Kendall correlations were performed using principle component (PC) analysis in PCORD version 6.0 (MJM Software, Gleneden Beach, OR, USA). Only PLFAs assigned to taxonomic microbial groups according to Bartelt-Ryser *et al.* [19] were utilized for PC analysis.

5. Summary and Conclusions

Considerable research efforts have been devoted to determining optimal feedstocks and application rates of biochar to soils for the improvement of soil fertility. These studies have often focused on the role that biochar amendment plays on the improvement of physical and chemical characteristics of the soil series to which they are applied. There is not, however, a commensurate amount of data examining the effects these soil amendments have on microbial community composition, with a particular focus on how soil types modulate those responses. Our study focused on the response of microbial community composition in two soil series to four separate biochar amendments; a mixture of pine chip and poultry litter in varying ratios, with a 2% application rate. We reported significant increases in a number of Mehlich-1 extractable nutrients, several of which significantly correlated with changes in microbial community composition. Soils amended with 100% poultry litter (PC:PL 0:100) biochar, based on PC analysis, were most dissimilar to their respective control soils; however, all indicators of stress, as measured by PLFA, remained low.

Acknowledgments

The information in this document has been funded in part by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory's Western Ecology Division and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. We thank Donald W. Watts for laboratory assistance.

Author Contributions

Thomas F. Ducey, Jeffrey M. Novak, and Mark G. Johnson were each responsible for planning and managing the study, collecting and analyzing the data, as well as writing and editing the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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