Locally produced wood biochar increases nutrient retention in agricultural soils of the San Juan Islands, WA, USA

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Abstract

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Biochar additions to agricultural soil have been shown to result in many benefits; however, most studies have been conducted in greenhouse or laboratory trials with few being conducted in the field and particularly in association with organic farming systems. Herein, this gap was addressed by conducting on-farm studies on the efficacy of locally produced biochar as a soil amendment in small-scale organic agriculture on 10 farms in San Juan County, WA. Biochar produced from local timber harvest residues in the San Juan Islands was applied in factorial combination with a poultry litter based fertilizer to replicated plots on all 10 farms. Dry beans (*Phaseolus vulgaris L*) were grown on eight of the farms with green beans and cauliflower being grown on the other two. Soils were examined for nitrogen (N), phosphorus (P), and carbon (C) pools during the growing season. Dry bean samples were evaluated for metal uptake. Results showed that biochar additions enhanced soil total C by 32-33%, soil

available NH_4^+ by 45-54%, soil active organic N by 48-110%, and active inorganic P by 29%; biochar additions enhanced soil NO_3^- -N, NH_4^+-N, and P retention by 33%, 53% and 39% respectively. Increased availability of soil P, Fe, Mg, Zn was reflected in nutrient density of harvested dry beans. This study demonstrates that locally produced wood biochar has the potential to increase soil nutrient availability and nutrient uptake. By producing biochar from timber harvest residues and applying them on neighboring organic farms on the San Juan Islands, WA, this study leveraged local resources and community readiness to drive forest restoration and sustainable agricultural practices on the sandy soils of the San Juan Islands.

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Chapter 1. Literature Review

1.1 The Need for Biomass Removal and Biochar Production

The potential for wildfire is the main driver of many biomass removal projects in forest ecosystem. During the past century, inappropriate fire management has led to overstocked forests and excess woody biomass, resulting in increased susceptibility to catastrophic fires (Kauffman 1990). Therefore, residual woody biomass removal is significant for hazardous fuel reduction and forest health improvement, especially in the Pacific Northwest (PNW), where the national forests are overgrown and vulnerable to wildfires and attacks by insects and disease (Agee 1996).

In much of the western United States, drought and conditions associated with climate change have exacerbated the forest management problem and further contribute to deteriorating forest health (Allen et al. 2010). In addition, although government and companies are trying to make highly utilization of forest biomass, the costs are high for those treatments to thin the forests, decrease fuel loads, and reduce the density of insect- and disease-killed trees. There is almost few market for small round wood and nearly no markets for residual material (tops, limbs, etc.) (Evans 2008). Data from Rummer et al. (2003) showed that the cost of managing forestry residues ranges from $0.10 to $40 per ton for chipping, the median cost of bringing biomass to the roadside was $680 per acre for only mild slopes, not including costs for haul distances (Rummer et al. 2003). Therefore, the highly variable costs of transporting and utilizing woody biomass depending on stand conditions, locations, and markets are also difficult to estimate (Lynch & Mackes 2003).

Due to the low value of biomass and limited accessibility, the majority of the woody residues are left on site. Piling and burning of forest residuals releases air pollutants (CO_2, CO, NO_x and particulate matter) and results in the loss of nutrients (volatile elements such as C, N, P and S, which are frequently of limited availability in forest environments) from the ecosystem (Binkley 1986; Fox et al. 2007). Woody biomass can be considered as a local, renewable resource that can be used for transportation fuel, heat and even power, the energy produced

from woody biomass could provide a sustainable way to reduce greenhouse gases and reduce energy waste (McElligott et al. 2011).

Besides energy production, another way to utilize woody residues is to convert them into a value-added material through pyrolysis, a process in which organic matter is heated rapidly to high temperatures with limited or no oxygen (Manyà 2012). Products from woody biomass generally include: 1) Bio-oil; 2) Synthesis gas, which could be used for energy production and serve as feedstock for fuels production (Anderson et al. 2013); 3) Biochar, a solid material and by-product, it could serve as a soil amendment and a precursor for secondary carbon products. Biochar production from pyrolysis process provides an economic way to utilize woody biomass residues. Bioenergy production system represents a means of extracting energy from the biomass, but it could not return the nutrients from biomass back into the ecosystem. Instead, biochar, the byproduct from the pyrolysis system/unit has a market value of its own, and nutrients can be recycled back to the soil as an amendment and a means of soil C sequestration. Many related approaches have been put into practice in agriculture and forest systems, primarily in agriculture ecosystem (Lehmann & Joseph 2015).

1.2 Biochar as a Soil Amendment

The term ‘biochar’ is relatively new as it was introduced only recently, first as a term to distinguish from activated C, and to replace the term “charcoal”, and to distinguish it from coal (Bapat et al. 1999). However, biochar is not a new substance. Biochar has been utilized in agriculture since long before the arrival of modern sciences and it has a history of at least 2,000 years (Kern et al. 2003; O’Neill et al. 2009). In the Amazon Basin, the occurrence of fertile dark-colored soils, known as ‘Terra Preta de Indio’, had been illustrating an extensive and native use of biochar. This idea was supported by the observations of charcoal pieces and artifacts within these soils, and the region still remains highly fertile till today. Therefore, biochar has been increasingly studied and discussed as a soil amendment to improve soil fertility and contribute to C sequestration recently.

1.2.1 Biochar Composition and Nutrient Content

Biochar composition can be divided into relatively recalcitrant C, labile or leachable C and ash (Lehmann et al. 2011). Biochar generally has a much larger fraction of recalcitrant C than other organic matter, especially more fused aromatic C structures (Schmidt & Noack 2000). Aromatic C can have different forms – amorphous C forms at lower pyrolysis temperature, and turbostratic C dominates at higher temperatures, as biochar exhibit higher fractions of crystallinity under higher HTT (highest treatment temperature) (Keiluweit et al. 2010; Nguyen et al. 2010; Chia et al. 2015). These C structures can explain the high stability of biochar across a long period of time (Nguyen et al. 2010). Unlike the recalcitrant C which microorganisms will less likely to use as an energy source, labile or leachable C in biochar has been proved to stimulate microbial activity and increase abundance (Steiner et al. 2008; Lehmann et al. 2011). In addition to recalcitrant C and labile C, the third fraction of biochar is minerals present in ash. These macro- and micro-nutrients are mostly coming from the produced feedstock, and the fraction or concentration is also influenced by the pyrolysis conditions. Some of these nutrients could be accessible to plants and microorganisms (Lehmann et al. 2011).

Since biochar is produced from biomass, it is expected to have large proportions of C and contain a range of macro- and micro-nutrients. However, the composition and structure depends on the nature of the feedstocks and the conditions of pyrolysis, even pre- or post-treatment steps such as chemical activation (Chia et al. 2015). Feedstock biomass can be forestry products, agricultural residues, animal wastes, etc. The original biomass structure can primarily influence the final biochar structure associated with its physicochemical properties, as supported by the fact that the pore structure of biochar is similar to the cellular structure of wood or plant-based feedstocks (Yao et al. 2011). Pyrolysis conditions also vary widely, including heating rate, HTT (highest treatment temperature), reaction pressure, reaction residence time, etc. However, most published experiments and studies only report the characteristics of the biochar used in their specific studies, an overall and better understanding of the biochar properties in relation to their feedstock and pyrolysis conditions is further needed. Some typical properties of biochar reported in recent studies are categorized and listed in Table 1.

Based on the existing literature on biochar properties from different feedstocks and pyrolysis conditions, it is evident that the composition of C content can vary widely. For instance, wood char has higher C content than biochar created from agricultural residues or other feedstocks. The actual C content can range between 112 g kg^{-1} and 905 g kg^{-1} (Chan et al. 2008). Additionally, it has been widely reported that the proportion of carbon in the biochar increases with increasing pyrolysis temperature, indicating carbonization degree increases, because of the conversion of labile C in the feedstock and formation of aromatic and more stable forms of carbon (Zheng et al. 2010).

N contents are in the range of 0.3 – 3.3% for biochars produced from agricultural residues, while it is 0.06 – 1.2% for wood chars (Xie et al. 2016). This trend indicates that the N content is also a function of the feedstocks, especially it will be higher when a high N feedstock is being used (Joseph et al. 2010). In fact, no matter how much total N there is in biochar, the extractable N concentrations (NO_3^-, NH_4^+, NO_2^-) in biochars have been reported relatively low, and are almost negligible (Belyaeva & Haynes 2012).

Total P content of charcoal ranges from 2.7 g kg^{-1} to 480 g kg^{-1}, total K from 1.0 g kg^{-1} to 58 g kg^{-1} (Chan et al. 2008). Biochar produced from animal waste tends to have higher total P content than wood char (Chan & Xu 2009). Increased temperatures tend to yield charcoal with a higher concentration of total P and total K (Feng et al. 2012; Peng et al. 2011). In total P, about 0.4% to 34% are available, which is relatively a high value. Similar to P, K is highly available and water-soluble in biochars (Cantrell et al. 2012; Ippolito et al. 2015). Anaerobic digestion process will contain more N, H, Mg and Ca, less O and K, this indicates the impact of feedstock pretreatment (Yao et al. 2011). Recent studies including typical properties of biochars produced from various feedstocks and conditions are presented in Table 1.

1.2.2 Physical and Chemical Properties

The physicochemical properties of biochar are key to understand the biochar functions in soil. Biochar porosity, which determines its surface area, has shown that the pore size distribution of biochar is widely variable: micro-pores (less than 2 nm), meso-pores (2-50 nm) and macro-pores (more than 50 nm) (Chia et al. 2015; Downie et al. 2009). As desired HTT (highest treatment temperature) increases, the surface area of the biochar increases as more pores are generated (Keiluweit et al. 2010), especially micro-pores which are reported to favor the colonization of microbes (Verheijen et al. 2010). Overall, the porous nature and surface area of biochar can alter soil hydrology, aeration and nutrient cycling, microbial activity and abundance, and the presence of certain toxic compounds such as pesticides, herbicides, and PAHs.

Biochar is often associated with a range of pH values from slightly acidic to highly alkaline, across large varieties of feedstock and operating conditions (Table 1). Commonly, pyrolysis temperature is the main factor influencing biochar pH, as acidic functional groups tend to be removed under higher temperatures (Novak et al. 2009a). Because of this, biochar has been used as a liming agent, thus reduce the acidic soil conditions (Yuan et al. 2011).

The cation-exchange capacity (CEC) of biochar represents its ability to electrostatically sorb or attract cations. It is developed when biochar is exposed to oxygen and water, creating oxygenated surface functional groups (Ippolito et al. 2015). Increasing pyrolysis temperature will decrease biochar CEC, as the high temperature will remove organic functional groups (Gaskin et al. 2008). Fresh biochar can have neutral or slightly negative charge thus low CEC compared to soil organic matter on a mass basis (Lehmann 2007; Lehmann et al. 2011), however, studies have illustrated that biochar's anion exchange capacity (AEC) will disappear over time, thus attain greater CEC by aging in the soil environment (Cheng et al. 2008; Quilliam et al. 2012).

Table 1. Typical properties of biochar from different feedstocks and production conditions.

Feedstock	Production conditions			Elements (%)							Ash (%)	Volatile matter (%)	Surface area (m^2 g^{-1})	pH	CEC (cmol kg^{-1})	References
	Pyrolysis temperature (^oC)	Duration (h)	Sieve	C	H	N	O	Ca	Mg	K						
Hickory woodchips	350	5	0.5-1 mm	71.60	3.88	0.25	23.20	0.63	0.17	0.23	-	-	< 0.5	-	42.7	
Hickory woodchips	450	3	0.5-1 mm	77.60	3.52	0.27	17.30	0.77	0.22	0.27	-	-	1.60	-	41.9	(Ding et al. 2016)
Hickory woodchips	600	2	0.5-1 mm	84.70	1.83	0.30	11.30	1.17	0.29	0.28	-	-	256.00	-	45.7	
Eichornia crassipes	300	2	0.150 mm	17.17	3.58	2.12	47.13	-	-	-	15.89	-	3.52	7.98	-	
Eichornia crassipes	500	2	0.150 mm	53.39	1.99	1.82	42.80	-	-	-	27.18	-	6.71	10.96	-	(Li et al. 2016a)
Eichornia crassipes	700	2	0.150 mm	51.34	1.10	0.73	46.83	-	-	-	37.25	-	175.00	11.54	-	
Douglas-fir wood	623	0.5		70.50	5.50	0.25	23.50	-	-	-	0.60	49.82	-	8.30	50-55	
Douglas-fir wood	873	0.5	2 mm sieve and ball milled to a particle size of about 590 µm	87.80	3.80	0.30	7.50	-	-	-	1.13	15.72	500.00	8.70	50-55	
Douglas-fir bark	623	0.5		66.10	4.80	0.65	24.00	-	-	-	4.72	43.78	-	7.90	30-35	(Suliman et al. 2016)
Douglas-fir bark	873	0.5		78.10	3.30	0.70	9.50	-	-	-	8.85	17.25	-	9.80	30-35	
Hybrid poplar wood	623	0.5		69.90	5.30	0.42	21.00	-	-	-	3.50	42.15	-	9.30	30	
Hybrid poplar wood	873	0.5		83.10	3.80	0.50	7.50	-	-	-	7.17	17.81	200.00	10.00	20	
Sewage sludge	500	5	-	20.60	2.01	2.83	14.20	-	-	-	-	-	4.07	7.43	-	(Khan et al. 2015)

Soybean straw	500	5	-	65.60	3.17	1.72	15.60	-	-	-	-	-	2.63	10.06	-	
Rice straw	500	5	-	47.40	3.13	1.65	14.70	-	-	-	-	-	2.21	10.05	-	
Peanut shell	500	5	-	52.50	3.16	1.58	11.80	-	-	-	-	-	4.25	9.68	-	
Pine sawdust	680	-	2 mm	90.90	1.31	0.11	6.10	-	-	-	1.01	-	795.00	9.70	148	
Paunch grass	680	-	2 mm	86.79	1.89	1.30	10.76	-	-	-	11.16	-	6.96	10.10	0.91	
Broiler litter	680	-	2 mm	64.79	2.28	1.80	12.40	-	-	-	28.73	-	1.96	8.80	3.39	(Srinivasan et al. 2015)
Sewage sludge	680	-	2 mm	77.98	2.10	0.50	19.33	-	-	-	12.61	-	-	7.90	1	
Dewatered pond sludge	680	-	2 mm	66.87	1.90	0.70	30.53	-	-	-	4.56	-	-	8.30	0.8	
Dissolved air-floatation sludge	680	-	2 mm	78.67	2.33	0.30	18.56	-	-	-	3.98	-	-	7.70	0.92	
Willow	450	-	-	78.40	2.03	0.82	-	-	-	-	4.30	11.20	-	7.30	33.4	
Willow	650	-	-	84.80	1.14	1.00	-	-	-	-	4.90	6.00	-	8.10	59.1	
Pine	450	-	-	86.80	2.80	0.19	-	-	-	-	0.90	12.10	-	6.70	38.6	(Nelissen et al. 2014)
Pine	650	-	-	92.60	1.68	0.15	-	-	-	-	1.10	6.00	-	7.70	68.8	
Maize	350	-	-	67.30	4.25	1.47	-	-	-	-	7.70	32.60	-	8.30	55.2	
Maize	550	-	-	72.10	2.21	1.52	-	-	-	-	10.90	12.10	-	9.80	61.9	

Wood mixture	-	-	-	68.10	1.50	0.40	-	-	-	-	8.30	12.00	-	8.60	46.3	
Hickory wood	300	-	0.5-1 mm	69.13	4.85	0.39	24.36	0.58	0.21	0.36	-	-	-	7.10	-	
Hickory wood	450	-	0.5-1 mm	83.62	3.24	0.17	11.46	0.92	0.18	0.33	-	-	12.90	7.90	-	
Hickory wood	600	-	0.5-1 mm	81.81	2.17	0.73	14.03	0.82	0.13	0.24	-	-	401.00	8.40	-	
Bagasse	300	-	0.5-1 mm	69.50	4.20	0.90	24.36	0.46	0.14	0.27	-	-	5.20	7.30	-	
Bagasse	450	-	0.5-1 mm	78.60	3.52	0.92	15.46	0.83	0.18	0.25	-	-	13.60	7.50	-	(Sun et al. 2014b)
Bagasse	600	-	0.5-1 mm	76.45	2.93	0.79	18.33	0.91	0.21	0.15	-	-	388.30	7.50	-	
Bamboo	300	-	0.5-1 mm	66.20	4.70	0.40	27.72	0.22	0.14	0.30	-	-	1.30	7.90	-	
Bamboo	450	-	0.5-1 mm	76.89	3.55	0.23	18.11	0.29	0.19	0.35	-	-	10.20	8.50	-	
Bamboo	600	-	0.5-1 mm	80.89	2.43	0.15	14.87	0.34	0.23	0.52	-	-	375.20	9.20	-	
Wheat straw	400	5	-	65.70	4.05	1.05	-	-	-	-	9.70	-	4.80	9.10	161.6	
Wheat straw	460	5	-	72.40	3.15	1.07	-	-	-	-	12.00	-	2.80	8.70	117	
Wheat straw	525	5	-	74.40	2.83	1.04	-	-	-	-	12.70	-	14.20	9.20	97.7	(Kloss et al. 2012)
Spruce wood	400	10	-	63.50	5.48	1.02	-	-	-	-	1.90	-	1.80	6.90	73.5	
Spruce wood	460	10	-	79.60	3.32	1.24	-	-	-	-	3.00	-	14.20	8.70	54.7	
Spruce wood	525	10	-	78.30	3.04	1.17	-	-	-	-	4.70	-	40.40	8.60	52.2	

Poplar wood	400	10	-	67.30	4.42	0.78	-	-	-	-	3.50	-	3.00	9.00	144	
Poplar wood	460	10	-	70.00	3.51	0.95	-	-	-	-	5.70	-	8.20	9.20	128.3	
Poplar wood	525	10	-	77.90	2.66	1.07	-	-	-	-	6.80	-	55.70	8.70	107.6	
Ponderosa pine	400	1	0.25 mm	74.10	4.95	0.06	20.90	-	-	-	1.40	36.40	28.70	-	-	(Keiluweit et al. 2010)
Ponderosa pine	500	1	0.25 mm	81.90	3.54	0.08	14.50	-	-	-	2.10	25.20	196.00	-	-	
Peanut shell	400	1-3	0.25 mm	74.80	4.50	2.70	9.70	-	-	-	8.20	38.40	0.52	7.90	-	
Peanut shell	500	1-3	0.25 mm	81.80	2.90	2.70	3.30	-	-	-	9.30	18.10	1.22	8.60	-	
Pecan shell	350	1-3	0.25 mm	64.50	5.30	0.26	27.60	-	-	-	2.40	61.60	1.01	5.90	-	
Pecan shell	700	1-3	0.25 mm	91.20	1.50	0.51	1.60	-	-	-	5.20	9.70	222.00	7.20	-	(Novak et al. 2009a)
Poultry litter	350	1-3	0.25 mm	46.10	3.70	4.90	8.60	-	-	-	35.90	36.70	1.10	8.70	-	
Poultry litter	700	1-3	0.25 mm	44.00	0.30	2.80	<0.01	-	-	-	52.40	14.10	9.00	10.30	-	
Switchgrass	250	1-3	0.25 mm	55.30	6.00	0.43	35.60	-	-	-	2.60	74.40	0.40	5.40	-	
Switchgrass	500	1-3	0.25 mm	84.40	2.40	1.07	4.30	-	-	-	7.80	13.40	62.20	8.00	-	

1.3 Biochar and Soil Biota

Soil biota is one of the most complex biologically active biotic community in our earth, represented by a diversity of soil organisms (e.g. bacteria and fungi) and soil animals (Paul & Kandeler 2015). Soil organisms can interact with organic matter, obtain energy, and therefore large quantities of soil reactions associated with decomposition and nutrient storage are processing in soil biota (Barrios 2007). Generally, biochar can alter soil biota through many aspects, as reviewed by Lehmann et al. (2011) and Thies et al. (2015), either serving as a potential habitat or substrate (Lehmann et al. 2011; Thies et al. 2015). The effect of biochar on soil biota include: (1) Alterations in soil enzyme activities and nutrient cycles (e.g. C and N); (2) changing in soil microbial abundance, diversity, and structure (Thies et al., 2015).

The alteration of soil enzyme activities by biochar additions have been observed in many studies (Masto et al. 2013; Maestrini et al. 2014; Bandara et al. 2015; Wang et al. 2015; Elzobair et al. 2016) . The effects are generally due to a number of factors including: (i) shift in soil pH by biochar; (ii) biochar itself as a preferred substrate; and (iii) adsorption and reaction with specific compound on biochar surfaces that may act as an enzyme enhancers or inhibitors (Thies et al. 2015). More importantly, biochar has been widely reported to have impact on soil microbial C cycles in terms of observations of increased soil CO_2 evolution (Deenik et al. 2010; Dempster et al. 2012; Masto et al. 2013; Maestrini et al. 2014; Keith et al. 2015). This enhancement might be explained by several mechanisms: (i) Biochar stimulates the decomposition of existing soil organic matter (Wardle et al. 2008); (ii) biochar caused changes in soil physical properties leading to changes in CO_2 flux; and (iii) breakdown of organic C or release of inorganic C within biochar itself (Kuzyakov et al. 2009; Smith et al. 2010; Cross & Sohi 2011; Jones et al. 2011; Zimmerman et al. 2011). Jones et al. (2011) conducted long-term field and lab studies (3 years) using ^{14}C-labeled SOM to identify a range of mechanisms by which biochar can result in net changes in soil CO_2 efflux, results showed that the observed increase in soil CO_2 emission is mostly derived from the breakdown of organic C and the release of inorganic C contained in biochar itself (Jones et al. 2011). Evidences also exist that biochar significantly repressed the turnover of native SOM (Herath et al. 2015; Jones et al. 2011), in contrast to Wardle et al. (2008)

who illustrated that charcoal caused loss of soil humus (Wardle et al. 2008). In addition, it is important to know that the amount of the net loss of CO_2 after biochar addition is relative small compared to the sequestered C in soil, illustrating a long-term C sequestration (Spokas et al. 2009; Major et al. 2010a; Jones et al. 2011). Biochar effects on soil N cycles are discussed in the following section.

A large quantity of recent studies has examined the soil microbial biomass and diversity after biochar additions (Dempster et al. 2012; Plaza et al. 2015; Sun et al. 2015; Jiang et al. 2016). Microbial biomass could be measured by chloroform fumigation method (Vance et al. 1987; Joergensen & Brookes 1990; Dempster et al. 2012), phospholipid fatty acid (PLFA) analysis (Paul & Kandeler 2015; Gomez et al. 2014), or by substrate induced respiration method (Beare et al. 1990; Gundale et al. 2015). Gomez et al. (2014) and Watzinger et al. (2014) have both reported a quick response (an increase) in soil G^- bacteria after biochar addition, illustrating that G^- bacteria contains members that are able to accommodate new C or other nutrient energy source (Gomez et al. 2014; Watzinger et al. 2014; Thies et al. 2015). Researchers have also indicated that biochar addition will lead to shift toward a bacterially dominated community, with evidence of a decrease of fungal to bacterial ratio (Jones et al. 2012; Chen et al. 2013b; Rousk et al. 2013;). Anderson et al. (2011) used molecular methods and found a significant change in soil bacterial communities under pine wood biochar addition, linking biochar-associated soil microbial communities to soil C, N and P cycles (Anderson et al. 2011). Overall, compared to soil microbial biomass, the diversity of microbial communities associated with biochar addition has rarely been studied; most of the studies are short-term observations, few are mechanistic. Further research and meta-analysis are needed to get a clear picture of the biochar effect on soil microbial communities.

1.4 Biochar and Soil Nutrient Transformations

1.4.1 Nitrogen

Nitrogen is the most limited nutrient in temperature ecosystem, especially in agricultural system. Most agricultural plants primarily choose to uptake inorganic nitrogen, which came from the organic nitrogen mineralization process, although a few species were observed to straightly use organic nitrogen for energy and growth (Stevenson 1999; Schimel & Bennett 2004). Biochar has been widely reported and discussed to influence nitrogen cycles. Several primary nitrogen transformation processes associated with biochar addition (N fixation, mineralization, immobilization, denitrification, ammonia volatilization) are discussed below.

1.4.1.1 Nitrogen fixation

Biological N fixation (BNF) is a main natural input of N to terrestrial ecosystems, and it plays an essential role in the N cycles of agricultural system (Peoples et al. 1995; Cleveland et al. 1999; Vitousek et al. 2002). The process of BNF is conducted by bacteria that are either free-living associative, or symbiotic living in an obligate arrangement with host plants (e.g. legumes) or fungal partners (e.g. lichen) (Paul et al. 2015). In recent years, a couple of agronomic studies have been reported that biochar had the capacity to influence BNF process of leguminous plants (Quilliam et al. 2013; Mia et al. 2014; Güereña et al. 2015; Van Zwieten et al. 2015); however, mechanisms remain unclear. A possible mechanism related to the biochar-associated increased N fixation could be the effect of nutrient availability. Rondon et al. (2007) conducted a short-term study investigating biochar effect on the BNF of common beans (*Phaseolus vulgaris*), results showed significant increase in BNF after biochar addition compared to the control (Rondon et al. 2007). They illustrated that the positive result could be attributed to the observed greater availability of trace metals brought by biochar, such as molybdenum (Mo), which is a constituent of the Mo-Fe protein nitrogenase, that can stimulate nodulation (Rondon et al. 2007). In addition to trace metals, it is also likely that the enhanced BNF is correlated with higher macro- or micro-nutrient availability, such as K (Mia et al. 2014), P (Vitousek et al. 2002; Nelson et al. 2011; Güereña et al. 2015), Ca and Mg (Major et al. 2010b), Fe and Mn (Hass et al.

2012). However, inhibitory effect has been also observed, since (i) nodulation is reported more likely to happen under the addition of nutrient-rich biochar (Tagoe et al. 2008; Wurst & van Beersum 2009). (ii) The adsorption of soil signaling compounds to biochar. Nodule formation in leguminous plants is initiated by the release of signaling compounds (e.g. flavonoid) (Koes et al. 1994). Gundale and DeLuca (2006) indicated that such polyphenolic compounds could be adsorbed by biochar, leading to a reduction of nodulation process (Gundale & DeLuca 2006). However, it is also important to know that nodule numbers may not represent the activity of N fixation, as Quilliam et al. (2013) found a reduced numbers of nodules, but the mass of nodules was increased as was nitrogenase activity (Quilliam et al. 2013).

Compared to the symbiotic N-fixing bacteria that live with leguminous plants, only a few studies have been conducted to examine the biochar effect on free-living N fixation bacteria. DeLuca et al. (2015) indicated that one methodology issue might influence the accuracy of the interpretation of N_2 fixation activity, since nitrogenase activity is commonly measured using the acetylene reduction assay, but biochar itself could release ethylene when applied to soil (Spokas et al. 2010; DeLuca et al. 2015b). Other than this, biochar could enhance activity of free-living N-fixing bacteria by influencing systematic N availability. Similar to post-fire BNF process, a decrease of N availability through N immobilization could possibly lead to the stimulation of BNF process (Lehmann et al. 2003; Steiner et al. 2007; Nelissen et al. 2012).

1.4.1.2 Nitrogen mineralization

Nitrogen mineralization is defined as the process by which organic N is converted to inorganic forms (primarily NH_4^+-N and NO_3^--N). The conversion of organic-N to NH_4^+-N is defined as ammonification. The conversion of NH_4^+-N or organic-N into NO_3^--N by autotrophic bacteria, archaea or certain fungi is defined as nitrification. Many studies in temperate or boreal forest soils have shown an increased net nitrification rates in forest soils by biochar additions; however, few studies had found out such results in agriculture system where may already accommodate an active nitrifying community (DeLuca et al. 2006; Rondon et al. 2007; DeLuca et al. 2015b) (Table 2).

Ammonification is a primary component of the N cycle that occurs in agriculture systems, especially in organic farming system. This process is driven by a broad consortium of organisms that are capable of enzymatic denaturation of proteins and the removal of amide groups from organic compounds (e.g. amino acids and amino sugars). It is typically measured by extracting NH_4^+-N from soil at different points in time using a high concentration salt solution, typically potassium chloride (KCl). The NH_4^+-N indicates the N mineralized or ammonified from the organic N pool over a given period of time that is free in the soil solution or exchangeable with K^+ on cation exchange sites when being extracted. Generally, the capacity of biochar to hold NH_4^+-N depends on the cation exchange capacity of the biochar. Therefore, NH_4^+-N extracted as a measure of nitrogen mineralization may actually represent the cat-ion exchange capacity of biochar, and vice versa.

Studies have shown increases, decreases and no change in N mineralization (Table 2). Maestrini et al. (2014) found an increase of NH_4^+-N content and gross N mineralization at the first week of rye-grass charcoal application, but then decrease over time (Maestrini et al. 2014). A recent study from Pereira et al. (2015) also illustrated an increase in N mineralization nearly two times greater than the control after biochar addition at an organically managed lettuce farm (Pereira et al. 2015). The gross mineralization rate was positively correlated with biochar H/C ratio, they suggested that less recalcitrant chars presenting high H/C ratios increased mineralization rates, since they are more likely to decompose and thereby free up N into the mineral pool (Pereira et al. 2015). Gundale et al. (2015) found enhanced net soil N mineralization rates and soil NH_4^+-N concentrations regardless of the soil mixing treatment after two growing seasons in northern Sweden, they attributed this more to the promotion of net N mineralization rather than the ash input from biochar itself (biochar serves as a NH_4^+-N source) (Gundale et al. 2015). Dempster et al. (2012) indicated that N mineralization might relate to biochar feedstock. They amended soil with biochar produced from low N feedstocks, such as wood or cotton stalks, resulted in a decreased nitrogen mineralization after application to soil (Dempster et al. 201 2). Whereas Prommer et al. (2014) and Ulyett et al. (2014) on the other hand found no significant change in N mineralization with low N feedstock biochar application (Prommer et al. 2014; Ulyett et al. 2014). A decrease of total net N mineralization

was observed in both an Aridisol from Colorado and an Alfisol from Virginia, after 18 days of incubation with switchgrass biochar (Kelly et al. 2015), they attributed it to the decline in microbial activity due to the presence of phytotoxic materials such as ethylene, a known nitrification inhibitor, as well as harmful salts such as Na or Cl. Güereña et al. (2013) did a biochar field study in a temperate North America maize-based production system, they found no change in N mineralization potential but an increase in microbial biomass, finally concluded that the mechanism for N retention is the incorporation into microbial biomass N and cycling into the organic N pool and possibly subsequent adsorption of organic N to biochar and minerals (Güereña et al. 2013). In general, these studies suggest that the biochar production feedstock and condition, characterizations, time scale, capacity of biochar to adsorb NH_4^+, and maybe soil type, are all the factors that needed to be considered in N mineralization in response to biochar.

1.4.1.3 Nitrogen immobilization

Immobilization is the opposite process of N mineralization, it is defined as the conversion of inorganic N into organic N. Whether N mineralization or immobilization occurs with organic amendments to soil depends on the C/N ratio of the amendment, if the C/N ratio is high enough (generally more than 25:1), then the N tends to be immobilized (Robertson & Groffman 2007). Biochar generated from wood or N-limited feedstock generally has a high C/N ratio, whereas biochar generated from N-rich feedstock (such as agriculture waste) could serve as N source (Lehmann et al. 2006). It is therefore uncertain whether biochar provide enough carbon to stimulate nitrogen immobilization or not.

Biochar studies have found variable results in terms of N immobilization. Bruun et al. (2012) indicated that application of incompletely pyrolyzed biomass (fast pyrolysis at low temperature) may cause immobilization of soil N, as more N is needed by the developing microorganisms than is provided by the substrate (Bruun et al. 2012), in other words, low-temperature biochar contain more bioavailable C or surface functional groups that can serve as microbial substrates (Liang et al. 2006; Steiner et al. 2007; Nelissen et al. 2012). Novak et al. (2010) conducted a lab incubation study using switchgrass biochar (Novak et al. 2010). Results

showed a short-term N immobilization due to the wide C/N ratio of switchgrass (73:1); they also observed a significant increase in mean CO_2 flux implying biochar simulated switchgrass mineralization and accelerated resident soil carbon. Similar observations such as increased respiration rates have also been reported and discussed in studies from (Wardle et al. 2008; Spokas et al. 2009). However, Jones et al. (2011) suggested that the increased CO_2 evolution after biochar addition was actually came from the emission of inorganic C within biochar itself (Jones et al. 2011). Therefore, immobilization process needs to be further studied with focus on the bioavailable C and microbial activity.

1.4.1.4 Gaseous N emissions

Nitrogen loss can happen in the soil system through many ways, such as leaching, denitrification, volatilization, crop removals, soil erosion and runoff. Among these mechanism, NO_3^- denitrification and NH_3 volatilization are two primary processes of gaseous N emissions. Denitrification is the process by which bacteria convert nitrate to N gases that are lost to the atmosphere ($NO_3^- \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$). Many studies have focused their interests on N_2O because it contributes a large portion of greenhouse gas emissions (Cayuela et al. 2013). Biochar has been reported to influence N_2O flux in many studies (Table 2). Case et al. (2015) reported that biochar suppressed cumulative soil N_2O production by 91% in near-saturated, fertilized soils in a field study (Case et al. 2015). Another recent field study conducted by Ameloot et al. (2016) also observed a 50-90% N_2O reduction after 7 months in a loam textured cropland field with biochar addition, implying that biochar exerts an indirect physical control over soil denitrification several months after incorporation (Ameloot et al. 2016). Harter et al. (2013) illustrated that biochar addition enhanced microbial nitrous oxide reduction with enhanced transcript copy numbers of the nosZ-encoded bacterial N_2O reductase (Harter et al. 2013), similar with (Jones et al. 2012) and (Van Zwieten et al. 2014). A meta-analysis done by Cayuela et al. (2014) reported that biochar reduced soil N_2O emissions by 54% in laboratory and field studies, across 30 studies and 261 experimental treatments during 2007 to 2013 (Cayuela et al. 2014). Several explanations and mechanisms were generated: (1) the elevated pH of the biochar creating an environment where N_2O reductase activity is promoted (Šimek et al. 2002;

Van Zwieten et al. 2009). (2) Enhanced soil aeration inhibiting denitrification due to more oxygen being present (Yanai et al. 2007; Van Zwieten et al. 2010; Case et al. 2015). (3) Shortage of available C due to the adsorption of labile soil organic matter (SOM) compounds on biochar may also decrease the denitrification potential and lower N_2O emission rates (Van Zwieten et al. 2009). (4) A reduction of the availability of inorganic-N to denitrifiers, limiting denitrification potential (Clough et al. 2013; Van Zwieten et al. 2014).

NH_3 volatilization is another process of gaseous N loss to the atmosphere. It is well known that NH_3 volatilization can be enhanced in soil with a higher pH (Stevenson 1999). It is also been reported that biochar with white ash can act as a liming agent that can increase soil pH (Yuan et al. 2011). However, studies have illustrated that the pH biochar increased is usually not high enough to enhance NH_3 volatilization (DeLuca et al. 2015b). A recent study by Mandal et al. (2016) showed that NH_3 volatilization was reduced by 70% with the addition of poultry litter biochar and nut shell biochar, mostly due to the NH_3 adsorption at oxygen-containing surface functional group or biochar micro pores (Mandal et al. 2016). Table 2 provides a summary of findings on NH_3 emissions with biochar additions. Taghizadeh-Toosi et al. (2012) found a 45% reduction of NH_3 volatilization after addition of wood-derived biochar (Taghizadeh-Toosi et al. 2012); Doydora et al. (2011) found a 56-63% reduction of NH_3 loss using poultry litter biochar (Doydora et al. 2011). Studies have also illustrated that biochar could induce ammonium immobilization and nitrification that can reduce NH_3 volatilization potential (Steiner et al. 2010; Mandal et al. 2016). Further in-situ field trial and adsorption or desorption studies are needed to verify these results and fully understand the dynamics of NH_3 adsorption and release.

Table 2. Studies on soil microbial N cycle responses to biochar additions.

Microbial N cycle variables	Observations	Type of study	Biochar description	Application rate	Soil characteristics	Citations
N mineralization	↓	Lab incubation (46 d)	350°C peanut biochar, sieved under 2 mm	0, 1%, 3% (w/w)	Sandy loam	(Chang et al. 2016)
N mineralization	↑		Straw residues, wood chips	0, 0.5%, 1%, 2% (w/w)	Paddy soil	(Li et al. 2016b)
N mineralization	Mineralization to NO_3^- ↓, mineralization to NH_4^+ no change overall	Field (3 years, Mediterranean barley crop)	Pine (*Pinus pinaster + Pinus radiata*) chip gasifier biochar, 600-900°C	0, 12, 50 t ha^{-1}	Sandy loam	(Marks et al. 2016)
N mineralization	Mineralization to NO_3^-↑	Lab	Poultry litter 400, 600°C, swine manure 400, 600°C	0, 2% (w/w)	Sandy, silt-loam soil	(Subedi et al. 2016)
N mineralization	↓	Lab	Pine chips and poultry litter at 400°C and 500°C	0, 20 t ha^{-1}	Luvisols	(Ameloot et al. 2015)
N mineralization	↑	Field (wheat and oilseed rape)	Hardwood trees thinnings, slow pyrolysis 400°C, sieved < 2mm	0, 2% (w/w)	Sandy loam	(Case et al. 2015)
N mineralization	↑	Field (boreal forest)	P. sylvestris, wood and bark	0, 10 t ha^{-1}	Fine sandy Typic Haplocryod	(Gundale et al. 2015)
N mineralization	↓	Growth chamber	Switchgrass	0, 25, 50, 100 t ha^{-1}	Aridisol, Alfisol	(Kelly et al. 2015)
N mineralization	↑	Field (organic lettuce farm)	Douglas-fir wood pyrolyzed at 410°C; Douglas-fir wood pyrolyzed at 510°C, hogwaste wood pyrolyzed between 600-700°C	0, 10 t ha^{-1}	Loam	(Pereira et al. 2015)

N mineralization	↑ at the first 4 days, − after 4 days	Lab	Rye grass, pyrolysis at 450°C	0, 13 mg g^{-1}	Cambisol (forest)	(Maestrini et al. 2014)
N mineralization	−	Field (barley and sunflower)	Hardwood-derived biochar (mostly beech), 500°C for 2 h	0, 24, 72 t ha^{-1}	Sandy to loamy ^{silt}	(Prommer et al. 2014)
N mineralization	− organic, ↓ conventional	Field (organic and conventional)	A mix of sycamore, oak, beech, bird cherry, 600°C 16 h, crushed to a diameter of less than 15 mm	0, 30, 60 t ha^{-1}	Sandy loam (Luvisol, Cambisol)	(Ulyett et al. 2014)
N mineralization	−	Field (maize system)	Maize stover, slow pyrolyzed at 600°C	0,1,3,12,30 t ha^{-1}	Kendaia silt loam and Lima loam	(Güereña et al. 2013)
N mineralization	↓	Greenhouse	Eucalyptus marginata, Pyrolysis 24h 600°C	0, 5, 25 t ha^{-1}	Grey Orthic Tenosol	(Dempster et al. 2012)
N mineralization	↑	Lab	Swine manure, barley stover, carbonized 600-800°C, digest 30 min 320°C, cooled, filtered, dried	0, 2% (w/w)	Utisols (under paddy or pasture)	(Yoo & Kang 2012)
N mineralization	−, ↓ at 14 days	Field	Commercial horticultural charcoal (coppiced woodlands: beech, oak, hazel, and birch), pyrolysis 500°C	0, 3, 6 kg m^{-2}	Silty loam	(Castaldi et al. 2011)
N mineralization	↓	Lab	Four biochars: douglas fir pellets, doulgas fir bark, switchgrass straw, animal digested fiber, all pyrolysis at 600°C	0, 9.8, 19.5, 39.0 t ha^{-1}	Sand, silt loam	(Streubel et al. 2011)
N mineralization	↓	Lab	Macadamia integrifolia, flash pyrolysis, 300-800°C	0, 2.5% (w/w)	Ustic kanhaplohumult	(Deenik et al. 2010)
N mineralization	↑	Field (Scots pine forest, Sweden)	Activated carbon	1000 kg ha^{-1}	Typic or Entic Haplocryods	(DeLuca et al. 2002)
Nitrification	↑	Field (wheat and oilseed	Hardwood trees thinnings, slow pyrolysis 400°C, sieved < 2mm	0, 2% (w/w)	Sandy loam	(Case et al. 2015)

		rape)				
Nitrification	↑ at the first 18 days, − after 4 days	Lab	Rye grass, pyrolysis at 450°C	0, 13 mg g^{-1}	Cambisol (forest)	(Maestrini et al. 2014)
Nitrification	↑	Field (barley and sunflower)	Hardwood-derived biochar (mostly beech), 500°C for 2 h	0, 24, 72 t ha^{-1}	Sandy to loamy silt	(Prommer et al. 2014)
Nitrification	↓	Greenhouse	*Eucalyptus marginata*, Pyrolysis 24h 600°C	0, 5, 25 t ha^{-1}	Grey Orthic Tenosol	(Dempster et al. 2012)
Nitrification	−	Field	Commercial horticultural charcoal (coppiced woodlands: beech, oak, hazel, and birch), pyrolysis 500°C	0, 3, 6 kg m^{-2}	Silty loam	(Castaldi et al. 2011)
Nitrification	↑	Lab (used forest soils)	Lab biochar, ponderosa pine wood, homogenized, sieved < 2mm	1000 mg charcoal kg^{-1} soil	Sandy loam	(DeLuca et al. 2006)
Nitrification	↑	Lab	Activated carbon	2000 kg ha^{-1}	Typic or Entic Haplocryods	(Berglund et al. 2004)
N immobilization	NH_4^+ immobilization ↑, NO_3^- immobilization ↓	Field (wheat and oilseed rape)	Hardwood trees thinnings, slow pyrolysis 400°C, sieved < 2mm	0, 2% (w/w)	Sandy loam	(Case et al. 2015)
N immobilization	NO_3^- immobilization ↑, NH_4^+ immobilization −	Field (barley and sunflower)	Hardwood-derived biochar (mostly beech), 500°C for 2 h	0, 24, 72 t ha^{-1}	Sandy to loamy silt	(Prommer et al. 2014)
N immobilization	↑ during the 65 days of incubation	Lab	Wheat straw, 525°C, fast pyrolysis	0, 5% (w/w)	Sandy loam	(Bruun et al. 2012)
N immobilization	↑	Lab (column study)	Pecan shell biochar	0, 0.5%, 1.0%, 2.0% (w/w)	Loamy sand (fine-loamy, kaolinitic, thermic typic	(Novak et al. 2009b)

					Kandiudults)	
N_2O evolution	↓	Lab incubation	Swine manure digestate biochar 350, 700°C, willow wood biochar 350, 700°C	0, 10 t ha^{-1}	Loam (Alfisol)	(Ameloot et al. 2016)
N_2O evolution	↓	Field (wheat and oilseed rape)	Hardwood trees thinnings, slow pyrolysis 400°C, sieved < 2mm	0, 2% (w/w)	Sandy loam	(Case et al. 2015)
N_2O evolution	↓	Lab	Commercial green waste biochar, 700°C	0, 2%, 10% (w/w)	Loamy sand (calcaric leptosol)	(Harter et al. 2013)
N_2O evolution	↓	Lab (column study)	Commercial wheat straw biochar, 450°C, 4.5 h	0, 30 t ha^{-1}	Agricultural soil (silt clay), forest soil (loam)	(Sun et al. 2014a)
N_2O evolution	↓	Lab incubation	Oil mallee, wheat chaff, and poultry litter biochars, all produced at 500°C	0, 1% (w/w)	Vertosol (clay), Ferrosol (clay), Calcarosol (sandy clay loam) and Tenosol (sand)	(Van Zwieten et al. 2014)
N_2O evolution	↓ (pasture soil + barley stover biochar), ↑ (rice paddy soil + swine manure biochar)	Lab	Swine manure, barley stover, carbonized 600-800°C, digest 30 min 320°C, cooled, filtered, dried	0, 2% (w/w)	Utisols (under paddy or pasture)	(Yoo & Kang 2012)
N_2O evolution	↑ at first 3 days, − after 3 days	Field	Commercial horticultural charcoal (coppiced woodlands: beech, oak, hazel, and birch), pyrolysis 500°C	0, 3, 6 kg m^{-2}	Silty loam	(Castaldi et al. 2011)
N_2O evolution	↓	Lab	Municipal biosolids	0, 10% (w/w)	Loam	(Yanai et al. 2007)
NH_3 volatilization	↓	Lab incubation	Poultry litter biochar and Macadamia nut shell biochar	0, 5% (w/w)	Mawson Lakes Technology Park soil, Port Sunny Vale	(Mandal et al. 2016)

					soil, Port Wakefield soil, Mount Lofty soil, and Adelaide Hill soil	
NH_3 volatilization	↓	Lab incubation	Coconut shell biochar followed by steam activation	0, 1.5%, 3% (w/w)	Silty loam	(Jordan et al. 2015)
NH_3 volatilization	↑	Chamber	Commercial *Miscanthus giganteus* biochar, slow pyrolysis at 600°C	0, 3% (w/w)	Silt-loam, loam soil	(Subedi et al. 2015)
NH_3 volatilization	↑ (agricultural soil), ↓ (forest soil)	Lab (column study)	Commercial wheat straw biochar, 450°C, 4.5 h	0, 30 t ha^{-1}	Agricultural soil (silt clay), forest soil (loam)	(Sun et al. 2014a)
NH_3 volatilization	↑ when under low pH (pH=5), ↓ when under medium pH (pH=7-8)	Lab incubation	Green waste biochar	0, 1%, 5%, 10%, 20% (w/w)	Bauxite residue sand	(Chen et al. 2013a)
NH_3 volatilization	↓	Lab	Monterey Pine biochar, 300, 350, 500°C, sieved < 2mm	0, 2% (w/w)	Temuka silt loam	(Taghizadeh-Toosi et al. 2012)
NH_3 volatilization	↓	Lab incubation (21 d)	Pine chips and peanut hulls biochar, slow pyrolysis 400°C, 1 h	0, 5 t ha^{-1}	Pasture soil (Cecil)	(Doydora et al. 2011)

1.4.2 Phosphorus

Phosphorus is another macro-nutrient for soils following nitrogen, especially in agricultural systems. Phosphorus exists in soils in organic and inorganic forms. P is reported almost inaccessible to plants in the organic form, thus need to be mineralized into inorganic P (mostly as $H_2PO_4^-$ and HPO_4^{2-}) prior to plants uptake (Ryan et al. 2001). Inorganic P is negatively charged in most soils, therefore it tends to react readily with positively charged ions to form mineral precipitates such as Ca-P, or strongly sorbed to the mineral phase (e.g. on Fe and Al oxy-hydroxide surfaces) thus will reduce the solubility of phosphorus (DeLuca et al. 2015b). Until now, biochar is reported to alter soil available P in three large aspects: (i) by acting as a P source providing available P for soils and plants; (ii) by altering P solubility, through the alteration of soil pH, adsorption of specific chelates or formation of specific compounds, and P solubilizing bacteria, etc.; and (iii) by altering the process of P mineralization and phosphatase enzyme activities.

Biochar can be a source of P because P does not volatilize until 700 ^oC (Knoepp et al. 2005), and it has been documented that available P ranges from 0.4% to 34% of total P in biochar, thus biochar can serve as a P source in soil (Ippolito et al. 2015). Wang et al. (2012) conducted a study to explore the bioavailability of P in biochars associated with feedstocks (dairy manure and biosolids), results showed that P in feedstock was fully recovered in the biochars by 98% to 119% (Wang et al. 2012). Therefore, the proportion of different P pools in biochar and total available P levels are highly dependent on original feedstocks. For instance, wood-derived biochar usually contains lower concentration of P, whereas manure- or biosolid-derived biochar has relatively higher levels of P that is plant available (Gaskin et al. 2008; Jin et al. 2016). As pyrolysis can cleave the organic P bonds present in the feedstock; pyrolysis can also lead to the formation of a range of mineral P forms which complexes with Fe, Al, Ca and Mg predominate, biochar therefore contains three pools of P: (i) free soluble; (ii) strongly bond to Fe and Al; (iii) organically bound as a residue of the original feedstock (DeLuca et al. 2015b).

Biochar can alter soil P solubility through several mechanisms. Biochar can influence P precipitation by altering soil pH and thus the strength of ionic P interactions with Al^{3+}, Fe^{3+}, and

Ca^{2+}; or by adsorbing organic molecules that act as chelates (such as phenolic acids, complex proteins and carbohydrates) of metal ions that otherwise precipitate P (Gundale & DeLuca 2007; Soinne et al. 2014; DeLuca et al. 2015b; Madiba et al. 2016). Hydrophobic or charged biochars are more efficient in sorbing these organic molecules onto their surfaces, and forming organo-biochar or organo-mineral-biochar complexes over time, leading to an enhanced P solubility, retention and availability (Joseph et al. 2013; Yang et al. 2016). Soil microorganisms are also helpful in releasing soil P through solubilization process (Rodríguez & Fraga 1999). For instance, Suksabye et al. (2016) reported that PO_4^{3-} solubilizing bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* are effective in solubilizing considerable amounts of tricalcium PO_4^{3-} (Suksabye et al. 2016). Promoted growth of bacteria that correspond to producing P solubilizing compounds in the presence of biochar could influence inorganic P bioavailability (Anderson et al. 2011).

Phosphorus in organic forms is released by mineralization process involving soil organisms. Biochar can alter the activity and abundance of these microbes thus P availability. Phosphatase is an enzyme that can hydrolyze compounds of organic P and transform them into different forms of inorganic P, which are assimilated by plants (Amador et al. 1997). It is been widely illustrated that biochar can enhance phosphatase activity (Yoo & Kang 2012; Oleszczuk et al. 2014; Bhaduri et al. 2016), whereas some studies reported no change (Mackie et al. 2015; Pandey et al. 2016). However, most of them are observation reports, not many are related to mechanisms.

1.5 Biochar and Soil Nutrient Leaching

Nutrient leaching is a part of nutrient cycling in agricultural system, it occurs when mobile nutrients are flushed down by percolation water to an area below the rooting zone that is unable for plant roots to utilize (Major et al. 2009). Biochar has been widely reported to have the potential to reduce nutrient leaching in most cases in agricultural systems, herein, a variety of observations and results from recent lab and field studies related to soil nutrient leaching are listed in Table 3.

In general, biochar could affect soil nutrient leaching under these following mechanisms (Major et al. 2009):

(1) Biochar surface chemistry and nutrient retention. Biochar can lead to the retention of most nutrients by cation exchange, associated with acidic functional groups formed during oxidation process on biochar surfaces; therefore retain most cations like Ca, Mg, K, and Na. The cation exchange capacity (CEC) of biochar has been considered one of the most essential surface chemistry properties that can enhance nutrient retention (Van Zwieten et al. 2010; Clough et al. 2013; Takaya et al. 2016), and it is been reported to increase as ageing (Cheng et al. 2006; Mukherjee et al. 2014; Heitkötter & Marschner 2015).

(2) Biochar affects soil solution chemistry and soil physical properties, thus altering nutrient retention. Biochar generally has a higher pH value and is known to be used as a liming agent in many agricultural cases, therefore it can indirectly alter soil nutrients solubility through changes in soil pH (Rogovska et al. 2016). Biochar can also affect soil physical properties such as soil bulk density, water retention, soil structure, aggregate stability, and total porosity (Sun & Lu 2014), thus nutrient retention. A recent study from Andrenelli et al. (2016) reported a significant increase of soil water retention properties with total water stored in soil pores increased up to 18-25%, and a decrease in soil bulk density after pelletized biochar addition, implying a nutrient retention potential through reduction of water mobility (Andrenelli et al. 2016).

(3) Soil microbial activities affected by biochar will alter soil nutrient retention. Studies have illustrated that biochar have greater potential to lead changes in microbial abundance,

community structure and activities (Gul et al. 2015; Jaafar et al. 2015). Pore spaces within biochar structure could provide suitable habitat for soil microorganisms (bacteria, fungi, protozoa) (Quilliam et al. 2013; Gul et al. 2015). The nutrients and DOC that are desorbed from biochar surface are responsible for the microbial growth, and will lead to alterations of nutrient cycling thus nutrient retention (Deenik et al. 2010; Spokas et al. 2010; Nelissen et al. 2012). Biochar may also induce soil N immobilization to some degree as it is N limited and has a high C:N ratio (DeLuca et al. 2015b). A biochar pot experiment on soil bacterial community structure from Anderson et al. (2011) indicated that, the addition of biochar could potentially enhance the growth of organisms that will produce NH_4^+-N from NO_3^--N that can then be adsorbed to biochar (Anderson et al. 2011). However, further studies related to direct evidences for the impact on microbial processes are needed.

Table 3. Studies on soil nutrient leaching responses to biochar additions.

Biochar	Type of Study	Soils Characteristics	Observations	Citations
Corn stalks, 350 °C	Lab	Loam with low SOC level (0.79%)	29% decrease in NO_3^- leaching	(Kanthle et al. 2016)
Sewage sludge, 300 °C	Lab	Clay loam (Ultisol)	6.8%, 8.5%, 7.9% decrease in NH_4^+, PO_4^{3-}, K^+ leaching, respectively; 0.2% increase in NO_3^- leaching	(Yuan et al. 2016)
Sewage sludge, 500 °C	Lab	Clay loam (Ultisol)	19.4%, 6.4%, 12.9%, 12.1% decrease in NH_4^+, NO_3^-, PO_4^{3-}, K^+ leaching, respectively	(Yuan et al. 2016)
Sewage sludge, 700 °C	Lab	Clay loam (Ultisol)	35.9%, 9.7%, 23.7%, 23.4% decrease in NH_4^+, NO_3^-, PO_4^{3-}, K^+ leaching, respectively	(Yuan et al. 2016)
Filtercake biochar, 575 °C	Lab	Sandy clay loam	No biochar effect on NO_3^- leaching	(Eykelbosh et al. 2015)
Acacia whole-tree greenwaste biochar, 550 °C	Field	Loamy sand	No significant effect on NO_3^-, K^+ leaching, but significantly increased the concentration (34%) and flux (103%) of PO_4^{3-} leaching	(Hardie et al. 2015)
Pig manure biochar and wood biochar, 600 °C	Lab	Sandy loam	24-26% decrease of NO_3^- leaching, no biochar effect on NH_4^+ leaching	(Troy et al. 2014)
Commercially produced from mixed feedstock of fruit trees, ~500 °C	Field	Silty clay loam	72% decrease in NO_3^- leaching, no effect on NH_4^+ leaching	(Ventura et al. 2013)
Maize stover, 600 °C	Field	Aeric Endoaquepts, fine-loamy	82% reduction in NO_3^- leaching at 100% recommended fertilization rate; no effect at 50% fertilization rate	(Güereña et al. 2013)
Peanut hall, 600 °C	Lab	Sandy	34 and 14% reduction in NO_3^- and NH_4^+ leaching, repectively; 39% increase in P leaching	(Yao et al. 2012)
Brazilian pepperwood, 600 °C	Lab	Sandy	30 and 35% reduction in NO_3^- and NH_4^+ leaching; 21% reduction in P leaching	(Major et al. 2012)

Locally produced mixed wood, ~500-700 ^{o}C	Field	Typic Haplustox clay soil	Leaching varied within the rooting zone: at 1.2 m depth Ca^{2+}, Mg^{2+}, K^{+}, NO_3^- and Sr^{2+} leaching decreased by 14, 22, 31, 2 and 14%, respectively, while no biochar effect on NH_4^+ and P	(Major et al. 2012)
Switchgrass at 250 ^{o}C	Lab	Xeric Haplocalcids loamy soil	27, 27, and 88% reduction in cumulative leaching of Ca, Mg and NO_3^-, respectively; 47% increase in K leaching; no effect on P leaching	(Ippolito et al. 2012)
Switchgrass at 500 ^{o}C	Lab	Xeric Haplocalcids loamy soil	67% reduction in cumulative leaching of NO_3^-, 267 and 172% increase in K and P, respectively; no effect on Ca and Mg leaching	(Ippolito et al. 2012)
Switchgrass at 250 ^{o}C	Lab	Xeric Haplocalcids silty soil	32, 28 and 72% reduction in Ca, Mg and NO_3^-, respectively; no effect on K and P leaching	(Ippolito et al. 2012)
Switchgrass at 500 ^{o}C	Lab	Xeric Haplocalcids silty soil	10, 11 and 152% increase in Mg, K and P leaching, respectively; 37% reduction in NO_3^- leaching	(Ippolito et al. 2012)
Bagasse at 800 ^{o}C	Lab	Clay soil	5% reduction in NO_3^- leaching	(Kameyama et al. 2012)
Mixed wood at 475 ^{o}C	Lab	Silty and sandy soils	No effect on P and NO_3^- leaching	(Borchard et al. 2012)
Jarrah wood at 600 ^{o}C	Lysimeter pots	Sandy soil	28% reduction in NO_3^- leaching	(Dempster et al. 2012)
Bamboo at 600 ^{o}C	Lab	Sandy silt	15% reduction in NH_4^+ leaching at the subsurface 10-20 cm depth	(Ding et al. 2010)
Mixed wood at ~550 ^{o}C	Lab	Typic Hapludolls fine loamy soil	74, 14, 28, 35, and 26% increase in leaching of K, Mg, Zn, Ca, and total N, respectively; no effect on P, Cu, Mn, Na, B and Si leaching	(Laird et al. 2010)
Pecan shells at 700 ^{o}C	Lab	Typic Kandiuduls fine loamy soil	206 and 110% increase in K and Na leaching, respectively; 35 and 78% decrease in P and Zn leaching; no effect on Ca, Mg and S leaching	(Novak et al. 2009b)

1.6 Biochar Effect on Plant Growth and Crop Yield

A large number of studies have focused on the influence of biochar on crop yield under both greenhouse and field environments (Lehmann & Joseph 2015). The response varies with biochar application rates, crop types, soil types, biochar types including feedstock and pyrolysis conditions, and combinations of these factors (Jeffery et al. 2011). Generally, increasing biochar application rate (within 5-150 t ha^{-1}) led to a greater increase in crop production or yields; however, this trend is only observed in short-term studies (generally within a year) (Jeffery et al. 2015), indicating that extra attention should be paid when interpreting these results. From 60 studies that are associated with biochar and crop production, commercial crops such as rice, wheat, maize and soybean all showed significant higher crop production after biochar additions (Jeffery et al. 2015). However, more field studies for specific species, either short- or long-term, are needed to increase the persuasiveness of this evidence and the accuracy for further reference. Besides, studies conducted on acidic soils or coarse textured soils tended to have greater biochar effect on crop productivity, suggesting liming effect and enhanced soil water storage are the two main reasons improving crop nutrient availability and thus yields (Lehmann et al. 2003; Chan et al. 2008; Gaskin et al. 2010; Major et al. 2010b; Jeffery et al. 2011).

Enhanced crop production with biochar additions may be observed as change in: plant growth, nutrient uptake and crop yields (Jeffery et al. 2015; Kammann & Graber 2015; Thies et al. 2015). First, biochar can alter soil nutrient pools and availability. Biochar itself can serve as a source of nutrients (Ippolito et al. 2015), and its structure and surface chemistry can enhance the capacity to hold nutrient ions thus increase availability (Kleber et al. 2015). The second most common mechanism for increased crop production is alteration of plant-soil water storage and status (Kammann et al. 2011). Biochar can alter the pore size distribution of soil in a long term due to its porous structure (Andrenelli et al. 2016; Yu et al. 2016), thus the addition of biochar may help improve topsoil water holding capacity and storage by the plant delivery of ground water to the topsoil through root hydraulic conductivity (Abel et al. 2013; Kammann & Graber 2015; Hansen et al. 2016). Although people are arguing that the pores in biochar are too small (usually less than 0.2 μm) for water molecule to percolate or stay (Sun et al. 2012), the

micro-pores of biochar can still be the source of water vapor that can move within the soil under different temperatures, especially for sandy soils in arid environments (Kammann & Graber 2015).

Besides the aspect of soil water storage, biochar itself can release other volatile organic compounds (VOCs) that will promote plant growth (Spokas et al. 2011; Bailly & Weisskopf 2012; Hofmann 2013); although opposite views exist (Buss & Mašek 2014; Dutta et al. 2016). As a common VOC, ethylene produced from biochar may count for another possible reason improving plant growth (Spokas et al. 2010). Ethylene (C_2H_4) is a natural product of plant metabolism (Beyer 1976), and it has been found to impact the soil microbial and plant processes, for instance, fine root hair growth, increased seed germination, leaf and flower senescence, and increased crop yield in some cases (Eplee 1975; Beyer 1976; Abeles et al. 1992; Spokas et al. 2010). Spokas et al. (2010) observed an increase of ethylene production under biochar-incorporated soils compared to the control, while the rate of ethylene production varied with biochar production temperature and source materials (Spokas et al. 2010).

In addition, biochar can also alter plant growth and nutrient uptake by altering the growth of roots and rhizosphere microbial activities (Kammann & Graber 2015). Joseph et al. (2010) indicated that plant roots or root hairs could enter the water-filled macropores or bond onto the biochar surface, causing a wide range of reactions that help the uptake of nutrient (Joseph et al. 2010). However, the diameter of typical root hairs (5-20µm) may not match the size of large macro-pores of biochar (wood-derived biochar: 10µm or more, cellulosic straws-derived biochar: 1-10µm), limiting the habitat of root hairs in biochar particles (Sun et al. 2012; Kammann & Graber 2015). In contrast, fungal hyphae may have more access to biochar, and influence plant nutrient uptake through participation in mycorrhizal functioning (Warnock et al. 2007).

It is also been reported that biochar can induce plant protection against soilborne diseases (Graber et al. 2014); and induce systemic plant resistance responses to foliar fungal pathogens (Elad et al. 2012; Bonanomi et al. 2015). However, further studies are needed to directly prove these points. Deeper exploration of biochar effect on plants is essential in understanding the potential value of biochar.

Chapter 2. Locally Produced Wood Biochar Increases Nutrient Retention in Agricultural Soils of the San Juan Islands, WA, USA

2.1 Introduction and Research Questions

Fire is a major form of ecosystem disturbance in western forest ecosystems; however, active fire suppression and a noted shift in forest management objectives have resulted in the occurrence of heavily stocked forests that are subject to stand replacing wildfire (Naficy et al. 2010; Hessburg et al. 2015). Fuel reduction and forest restoration treatments have been promoted as a means of reducing fire hazard and returning forest stand structure and composition to a more resilient form (Hessburg et al. 2015). Forest residues from timber harvests and fuel reduction treatments are normally piled and burned resulting in generation of air pollutants (CO_2, CO, NO_x and particulate matter), loss of nutrients, and incursion of exotic plant invasion (Kauffman 1990).

Biochar or charcoal obtained from the thermochemical conversion of forest residues may represent a means of creating a low emission, value added product from forest residuals while offering an innovative approach to improving soil fertility and crop productivity (Lehmann & Joseph 2015). Biochar is a C rich, recalcitrant solid material that is generated from the pyrolysis or thermochemical decomposition of organic material in an oxygen limited environment under controlled conditions.

The fertility and productivity of Amazonian dark earth soils (Terra Preta soils) are attributed to the heavy presence of charcoal in these soils (Glaser et al. 2002). The persistence of the dark color, high C content and noted productivity of these human manufactured soils hundreds of years after their establishment has generated a great deal of interest in biochar as a soil amendment for C sequestration and agronomic improvement (Lehmann et al. 2006; Laird et al. 2010; Brantley et al. 2015). The application of biochar to soils has been shown to increase soil nutrient retention, improve nitrogen fixation in cover crops, decrease the need for irrigation, and sequester C from the atmosphere (Lehmann & Joseph 2015). Studies in Midwestern soils, for example, illustrate that biochar decreased N and P leaching by 11% and

69% respectively (Laird et al. 2010). More recently, Ventura et al. (2013) reported a 72% reduction of NO_3^- leaching in sub-alkaline soils in an apple orchard (Ventura et al. 2013). Biochar has also been found to increase nitrogenase activity in leguminous crops and cover crops by 0 – 515% (DeLuca et al. 2015b). Some biochar studies have illustrated even greater benefits with calcium (Ca) and magnesium (Mg), increasing uptake by between 77-320% (Major et al. 2012). Biochar may even help decrease irrigation needs by increasing soil-water retention (Karhu et al. 2011). Biochar can also serve as an effective soil C sink as it has high proportion of recalcitrant C with thousands of years of stability (Lehmann & Joseph 2015). Its highly porous structure, large surface area may offer appropriate habitat for beneficial microorganisms to flourish; other physico-chemical properties such as high ion-exchange capacity can also impact a number of processes in the soil N cycle associated with enhanced soil fertility (Clough et al. 2013).

Improvements in soil fertility by biochar addition have also led to increased crop yield and productivity, the magnitude of response varies with biochar feedstocks (Gaskin et al. 2010; Agegnehu et al. 2016a), biochar activation or inoculation process (Hansen et al. 2015; Ingold et al. 2015), application rates (Major et al. 2010a; Wisnubroto et al. 2011; Nguyen et al. 2016), crop species (Bhattacharjya et al. 2015; Bass et al. 2016; Pandey et al. 2016), soil types (Manickam et al. 2015) and other soil inputs (Agegnehu et al. 2016b), as well as combination of these factors (Jeffery et al. 2015). A meta-analysis of crop production from Jeffery et al. (2011) showed that biochar application rate lower than 1-5 t ha^{-1}, or more than 150 t ha^{-1} did not simulate significant yield increases (Jeffery et al. 2011). Crops such as rice, wheat, maize and soybean showed relatively higher increases in crop yield and production when growing with biochar addition. Enhancement in crop production by biochar addition are generally attributed to the alteration of soil nutrients availability, liming effect, soil hydrological effects, as well as biotic interactions such as enhanced biological nitrogen fixation or mycorrhizal fungi colonization (DeLuca et al. 2015b; Jeffery et al. 2015).

In San Juan County, WA, approximately seventy percent of the land cover is considered overstocked with second growth Douglas-fir (*Pseudotsuga menziesii*) forests (San Juan Conservation District, personal communication). Thinning treatments geared toward improving

forest health result in residue generation can actually increases the potential for catastrophic fires. Importantly, a critical part of San Juan County's economy is agriculture and organic farming on well drained sandy loam soils formed in glacial till and outwash. Growing seasons are relatively short and dry due to the "rain shadow" effect created by Olympic Mountains and Vancouver Island. Therefore, biochar production from local timber harvest residues in San Juan County may offer a sustainable means of reducing wildfire hazard fuel loading while improving soil health and reducing nutrient loss on neighboring organic farms.

Numerous short-term studies have examined the influence of biochar on crop productivity and soil fertility, a comprehensive review of these results can be found in Lehman and Joseph, 2015. Many of the short-term studies have been conducted in pot and column trials in the greenhouse or laboratory environment. Longer-term field trials have often been conducted at agricultural experiment stations using conventional agricultural production approaches. To date, very few studies have been conducted in the field in active organic farming operations and as a part of a holistic closed loop system. Herein, we address this gap by evaluating the efficacy of locally produced wood biochar as a soil amendment in small-scale organic agriculture. We conducted these studies at ten independent organic farms in San Juan County, WA to examine whether locally produced wood biochar would:

(1) Increase soil nutrient availability;

(2) Improve soil nutrient retention;

(3) Increase nutrient uptake by dry beans.

By producing biochar from on-site logging residues that would otherwise be pile burned with no benefit, we recapture the value of the residues and potentially improve farm soil productivity. Importantly, this is a community cooperative effort that represents operational, on-farm research trials that are of value to the broader research community and regional farming community; as well as leverages the existing resources and community readiness to drive forest restoration and sustainable agricultural practices.

2.2 Materials and Methods

2.2.1 Study Site Description

The study reported herein was performed at 10 organic farms located on three islands in San Juan County, WA, USA (Figure 1). These include: the Morning Star Farm (48.613°N, 122.925°W), Emmet and Brooke Farm (48.629 °N, 123.013 °W), Oceanside Farm (48.622 °N, -122.828 °W), Maple Rock Farm (48,706 °N, 122.893 °W), CPA Farm (48.623 °N, 122.951 °W) and Cofelt Farm (48.673 °N, 122.939 °W) located on Orcas Island, WA; the Sweet Earth Farm (48.561 °N, 123.162 °W) located on San Juan Island, WA; the Huntley Farm (48.718 °N, 123.021 °W), Forage Farm (48.697 °N, 123.034 °W) and Blue Moon Farm (48.717 °N, 123.011 °W) located on Waldron Island, WA (**Figure 1**).

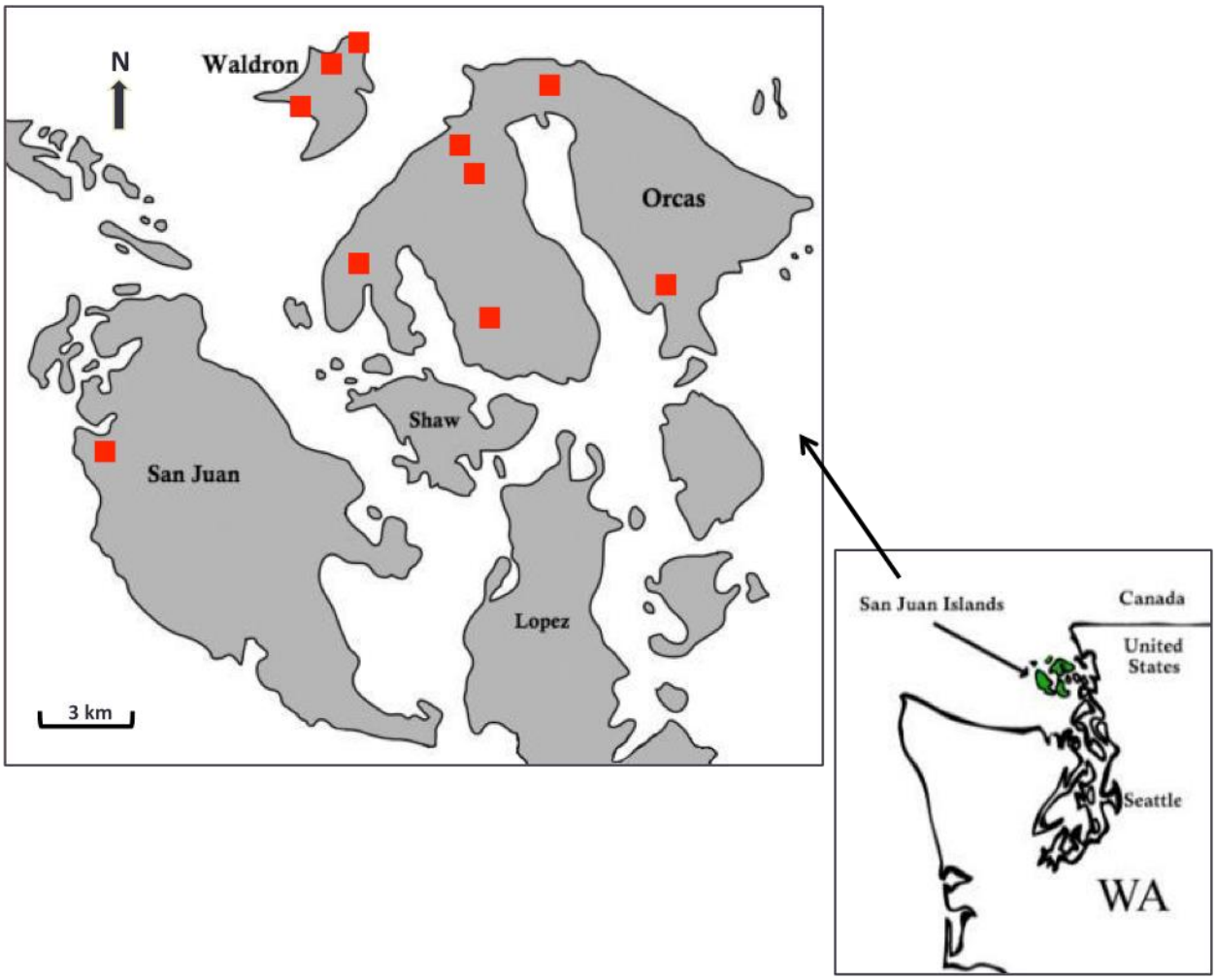

Figure 1. The location of 10 organic farms in San Juan County, WA, USA.

2.2.1.1 Climate

The climate of San Juan Islands is influenced by the Olympic Mountains and Vancouver Island, situated southwest and west northwest of the San Juan Islands respectively, which create a "rain shadow" effect producing less rainfall and experiencing significantly dryer and brighter weather than the surrounding locations. Summers are relatively short, cool and dry, average temperature is 15.2°C; winters are mild and moderately dry when compared to other portions of northern Puget Sound, with an average temperature of 5°C. The average annual total precipitation is about 713 mm. Of this, about 62% usually falls in March through November. The growing season for most crops falls within this period.

2.2.1.2 Land Cover

San Juan County's land cover is dominated by forests, which protect the shallow soils and provide abundant habitat for many species. Almost seventy percent of the county is covered by forest, consisting mostly of conifers such as Douglas fir (*Pseudotsuga menziesii*), Western hemlock (*Tsuga heterophylla*), and Western red cedar (*Thuja plicata*). Most of the remaining landcover in the county is cleared and largely used for agricultural.

From the 1800s to the 1930s the entire county was logged to produce lumber, provide fuel for lime kilns and steam-powered boats, or to clear land for agriculture. However, a large portion of the county's land cover has regenerated into second-growth woodlands.

2.2.1.3 Soils

According to the San Juan County Soil Survey (USDA, 2009), a large proportion of San Juan County's soils are defined as sandy and rocky, with a shallow rooting zone, and low moisture-holding capacity. Many of the soils are less than 30 inches in depth and sit atop a cemented glacial till layer that restricts the downward flow of water. The soils that are deeper than 30 inches are located above coarse layers of sand and gravel that allow water to drain through the soil very rapidly. The farms are found on gently sloping landscapes in glacial outwash, till, and alluvial deposits. The soils dominating these 10 study sites are Xerepts

(Oceanside, Maple Rock, Emmet & Brooke, and Huntley); Xeralfs (CPA, Cofelt, Morning Star, Sweet Earth, and Blue Moon) and Albolls (Forage).

2.2.2 Biochar Production and Experimental Design

Biochar was produced on-site using "Cylinder Burn" technique, a biochar production methods tested by a series of farmers and foresters at Rainshadow Counsulting and Northwest Natural Resource Group (Figure 2), using logging residues consisting of a mixture of 85% Douglas fir (*Pseudotsuga menziesii*), 15% White fir (*Abies concolor*), and 5% Western red cedar (*Thuja plicata*). In the summer of 2015, dry beans (*Phaseolus vulgasris L*) were planted on eight farms with green beans and cauliflower being grown on the other two.

Figure 2. Use of a cylinder burner for on-site production of biochar from forest harvest residuals on the San Juan Islands, WA, USA

Since these farms have been applying manure for decades, in addition to 'control' and 'biochar' treatment, we created a 'poultry litter' treatment and a 'charged biochar' treatment. Treatments consisted of: (1) Control: no additional amendments; (2) Poultry litter: 102 g "8:4:2 Nutri-Rich chicken litter" per plot (resulting 70 kg N ha^{-1}); (3) Biochar: applied at 20 t ha^{-1}; and (4) Charged biochar (poultry litter-amended biochar): 20 t ha^{-1} biochar charged/inoculated with 102 g "8:4:2 Nutri-Rich chicken litter" (70 kg N ha^{-1}) and local pond water (a moist mixture of biochar and poultry litter). Three to five replicated field plots were established on each farm site, four treatments were randomly applied within each replicated plot, resulting in a total of

136 treatment subplots. Each treatment was a 1m^2 (1m × 1m) subplot, with 30 cm buffer in between (Figure 3). All biochar treatments were incorporated into the top 15 cm of the soil (using gardening spade and rake) at the beginning of the growing season (May 2015), prior to planting dry beans (Figure 4). Replicate soil samples were collected on three separate occasions using a 1 cm^2 diameter soil core and compositing seven subsamples per m^2 treatment subplot.

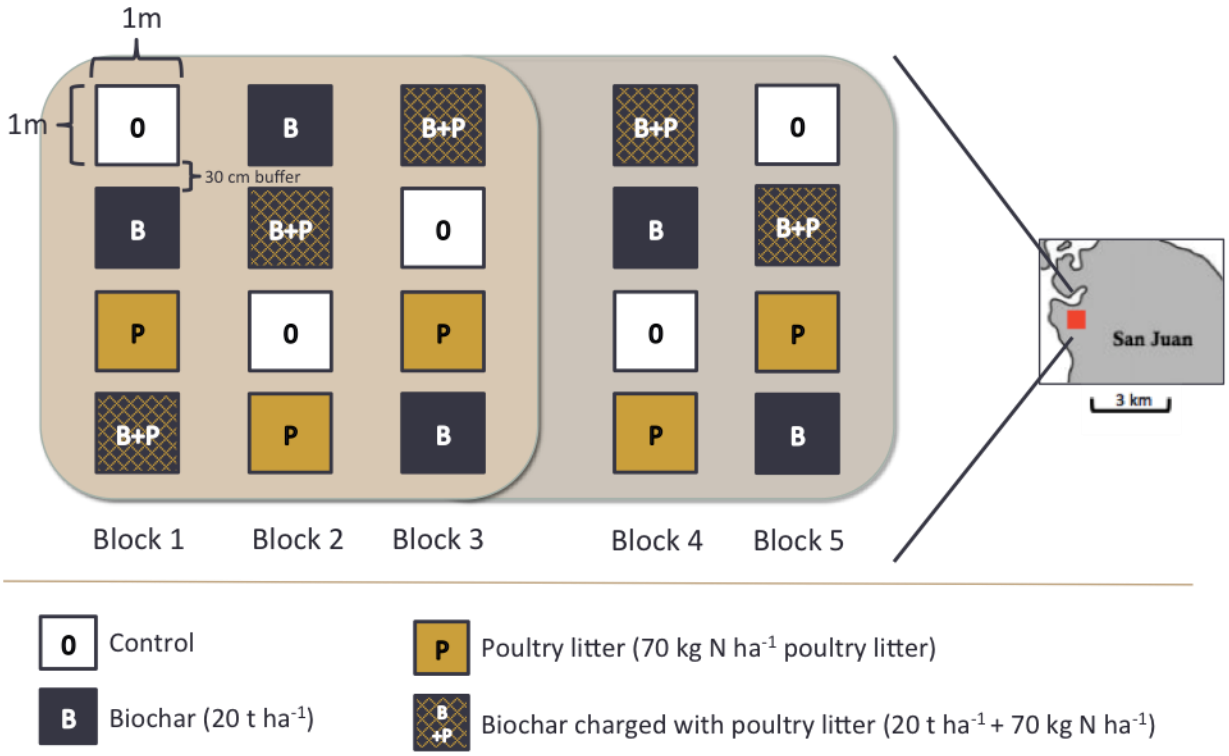

Figure 3. Example experimental layout with each farm receiving the same four treatments assigned randomly to three to five replicated blocks and each treatment applied to 1 m^2 plots with a 30 cm buffer in between at 10 organic farms on the San Juan Islands, WA. No additional amendment was applied in "control" plots; "poultry litter" was applied at 70 kg N ha^{-1}; "biochar" was applied at 20 t ha^{-1}; "charged biochar" was made up with a moist mixture of biochar (20 t ha^{-1}) and poultry litter (70 kg N ha^{-1}) (biochar was inoculated with poultry litter using local pond water, so that biochar was in moist contact with poultry litter).

Figure 4. Biochar application at the beginning of the growing season (May 2015).

2.2.3 Soil and Biochar Characterization

A composite surface soil sample (0-15 cm) was collected from each farm prior to biochar application. The soil was thoroughly homogenized and passed through a 2 mm thieve. Soil pH was determined in a 1: 1 soil to water suspension. Total C and N of soil and biochar samples was measured using a CHN analyzer (PE 2400 CHN Analyzer Waltham, Massachusetts USA). Bulk density was measured using a bulk density core that was pressed into the soil. Particle size analysis was conducted by the hydrometer method (Laker & Du Preez 1982). Water holding capacity was determined by gravimetry (Loveday 1974). Soils, biochar, and poultry litter properties are given in Tables 4 and 5.

Table 4. Soil physical and chemical properties at 10 organic farms in San Juan County, WA.

Farm Name	Replicated Plots	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Sand (%)	Clay (%)	pH	Bulk Density ($g\ cm^{-3}$)	Water Holding Capacity (%)
Oceanside	3	44.6	3.9	64	19	5.77	0.48	35.8
Maple Rock	3	43.5	3.9	48	22	6.20	0.53	33.7
CPA	3	60.3	5.6	49	15	5.98	0.41	34.2
Cofelt	3	59.4	4.9	70	17	6.00	0.47	44.6
Morning Star	3	54.7	4.4	45	19	5.86	0.34	41.7
Emmet & Brooke	3	48.1	3.2	49	23	5.99	0.32	47.4
Sweet Earth	5	38.9	2.9	75	15	5.84	0.52	32.3
Huntley	3	51.8	3.5	78	13	5.72	0.67	30.0
Blue Moon	5	41.3	3.5	77	14	5.98	0.88	31.3
Forage	3	49.4	2.6	54	17	5.58	0.64	53.5

Table 5. Total C and N of poultry litter and biochar treatments used in on-farm field trials on the San Juan Islands, WA.

Amendment	Treatment	Total C (%)	Total N (%)
Poultry Litter	2	40	8
Biochar	3	69.6	0.112
Charged Biochar	4	68.9	0.469

2.2.4 Soil Chemical and Biological Analyses

On two occasions during the growing seasons soil samples were collected for a suite of chemical analyses. Composite surface soil samples (0-15 cm) were collected from each treatment subplot at both mid-growing season (June 2015) and end-growing season (September 2015). Soils samples were taken back for analysis within three days of collection. Samples were shaken with 1 M KCl, filtered and analyzed for extractable NO_3^-, NH_4^+ using microplate-colorimetric technique, using the salicylate-nitroprusside method for NH_4^+ (Mulvaney et al. 1996) and the vanadium method for NO_3^- (Miranda et al. 2001). Soil P status was determined at the end of the growing season using the biologically based method recently described by DeLuca et al. (2015). Briefly, soil samples were extracted in parallel with 0.01 M CaCl2, 0.1 M citric acid, phosphatase enzymes, and 1 M HCl and analyzed for orthophosphate using the Malachite green method (DeLuca et al. 2015a). Soil microbial biomass N was determined by fumigation extraction with amino-N determination by reaction with ninhydrin (Brookes et al. 1985). Details of methods are described below.

2.2.4.1 Ammonium Determination in Soil Solutions by Colorimetric Method (modified from Mulvaney et al. 1996)

The NH_4^+ extracted from soil with *1M* KCl is determined by measuring the intensity of the emerald green color that form upon treatment of an aliquot of the extract with salicylate and hypochlorite at high pH. A catalyst (sodium nitroprusside) increases the rate and intensity of color development, and a chelating agent (EDTA) prevents the precipitation of divalent and trivalent cations as hydroxides. There is a three-step mechanism for color development by this method. In the first step, NH_3 reacts with hypochlorite to form monochloramine (NH_2Cl). Then, the monochloramine then reacts with salicylate to from benzoquinone monoamine, which couples with salicylate to give the colored indophenol dye at last.

For maximum sensitivity and accuracy in NH_4^+ analyses by this method, absorbance measurements are made at 667 nm. A pH of 13 is required for maximal color development in this method (Nelson 1983). A lower pH will lead to a loss of sensitivity. Standard ammonium

solution is ammonium sulfate ($(NH_4)_2SO_4$) solution, calibrate absorbance measurements by analysis of standards containing 0, 0.5, 1, 3, 5, 10, 20 ppm or µg/ml NH_4^+-N. Determine the NH_4^+ concentration of the sample using the equation obtained by linear regression of the concentration of the standards on the corresponding absorbance measurements. Alternatively, make this determination by reference to a calibration curve prepared from analyses of standards. Experiment included three DI blanks and three *1M* KCl blanks.

2.2.4.2 Nitrate Determination in Soil Solutions by Colorimetric Method (modified from Mulvaney et al. 1996)

The NO_3^- extracted from soil with *1M* KCl is determined by colorimetric method as well. In this method, a strongly pink azo dye is formed in acidic solution from the coupling of sulfanilamide and N-(1-naphthyl) ethylenediamine (N.E.D.) by nitrite. The reaction proceeds in three stages – NO_3^- is reduced by copperized cadmium to nitrite, which then forms a diazide with sulfanilamide. The diazide undergoes a substitution reaction with the aromatic ring of N.E.D., forming the para-substituted napthylene-dye. The intensity of pink color that develops is proportional to the concentration of NO_3^- in the soil extract.

For maximum sensitivity and accuracy, absorbance measurements are made at a wavelength of 540 nm, although, if necessary, any wavelength may be employed between 510 and 550 nm. Standard nitrate solution is potassium nitrate (KNO_3) solution. Calibrate absorbance measurements by analysis of standards containing 0, 0.5, 1, 3, 5, 10, 20 ppm or µg/ml NO_3^-N. Determine the NO_3- concentration of the sample using the equation obtained by linear regression of the concentration of the standards on the corresponding absorbance measurements. Alternatively, make this determination by reference to a calibration curve prepared from analyses of standards. Experiment included three DI blanks and three *1M* KCl blanks.

2.2.4.3 Determination of Potential Mineralizable N (PMN) by 14 day Anaerobic Incubation (modified from Scott et al. 1998 and Parfitt et al. 2005)

Potentially mineralizable nitrogen is a measure of the active fraction of soil organic N, which is chiefly responsible for the release of mineral N through microbial action. Mineralizable N is composed of a heterogeneous array of organic substrates including microbial biomass, residues of recent crops, and humus. An anaerobic incubation method for estimating mineralizable N was proposed by (Keeney & Bremner 1966). This anaerobic technique has significant practical and operational advantages over aerobic techniques in that the incubation period is relatively short (7 days) and the need for careful adjustment of soil water content is avoided. In theory, potentially mineralizable N is the amount of N that will mineralize in infinite time at optimum temperature and moisture. It is estimated by incubating soil under optimal conditions and measuring N mineralized as a function of time by periodically leaching mineral N from the soil. In order to get the best optional environment condition and assure homogeneity of the anaerobic condition, we revised this method by using 14 days instead of 7 days as incubation time. Generally, weigh around 6 g moist soil into 50 ml centrifuge tubes; add 12.5 ml of DI water; displace headspace of tube with N_2 gas after one minute of bubbling in water to displace oxygen and 30 seconds in the headspace; seal and incubate at room temperature (25-30^{o}C) for 14 days; after 14 days, add 12.5 ml of *2M* KCl to create a *1M* KCl solution; shake for 30 minutes and filter to get soil extract; determine NH_4^+-N by spectrophotometric method (Thermo-Multiskan Microplate photometer). NH_4^+-N in the soil before incubation is determined by extracting a separate sample with *1M* KCl. Potentially mineralizable N is calculated by subtracting initial NH_4^+-N (0 day) from that determined at the end of the incubation (14 days).

2.2.4.4 Soil Phosphorus Availability

Soil phosphorus status determination was conducted on the soils collected at end-growing stage (September 2015). Soil phosphorus analyses follow the methods from DeLuca et al. (2015). This method combined four established approaches to assessing different pools of bioavailable P thereby simultaneously assessing soil P as influenced by plant rhizosphere P acquisition mechanisms: (1) root interception; (2) organic acid complexation; (3) enzyme hydrolysis and (4) proton excretion induced acidification. Extractants are (1) 0.01 M $CaCl_2$ extractable P (soluble and weakly adsorbed inorganic P, emulates P accessed by root

interception and diffusion); (2) 10 mM citrate extractable P (active inorganic P pool sorbed to clay particles or weakly bound in inorganic precipitates); (3) 0.2 enzyme unit extractable P (organic P readily attacked by acid phosphatase and phytase enzymes, emulates enzyme release); and (4) 1 M HCl extractable P (soluble, active, and moderately stable inorganic P adsorbed to mineral surfaces or present in inorganic (Fe, Al, or Ca) precipitates, emulates proton extrusion by plants and microorganisms to access adsorbed and precipitated P).

Each P pool was measured in parallel by shaking 0.5 g of soil with each extractant (10 ml) in separate 15 ml centrifuge tubes for 3 h on a reciprocal shaker at 200 rev min^{-1}. Extracts were then centrifuged (3220 g, 30 min) to negate the need to filter the supernatant. An aliquot of the supernatant was then decanted and stored for no more than 3 d at 4 ^{o}C prior to analysis. Colorimetric method associated with the Malachite Green method was used to measure PO_4^{-3} in sample extracts (DeLuca et al. 2015a).

2.2.4.5 Soil Microbial Biomass N

Soil microbial biomass, which consists mostly of bacteria and fungi, is a measure of the mass of the living component of soil organic matter. The microbial biomass decomposes crop residues and soil organic matter to release carbon dioxide and plant available nutrients, such as nitrogen that is available for plant uptake. Generally, about half the microbial biomass is located in the surface 10 cm of a soil profile and most of the nutrient release also occurs here (Murphy & Sparling 1998). Plant production in organic farming systems mainly depends on nutrient release as a function of mineralization process in soils. The build-up of a large and active soil microbial biomass is important pool of accessible nutrients, therefore, is an important priority in organic farming.

Microbial biomass N (MBN) was determined using the fumigation extraction method (Brookes et al. 1985; Joergensen & Brookes 1990). Generally, separate field-moist soil into two subsamples (10 g for pre-fumigation and 10 g post-fumigation). Fumigate 10 g wet soil for 24 hours with $CHCl_3$ under vacuum. Extract pre-fumigated and post-fumigated soils with 30 ml *1 M* KCl and shake for 1 hour. MBN is determined by measuring ninhydrin reactive N and amino-N using the colorimetric method (Amato & Ladd 1988).

2.2.4.6 Soil Basal Respiration

Soil basal respiration is defined as the steady rate of respiration in soil, which originates from the mineralization of organic matter and represents the overall microbial activity (Pell et al. 2005). In our study, soil basal respiration was determined by tracking the CO_2 emissions. Generally, soil samples collected from field were incubated in glass jars containing a gas septum, adjusted to 60% water-holding capacity, incubated at room temperature for 72 hours, and sealed to trap respired CO_2 (Anderson 1982; Dempster et al. 2012). Headspace gas was analyzed for CO_2 with gas chromatography analysis (TRACE Ultra Gas Chromatograph, Thermo).

2.2.5 Soil Accumulation of Nutrients Below Rooting Zone

Ionic resin bags (UNIBEST Ag Manager) were installed at approximately 25 cm depth in each treatment subplot at mid-growing season (June 18th 2015). Generally, using a soil corer, resin capsules were buried at an angle instead of straight down around a crop plant so that the dry bean rooting system could be protected. Ideally, nutrients around the resin capsules that would potentially leach down or be lost below the rooting zone will be caught in the resin capsules. The resin capsule acted as a trap, continually exchanging ions for specific counter ions, thus all exchangeable nutrients could be monitored simultaneously (DeLuca et al. 2002). Resin capsules were retrieved at the end of the growing season (September 12th) after remaining in the soil for three months. Resin capsules were extracted sequentially with three 10 ml aliquots of 0.05 M HCl and analyzed for NO_3^--N, NH_4^+-N, PO_4^{-3} by colorimetry (as described above) and K^{+1}, Ca^{+2}, Mg^{+2}, Na^{+1}, Fe^{+3}, Mn^{+2}, Cu^{+2}, and Zn^{+2} were measured using an inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific 6300, Waltham, MA) as described elsewhere (Soltanpour 1991).

2.2.6 Nutrient Analysis of Dry Beans

Dry beans samples were collected from each treatment subplot, taken back to lab, washed with deionized water, dried in oven and ground in a domestic food processor resulting in a homogeneous sample. The nutrient concentration of dry beans was determined using ICP-

OES (as described above) following a dry-ashing digestion procedure (Soltanpour 1991; Santos et al. 2008).

2.2.7 Statistical Analyses

Each farm can be considered as a stand-alone, randomized complete block study that can be analyzed individually using analysis of variance (ANOVA). We can also analyze across all ten farms with each farm serving as a replicate of the whole experiment and with farm site as a factor. For every response variable (e.g. soil total C), measurements made within each treatment subplot (1 m^2) were always averaged to generate plot (farm) level values. All data were subsequently analyzed using analysis of variance (ANOVA), block (farm site) was initially included as a random factor within each ANOVA model and was removed whenever significant block effects were not present (Zar 1999). Whenever ANOVAs revealed significant interactive effects among factors, data were subsequently analyzed using post hoc Tukey's HSD tests to identify differences among treatments. All data were analyzed using R (Team 2013).

2.3 Results

2.3.1 Soil Response Variables

Soil physicochemical properties, total C and N content, available N and P in response to applications of poultry litter, biochar and charged biochar across 10 farms are reported in Table 6. Data from individual farms are presented in Appendix A-G.

2.3.1.1 Soil Total C

Biochar addition to soil (both 'biochar' and 'charged biochar' treatments) resulted in significantly greater soil total C content (Figure 5) compared to non-biochar treatments after four months field study when averaged across all farm sites ($n = 10$, $p<0.001$). The 'biochar' treatment (52.8 ± 5.5 g C kg^{-1}) increased soil C by 32% compared to 'control' (40.1 ± 3.9 g C kg^{-1}); and 'charged biochar' treatment (56.4 ± 4.9 g C kg^{-1}) increased soil total carbon by 33% compared to 'poultry litter' treatment (42.3 ± 3.5 g C kg^{-1}).

Table 6. Soil physicochemical properties, total C and N content, available N and P in response to biochar, poultry litter, and charged biochar treatments at 10 organic farms on the San Juan Islands. Data are presented as mean ± SE (n = 10). Data were compared among treatments using Tukey-HSD test following ANOVA. Numbers with the same letter are not significantly different at p = 0.05. No letters following the numbers indicate no significant difference (at p = 0.05) among treatments.

Parameters	Physicochemical properties				Soil total C and N			Soil available N (mg N kg^{-1})						Soil available P (mg P kg^{-1})			
	Soil pH	Water content (%)		WHC (%)	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N	NO_3^--N		NH_4^+-N		PMN		$CaCl_2$ extractable P	Citrate extractable P	Enzyme extractable P	HCl extractable P
Stage		Mid	End					Mid	End	Mid	End	Mid	End	End			
Control	5.88[a] ± 0.04	25.37 ± 0.03	30.55 ± 0.03	38.50[a] ± 2.50	40.1[a] ± 3.9	3.18 ± 0.37	13.3	3.82 ± 1.92	1.65 ± 0.63	9.79[a] ± 0.89	4.84[a] ± 0.66	7.16[a] ± 1.08	11.62[a] ± 2.79	10.99 ± 3.34	105.63[a] ± 31.93	48.81 ± 16.83	1129.62 ± 199.87
Poultry Litter	5.76[a] ± 0.03	26.24 ± 0.03	32.86 ± 0.04	40.20[a] ± 1.78	42.3[a] ± 3.5	2.95 ± 0.33	15.3	4.34 ± 2.34	1.82 ± 0.70	14.66[b] ± 1.62	6.74[bc] ± 0.90	9.38[ab] ± 1.79	16.44[ab] ± 4.34	12.64 ± 4.30	111.03[a] ± 32.70	51.05 ± 20.22	1192.52 ± 199.23
Biochar	6.88[b] ± 0.04	27.42 ± 0.03	32.80 ± 0.04	47.94[b] ± 4.65	52.8[b] ± 5.5	3.20 ± 0.35	17.2	4.82 ± 2.45	1.32 ± 0.51	15.10[b] ± 1.64	6.05[ab] ± 0.86	11.37[b] ± 1.79	17.23[bc] ± 3.80	10.34 ± 3.63	135.79[b] ± 40.64	51.64 ± 19.56	1238.98 ± 206.30
Charged Biochar	6.95[b] ± 0.03	28.89 ± 0.04	33.62 ± 0.04	48.33[b] ± 3.02	56.4[b] ± 4.9	3.19 ± 0.34	19.9	3.70 ± 1.53	1.73 ± 0.60	21.32[c] ± 2.40	7.68[c] ± 1.08	19.71[c] ± 2.67	21.00[c] ± 4.57	11.37 ± 3.87	143.63[b] ± 42.31	60.77 ± 22.23	1300.52 ± 193.90

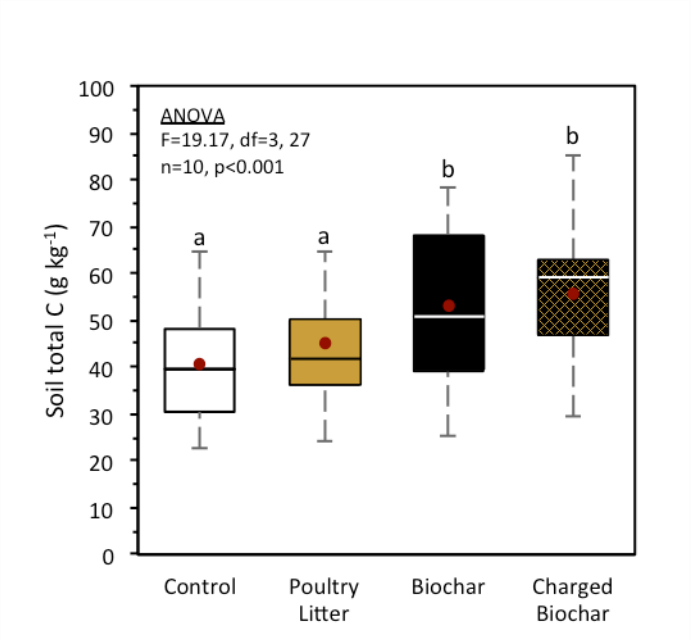

Figure 5. Soil total C content (g kg^{-1}) in response to application of biochar, poultry litter, or charged biochar treatments after four months across 10 organic farms on the San Juan Islands, WA. Data were compared using Tukey-HSD test following ANOVA. Soil total C content were significantly different among treatments (n = 10, $p<0.001$) after four months application of four treatments. Columns with the same letter are not significantly different at $p = 0.05$. Bars in the boxes indicate median, dots in the boxes indicate mean.

2.3.1.2 Soil Available N

Soil extractable NO_3^--N, NH_4^+-N, and PMN values were examined at both mid and end of the growing season. Comparing 'biochar' treatment to 'control', and 'charged biochar' to 'poultry litter' respectively allows one to consider the N added with the poultry litter and the N actually contained in biochar itself. In our study, the extractable NH_4^+-N content of biochar is 0.0004 mg g^{-1}, therefore, although the total N content in biochar is reported as 0.112%, a large percent of the total N is recalcitrant N with very little as extractable N (0.0004 mg NH_4^+-N g^{-1} biochar only accounts for 0.008 kg N ha^{-1}; NO_3^- was undetectable in the biochar)**.** Therefore, the NO_3^- and NH_4^+ contributed by biochar itself are considered negligible.

Biochar had no significant main effect on soil extractable NO_3^--N contents either during or after the growing season (Table 6). Soil available NH_4^+-N contents increased significantly at mid growth stage in both 'biochar' and 'charged biochar' treatments (Figure 6a, Table 6).

'Biochar' addition (15.10 ± 1.64 mg N kg^{-1}) increased soil extractable NH_4^+-N contents by 54% compared to 'control' plots (9.79 ± 0.89 mg N kg^{-1}); 'charged biochar' addition (21.32 ± 2.40 mg N kg^{-1}) increased soil extractable NH_4^+-N contents by 45% compared to 'poultry litter' treatment (14.66 ± 1.62 kg N ha^{-1}). Significant differences were also observed between treatments for soil available NH_4^+-N ($n = 10$, $p<0.05$) at end of the growing season (Figure 6b), with 'charged biochar' treatment reaching the relatively highest soil extractable NH_4^+-N level compared to the other three treatments.

Soil PMN levels (14 d anaerobic incubation) were enhanced by biochar additions as 'charged biochar' treatments resulted in the highest soil PMN levels at both mid-growing season and end-growing season sampling periods (Figure 6c, 6d). At mid-growing season, 'biochar' addition (11.37 ± 1.79 mg N kg^{-1}) increased soil PMN value by 59% compared to 'control' plots (7.16 ± 1.08 mg N kg^{-1}); compared to 'poultry litter' treatment (9.38 ± 1.79 mg N kg^{-1}), 'charged biochar' addition (19.71 ± 2.67 mg N kg^{-1}) improved soil PMN values by 110%. At the end-growing season the 'biochar' treatment had a PMN value of 17.23 ± 3.80 mg N kg^{-1} or 48% higher than the soil PMN values in 'control' plots (11.62 ± 2.79 mg N kg^{-1}). Soils under the 'poultry litter' treatment were elevated compared to the control, but relatively low (16.44 ± 4.34 mg N kg^{-1}) compared to the PMN of 'charged biochar' treatment (21.00 ± 4.57 mg N kg^{-1}). Overall, biochar significantly increased soil PMN levels in both mid- and end-of- growing season, which represented an active fraction of organic N that could be readily converted into inorganic N for plant up-take.

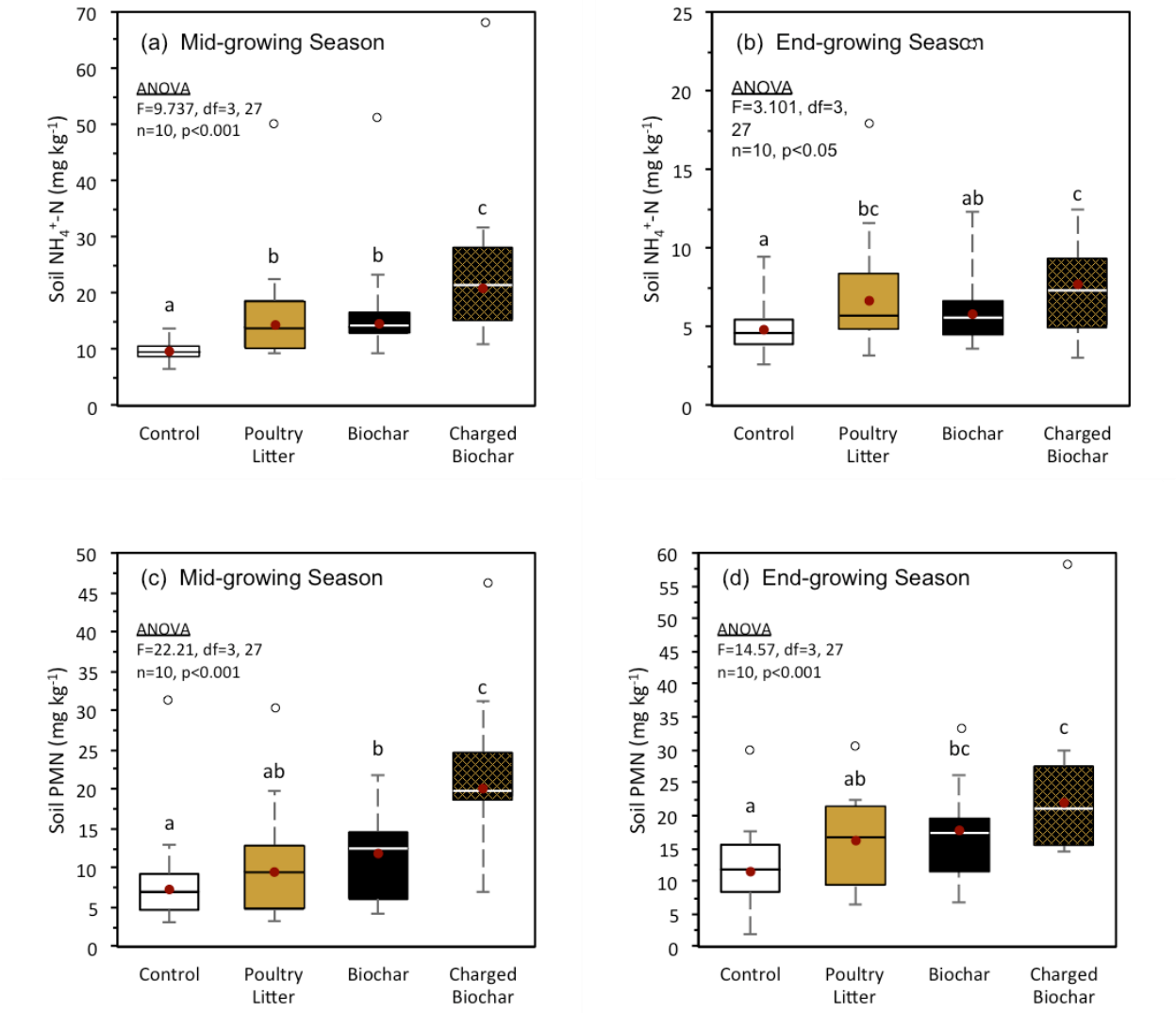

Figure 6. Soil (a) extractable NH_4^+-N at mid-growing season, (b) extractable NH_4^+-N at end-growing season, (c) potentially mineralizable nitrogen at mid-growing season, (d) and PMN (all in mg kg^{-1}) four months after application of biochar, poultry litter, and charged biochar treatments across 10 organic farms on the San Juan Islands, WA. Data were compared using Tukey-HSD test following ANOVA. Soil extractable NH_4^+-N were significant different among treatments at both mid-growing season (n = 10, $p<0.001$) and end-growing season (n = 10, $p<0.05$); soil PMN levels were significant different among treatments at both mid-growing season (n = 10, $p<0.001$) and end-growing season (n = 10, $p<0.001$). Columns with the same letter are not significantly different at p = 0.05. Bars in the boxes indicate median, dots in the boxes indicate mean. Small circles indicate outliers.

2.3.1.3 Soil Available P

We evaluated soil P using the BBP method (DeLuca et al. 2015a) wherein four different pools of soil available P are measured in parallel: active inorganic P (citrate extractable P), soluble P ($CaCl_2$ extractable P), organic labile P (enzyme extractable P), and more recalcitrant P (HCl extractable P). Results showed that biochar caused a significant increase (29%) in soil citrate extractable P that corresponds to the active pool of inorganic P sorbed to clay particles or weakly bound in inorganic precipitates which have been shown to be accessible to plants following the release of organic acids into soil (Figure 7). Soils under 'biochar' and 'charged biochar' additions have a relatively higher citrate extractable P levels (135.79 ± 40.64 mg P kg^{-1}, 143.63 ± 40.31 mg P kg^{-1}, respectively) than the 'control' soil (105.63 ± 31.93 mg P kg^{-1}) and soils under 'poultry litter' treatment (111.03 ± 32.70 mg P kg^{-1}). No significant differences were observed between treatments for soil soluble P ($CaCl_2$ extractable), organic labile P (enzyme extractable), and more recalcitrant inorganic P (HCl extractable) (Table 6).

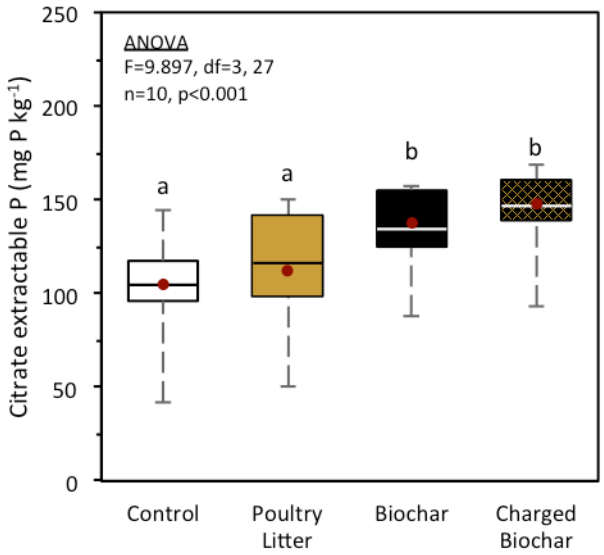

Figure 7. Soil citrate extractable P concentration (mg kg^{-1}) four months after application of biochar, poultry litter, or charged biochar treatments across 10 organic farms on the San Juan Islands, WA. Data were compared using Tukey-HSD test following ANOVA. Columns with the same letter are not significantly different at p = 0.05. Bars in the boxes indicate median, dots in the boxes indicate mean.

2.3.1.4 Soil Microbial Biomass N

Significant differences between treatments for microbial biomass N were observed and reported in Figure 8 (n = 10, p<0.001). Soils in plots treated with 'charged biochar' had higher microbial biomass N than controls. There was; however, no effect on 'biochar' compared to the 'control', and 'charged biochar' to 'poultry litter'.

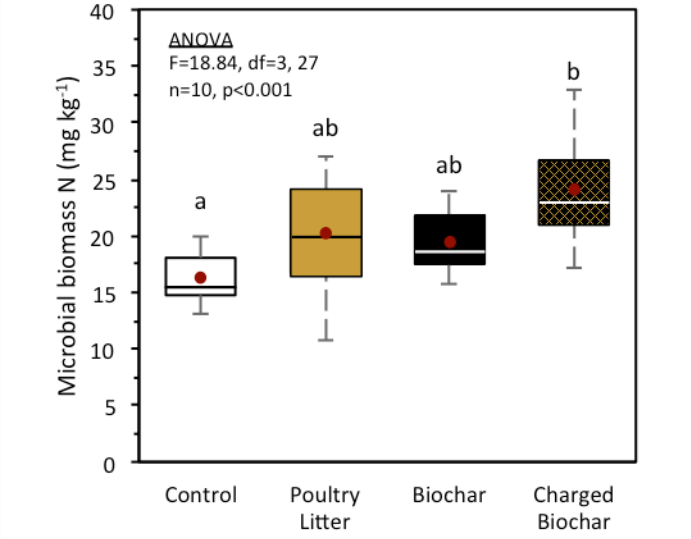

Figure 8. Soil microbial biomass N concentration (mg kg^{-1}) measured at the end-growing season after application of biochar, poultry litter, or charged biochar treatments across 10 organic farms on the San Juan Islands, WA. Data were compared using Tukey-HSD test following ANOVA. Columns with the same letter are not significantly different at p = 0.05. Bars in the boxes indicate median, dots in the boxes indicate mean.

2.3.2 Soil Nutrient Accumulation Below Rooting Zone

Accumulated NO_3^--N, NH_4^+-N and P below the rooting zone are reported in Figure 9, other nutrients are reported in Table 7. Potentially leached NO_3^--N, NH_4^+-N, and P were significantly lower in biochar-treated soils ('biochar' and 'charged biochar') compared to the no-biochar soils ('control' and 'poultry litter') during the four months of the experiment. 'biochar' addition treatment caused a 33%, 53% and 39% reduction in NO_3^--N, NH_4^+-N and P accumulation in resin capsules at 25 cm depth compared to the 'control' soils, respectively. 'Charged biochar' addition to soils caused a 28%, 50% and 46% reduction of potentially leached NO_3^--N, NH_4^+-N and P compared to the 'poultry litter' soils (Figure 9). It is also observed that biochar caused a retention of Ca, Fe, Mg, Cu, Mn, Ni, Zn after three months (Table 7). Overall, biochar treatments reduced the accumulation of nutrients that are otherwise being lost below the rooting zone from this one growing season experiment.

2.3.3 Nutrient Response in Dry Beans

Nutrient levels (10 elements) of dry beans with treatments are reported in Table 8. 'Biochar' treatment significantly increased P, Fe, Mg and Zn levels in harvested dry beans compared to the 'control' (Figure 10). Further, no significant differences were observed between 'charged biochar' treatment and 'poultry litter' treatment across 10 farms.

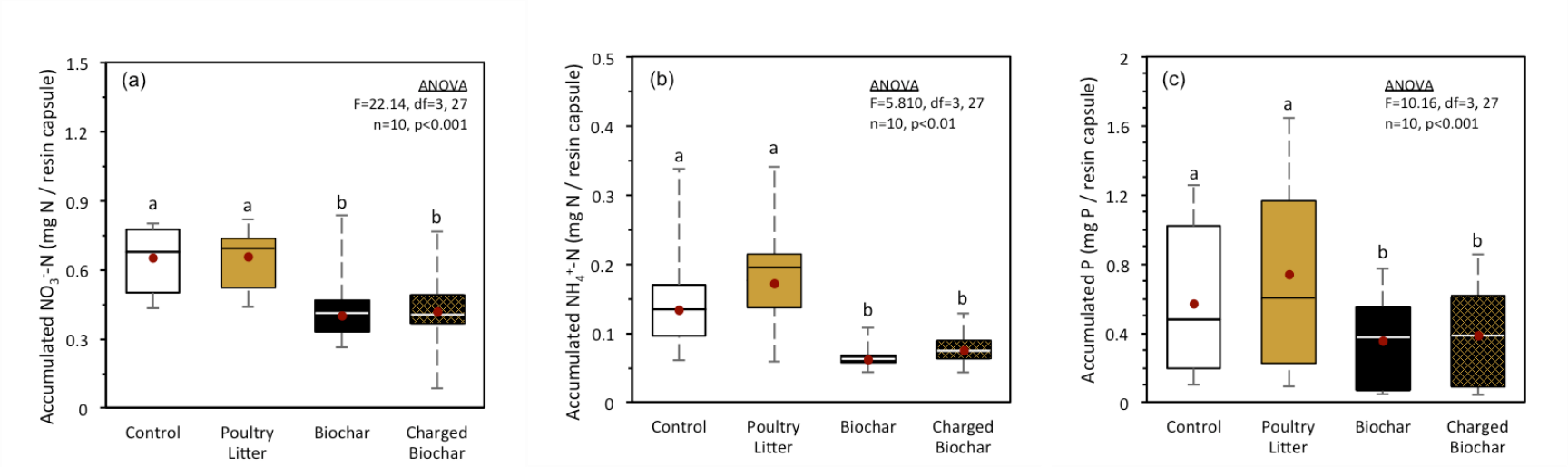

Figure 9. (a) Accumulated NO_3^--N, (b) accumulated NH_4^+-N, and (c) accumulated P of resin capsules below the rooting zone during the growing season following after application of biochar, poultry litter, or charged biochar treatments across 10 organic farms on the San Juan Islands, WA. Data were compared using Tukey-HSD test following ANOVA. Significant differences were observed among four treatments in accumulated NO_3^--N ($n = 10$, $p<0.001$), accumulated NH_4^+-N ($n = 10$, $p<0.01$) and accumulated P ($n = 10$, $p<0.001$) over the growing season. Columns with the same letter are not significantly different at $p = 0.05$. Bars in the boxes indicate median, dots in the boxes indicate mean.

Table 7. Nutrient accumulation (mean ± SE) below the rooting zone (3 months period) in response to biochar, poultry litter, and charged biochar treatments at 10 organic farms on the San Juan Islands, WA. Data were compared among treatments using Tukey-HSD test following ANOVA. Numbers with the same letter are not significantly different at p = 0.05. No letters following the numbers indicate no significant difference (at p = 0.05) among treatments.

Accumulated Nutrients	NO_3^--N	NH_4^+-N	P	Ca	Fe	K	Na	Mg	Cu	Mn	Ni	Zn
	mg per resin capsule								µg per resin capsule			
Control	0.69^a ± 0.13	0.14^a ± 0.03	0.57^a ± 0.16	1.11^a ± 0.08	0.03^a ± 0.01	1.06 ± 0.20	1.06 ± 0.22	0.54^a ± 0.10	0.5^a ± 0.1	8.1^a ± 1.6	0.6^a ± 0.1	5.6^a ± 0.9
Poultry Litter	0.70^a ± 0.12	0.16^a ± 0.03	0.73^a ± 0.20	1.18^a ± 0.03	0.02^a ± 0.00	1.65 ± 0.42	1.66 ± 0.43	0.58^a ± 0.11	0.6^a ± 0.0	9.6^a ± 1.5	0.5^a ± 0.1	5.1^a ± 0.5
Biochar	0.46^b ± 0.11	0.07^b ± 0.01	0.35^b ± 0.09	0.70^b ± 0.09	0.02^b ± 0.00	1.10 ± 0.15	1.22 ± 0.18	0.40^b ± 0.11	0.4^{ab} ± 0.0	6.6^b ± 1.1	0.7^a ± 0.0	4.6^b ± 1.0
Charged Biochar	0.49^b ± 0.11	0.08^b ± 0.01	0.39^b ± 0.11	0.95^b ± 0.04	0.02^b ± 0.00	1.04 ± 0.20	1.28 ± 0.36	0.42^b ± 0.01	0.4^b ± 0.0	6.7^b ± 1.1	0.3^b ± 0.0	4.6^b ± 0.9

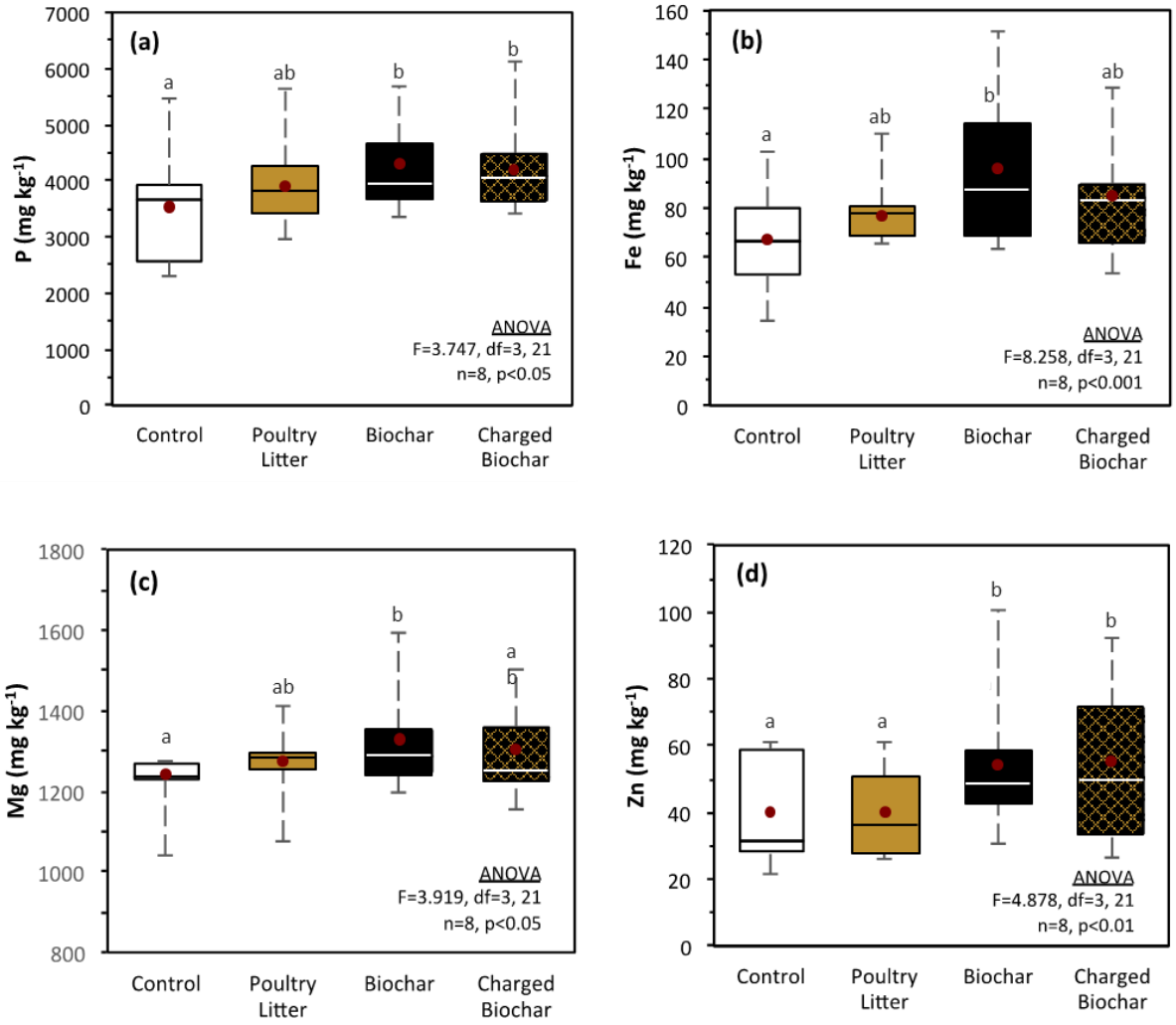

Figure 10. Nutrient concentrations in dry beans (mg kg^{-1}) for (a) P, (b) Fe, (c) Mg, and (d) Zn in response to biochar, poultry litter, or charged biochar applications across 8 organic farms (farms growing dry beans) on the San Juan Islands, WA. Data were compared using Tukey-HSD test following ANOVA. Significant differences among treatments were observed in P ($n = 8$, $p<0.05$), Fe ($n = 8$, $p<0.001$), Mg ($n = 8$, $p<0.05$) and Zn ($n = 8$, $p<0.01$) levels in harvested dry beans. Columns with the same letter are not significantly different at $p = 0.05$. Bars in the boxes indicate median, dots in the boxes indicate mean.

Table 8. Nutrient concentration of dry beans between treatments in on-farm field trials on the San Juan Islands, WA. Data were compared among treatments using Tukey-HSD test following ANOVA. Numbers with the same letter are not significantly different at p = 0.05. No letters following the numbers indicate no significant difference (at p = 0.05) among treatments.

Nutrients (mg kg^{-1})	Cu	Fe	Mn	Ni	Zn	Ca	Mg	Na	P	K
Control	17.67 ± 6.75	67.21^{a} ± 7.55	15.28 ± 0.73	1.590 ± 0.34	40.07^{a} ± 5.94	1256 ± 192	1228^{a} ± 25	233 ± 71	3606^{a} ± 344	12415 ± 1047
Poultry Litter	20.62 ± 8.24	78.89^{ab} ± 4.96	17.59 ± 1.29	1.340 ± 0.24	39.86^{a} ± 4.77	1244 ± 151	1273^{ab} ± 36	181 ± 33	3979^{ab} ± 285	12676 ± 985
Biochar	25.88 ± 7.86	94.75^{b} ± 9.88	16.66 ± 1.42	2.120 ± 0.29	54.69^{b} ± 8.87	1426 ± 235	1324^{b} ± 39	217 ± 40	4278^{b} ± 303	15718 ± 3445
Charged Biochar	36.99 ± 13.38	83.05^{ab} ± 8.47	16.01 ± 1.26	1.560 ± 0.35	54.91^{b} ± 8.68	1364 ± 218	1279^{ab} ± 36	284 ± 78	4349 ^{b} ± 323	13971 ± 1200

2.4 Discussion

2.4.1 Soil Response Variables

2.4.1.1 Soil Total C

The application of 'biochar' and 'charged biochar' to sandy mineral soils of the San Juan Islands resulted in a significant increase in total soil C (about a 32% increase) compared to no-biochar soils. These findings are consistent with that of numerous biochar studies where researchers evaluate soil C storage (Singh et al. 2012; Fang et al. 2014; Singh & Cowie 2014; Yang et al. 2016). Since the biochar was applied to surface soils and only incorporated to a shallow depth, the large increase in soil C in the top 10 cm of soil was expected. Given that a large fraction of biochar is in a recalcitrant form, it is unlikely that the applied biochar would decompose or leach to any degree during the study period. Biochar composition can be crudely divided into relatively recalcitrant C, labile or leachable C, and ash (Lehmann et al. 2011). Biochar produced from woody feedstocks usually has low ash content (Lee et al. 2006) and low labile C content (Ippolito et al. 2015), therefore, recalcitrant C (fixed C) is the dominant component of a high temperature wood biochar C such as that used in this study. A recent study from Yang et al. (2016) reported that soil minerals can interact with biochar in a manner that leads to interfacial reactions that enhance the oxidation resistance ability of biochar particles, improving biochar stability and thus C sequestration (Yang et al., 2016). And results from a recent meta-analysis of biochar stability suggested that mean residence time (MRT) of labile and recalcitrant biochar C pools were estimated to be about 108 days and 556 years, with pool sizes of 3% and 97% across 108 observations from 24 studies using stable (^{13}C) and radioactive (^{14}C) C isotopes (Wang et al. 2016). These results as well as numerous other studies cited by (Lehmann & Joseph 2015) indicated that only a small portion of biochar is available for microbial decomposition and most of the remaining recalcitrant C contributes directly to long-term C sequestration in soil. Generally, higher the pyrolysis temperatures yield a greater presence of turbostratic C in the biochar (Keiluweit et al. 2010). It has been indicated that the nature of these C structures (fused aromatic C structures) is the main reason for the high stability of biochars (Nguyen et al. 2010; Lehmann et al. 2011).

Compared to conventional farming, organic farming operations usually have higher levels of soil organic matter that have been built up over time (Gattinger et al. 2012). Organic farming practices occupy a large portion of agricultural production on the San Juan Islands. Our results confirmed the benefit of on-site produced biochar in increasing soil C storage and illustrated the value-add potential of converting forest harvest residuals to biochar offering an incentive to improved forest management and organic farming.

2.4.1.2 Soil Available N

Most of N inputs on organic farms on the San Juan Islands come from manure and poultry litter applications that consists mostly of organic N which must be mineralized to NO_3^- or NH_4^+ prior to plant uptake (Stevenson 1999). Our results showed no significant effect of biochar amendments on soil NO_3^--N, indicating that biochar additions did not stimulate nitrification, either during or after the growing season (Table 6). Unlike forest ecosystem where charcoal may enhance nitrification (DeLuca et al. 2006), agricultural ecosystems that receive manure additions and tillage normally have highly active nitrifying communities that do not further respond to charcoal (Ducey et al. 2013; DeLuca et al. 2015b).

Soil NH_4^+-N levels have been proposed to be an estimate for soil inorganic N availability in closed-loop organic farming systems (Mikkelsen & Hartz 2008). Unlike our NO_3^-N results, we found a significant increase in extractable NH_4^+-N in soils with 'biochar' treatments and 'charged biochar' treatments, regardless of whether soil samples were collected in the middle or at the end of the growing season (Figure 6a, 6b). This observation is similar to previous studies that demonstrate an increase in NH_4^+ with biochar additions (Dempster et al. 2012; Zheng et al. 2013; Agegnehu et al. 2015). Biochar treatments only accounted for 0.19% and 0.83% of the increased NH_4^+-N levels at mid- and end-of- growing season respectively (assuming an average soil bulk density of 0.53 g cm^{-3} and a depth of 15 cm, the increased NH_4^+-N concentrations caused by biochar addition scale up to 0.01 mg NH_4^+-N kg^{-1} soil). Therefore, the positive effect of biochar on NH_4^+ availability is likely attributed to the adsorption capacity of biochar and the retention of NH_4^+ rather than its N input (Dempster et al. 2012; Pluchon et al. 2014; DeLuca et al. 2015b). Previous studies have shown that biochar retains NH_4^+ in soils

through acid functional groups (e.g. carboxyl and hydroxyl) on its surface via cation exchange given biochar's moderately high cation exchange capacity (CEC) (Curtin & Condron 2010; Zheng et al. 2013). The CEC of biochar has been reported to have the potential to increase with residence time in soil, due to its unique surface oxidation with the formation of carboxylic functional groups (Cheng et al. 2006; Dempster et al. 2012). The sandy and even skeletal nature of the soils on the San Juan Islands increases the likelihood that the biochar additions would have an impact on total CEC and nutrient retention in these soils. It is possible that biochar applied during our field study would act as a "slow release fertilizer" that efficiently releases a steady stream of nutrients after most or all of the pores and negative charges are saturated with nutrients by adsorption process. Charging the biochar with a poultry litter and pond water slurry prior to field application may have accelerated the cation saturation process thereby increasing the NH_4^+-N concentrations in soils with the charged biochar treatments.

In addition to NH_4^+-N, PMN is used to roughly estimate the availability of organic N over a growing season (Doran 1987). In this study, we observed significantly greater PMN concentrations in soils with biochar additions than those without biochar (Figure 6c, 6d). It is possible that the biochar in this study adsorbed resident organic N compounds (such as amino acids, small proteins and peptides) that added to the total mineralizable N pool (DeLuca et al. 2015b). It is also possible that biochar additions altered mineralizable N by improving soil moisture retention (Table 6) associated with its high micro pore volume (Pluchon et al. 2014; Gundale et al. 2015) given that N mineralization is most active under appropriate soil moisture conditions (Curtin & Campbell 2008). It is well accepted that length of time that biochar resides in the soil environment influences the amount of organic matter adsorbed onto the biochar surface (Zackrisson et al. 1996).

Compared to conventional farming, N input in the form of manure in organic farming is mineralized more slowly and thus less readily available for plant uptake (Poudel et al. 2002; Kontopoulou et al. 2015). The enhanced N availability by biochar additions observed in our study, particularly PMN levels, illustrated active N turnover following organic N input, as well as greatly improved manure use efficiency in across the 10 organic farms on San Juan Islands.

2.4.1.3 Soil Available P

Phosphorus can be a primary limiting nutrient in agricultural systems as P tends to bond to soil minerals or complexed into mineral precipitates and organic forms that are not readily available for plant uptake. Accordingly, many plants have P acquisition strategies to cope with restricted P supply (Ryan et al. 2001). Dry beans (*P. vulgaris*), for example, is considered a "P efficient crop" that releases organic acids into the rhizosphere to enhance P acquisition during the growing season (Jones et al. 2003; Khademi et al. 2009). In our study we found that citrate extractable P (which represents a chelation based acquisition strategy), to be enhanced by biochar additions (Figure 7). Joseph et al. (2013) and Briones (2011) indicated that hydrophobic or charged biochar could adsorb organic molecules involved in chelation of Al^{3+}, Fe^{3+}, and Ca^{2+} ions on its surface, as it tends to attract polar or non-polar molecules and form organo-biochar or organo-mineral-biochar complexes (Briones 2011; Joseph et al. 2013). Input of N, P and K to organic farming systems tends to be notably lower (34 - 51% for P) than in the conventional systems (Mäder et al. 2002). Enhanced soil active inorganic P with biochar addition, especially in the 'charged biochar' treatment, clearly demonstrates the benefit of biochar in improving soil P availability across the 10 organic farms growing dry beans in our study.

2.4.1.4 Soil Microbial Biomass N

Similar to the recent results found in (Lanza et al. 2016), the biochar treatments had little influence on soil microbial biomass N (Figure 8). 'Poultry litter' and 'charged biochar' treatments resulted in higher levels of microbial biomass N than the 'control' (Figure 8). This is likely due to the N additions from the poultry litter treatment which provided the test plots with readily mineralizable organic N during the growing season (Gunapala & Scow 1998).

2.4.2 Soil Nutrient Accumulation Below Rooting Zone

Biochar treatments ('biochar' and 'charged biochar') appeared to reduce the amount of NO_3^--N, NH_4^+-N and PO_4^{-3} accumulated below the rooting zone in comparison to the 'control' and 'poultry litter' treatments (Figure 9). A reduction in nutrient leaching after biochar application has been reported in laboratory and greenhouse based studies (Laird et al. 2010;

Borchard et al. 2012; Dempster et al. 2012; Ippolito et al. 2012; Kameyama et al. 2012; Yao et al. 2012) and in a few field studies (Major et al. 2012; Güereña et al. 2013; Ventura et al. 2013). Soil NH_4^+ leaching has been found to be reduced with biochar additions in many cases (Ding et al. 2010; Laird et al. 2010; Singh et al. 2010) with the primary rationale being the high cation exchange capacity of biochar given the negative electrochemical charge on the biochar surface (Glaser et al. 2002). The observed 30% reduction in NH_4^+ lost below the rooting zone in our study fits well with previous findings, as well as further confirmed our observed results of enhanced top soil NH_4^+-N levels.

Being a soluble anion, NO_3^- is highly susceptible to leaching in agricultural soils. Our observation of a 30% reduction in NO_3^- -N accumulation on resin capsules buried below the rooting zone is consistent with findings from various field studies (Laird et al. 2010; Ventura et al. 2013). Although the specific mechanisms influencing NO_3^- retention by biochar remains unclear, several hypotheses have been proposed:

(1) Microbial immobilization of NO_3^- by biochar additions. The on-farm produced biochar is produced from softwood mix, which is N-limited, but C-rich. It is possible that biochar could increase net NO_3^- immobilization rates thereby reducing NO_3^- leaching (Lehmann et al. 2003).

(2) It has also been reported that biochar could increase soil water retention by altering soil physical properties such as enhancing micro- and meso-pores, thus reducing leaching by reducing water movement through the profile (Glaser et al. 2002; Brockhoff et al. 2010; Ventura et al. 2013). Although some authors observed the opposite results (Bell & Worrall 2011; Knowles et al. 2011).

(3) Anderson et al. (2011) indicated that biochar could enhance the growth of soil microorganisms involved in dissimilatory reduction of NO_3^- to NH_4^+, as well as inhibit the activity of *Nitrosomonadaceae* which are responsible for oxidation of NH_4^+ to NO_3^-, thus decreasing the highly mobile NO_3^- pool (Anderson et al. 2011).

(4) Direct adsorption of NO_3^- could happen due to the anion exchange reactions that exist on fresh biochar surfaces (Mukherjee et al. 2011; Zheng et al. 2013) thereby reducing net NO_3^- leaching.

Resin-sorbed phosphate is defined as freely exchangeable P that is available for plant uptake. Biochar has been reported to decrease (Novak et al. 2009b; Yao et al. 2012; Yuan et al. 2016), increase (Ippolito et al. 2012; Hardie et al. 2015) and have no effect on (Borchard et al. 2012; Iqbal et al. 2015) P leaching in mineral soils. We found a 30% decrease in the accumulation of P in resins below rooting zone, suggesting a potential benefit of biochar in P management in the sandy soils found on the San Juan Islands. Beaton (1959) demonstrated the capacity of charcoal to adsorb phosphate and proposed a mechanism of hydrogen bonding between orthophosphate and charcoal surfaces (Beaton 1959). However, it is been reported that the amount of P adsorbed to charcoal is relatively low compared to P adsorption on soil surfaces (Nelson et al. 2011). As noted above, dry beans are P-efficient plants, therefore it is possible that the beans solubilize some amount of P that accumulated at depth. Our study also showed that biochar can help increase the active pool of P that sorbed to soil minerals (Figure 7), thus partially explained the decrease of resin-sorbed P below the rooting zone.

2.4.3 Nutrient Concentrations in Dry Beans

Dry beans are an important part of the human diet in many countries throughout the world. They supply protein, complex carbohydrate, food fiber, essential vitamins and minerals, are low in fat and contain no cholesterol (Geil & Anderson 1994). The concentrations of magnesium (Mg), phosphorus (P) and zinc (Zn) in dry beans grown in the 'charged biochar' treatment were significantly higher than those in the 'control' (Figure 10) suggesting that biochar has the potential to help improve nutritional values of dry beans grown on organic farming systems in San Juan Islands. Increased soil available P following organic acid exudation, and decreased accumulation of P below rooting zone were both reflected in the P concentration in dry beans. The decreased resin-sorbed accumulations of metals below the rooting zone (Fe, Mg, Zn) were also reflected in the dry beans (Figure 10 and Tables 7, 8). As dry beans have a high P demands during the growing season it is possible that biochar additions accelerated the growth of arbuscular mycorrhizas which penetrate plant root cells and improve uptake of soil P, especially iron-bounded phosphorus (Vanek & Lehmann 2015). Charging or inoculating biochar with nutrients is reported to potentially lead to improved mycorrhizal

nutrient uptake (Hammer et al. 2015). It is possible that biochar serves as a “slow fertilizer” and “nutrient carrier” that adsorb nutrients into its micro-pores and exchanges nutrients onto its surface given its high cation exchange capacity. However, it is unclear why the mineral levels of dry beans (with the exception of Zn) growing on ‘charged biochar’ plots did not show a positive response when compared to those under ‘poultry litter’ treatment or the ‘control’ (Figure 10). The nutrient concentrations of beans grown on the ‘charged biochar’ plots showed a relatively wide concentration range, implying a great degree of variability across the 10 organic farms in terms of different beans species.

Nitrogen concentration in harvested dry beans were not examined in our study, however, it is important to note that dry beans can fix some amount of nitrogen that directly goes into its plant tissue (Broughton et al. 2003). Further studies on biological N fixation of beans (e.g. conduct acetylene reduction assay) are needed in order to fully understand the biochar effect in organic agricultural systems.

2.5 Conclusion

Soils of San Juan County, WA are dominated by sandy soils of glacial origin, which have a naturally high leaching capacity and limited water holding capacity. The area has an urgent need for forest health treatments to reduce fire risk on this isolated dry-forest ecosystem. The results from this short-term field study on ten organic farms in the San Juan Islands, WA suggest that locally produced biochar applied alone or when "charged" with chicken litter has the potential to improve N and P availability; increase nutrient retention; and increase dry bean nutrient density. By producing biochar from local timber harvest residues and applying them in neighboring agricultural soils, our study illustrated an overall positive benefit of an integrated agronomic and forest management strategy. Organic farming systems strive to create closed nutrient cycles that have lower immediately available nutrients compared to conventional farming. We believe on-site produced biochar used in our study could potentially improve nutrient cycling and availability to crops. Further studies are needed to explore the stability and long-term effectiveness of our on-site produced biochar on overall soil health.

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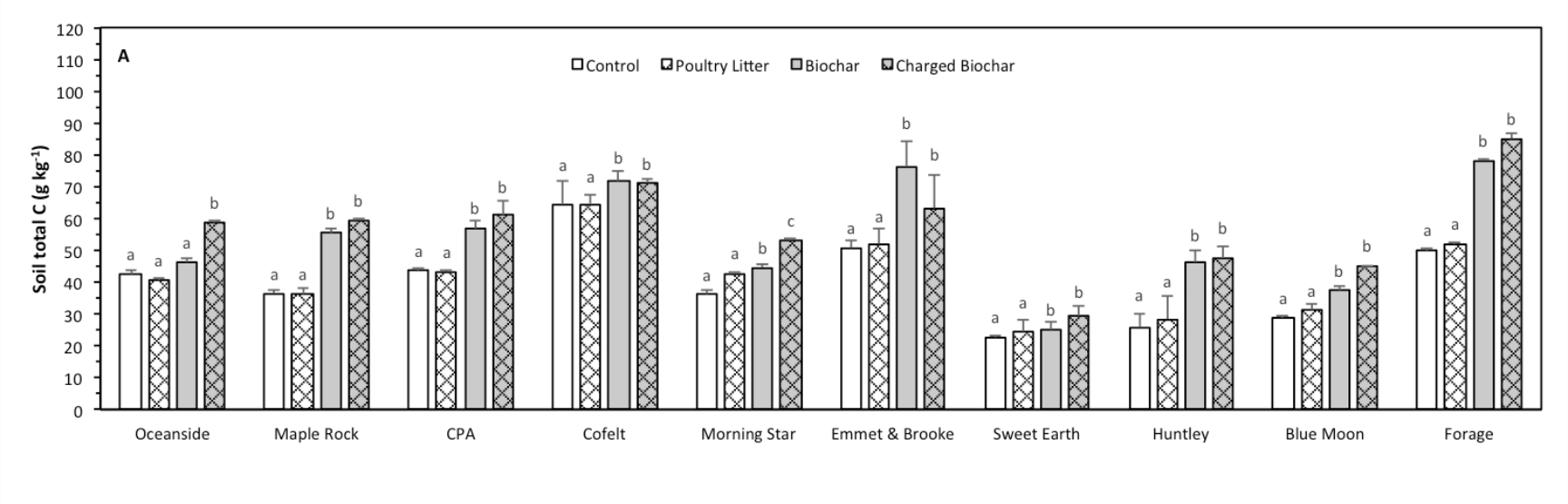
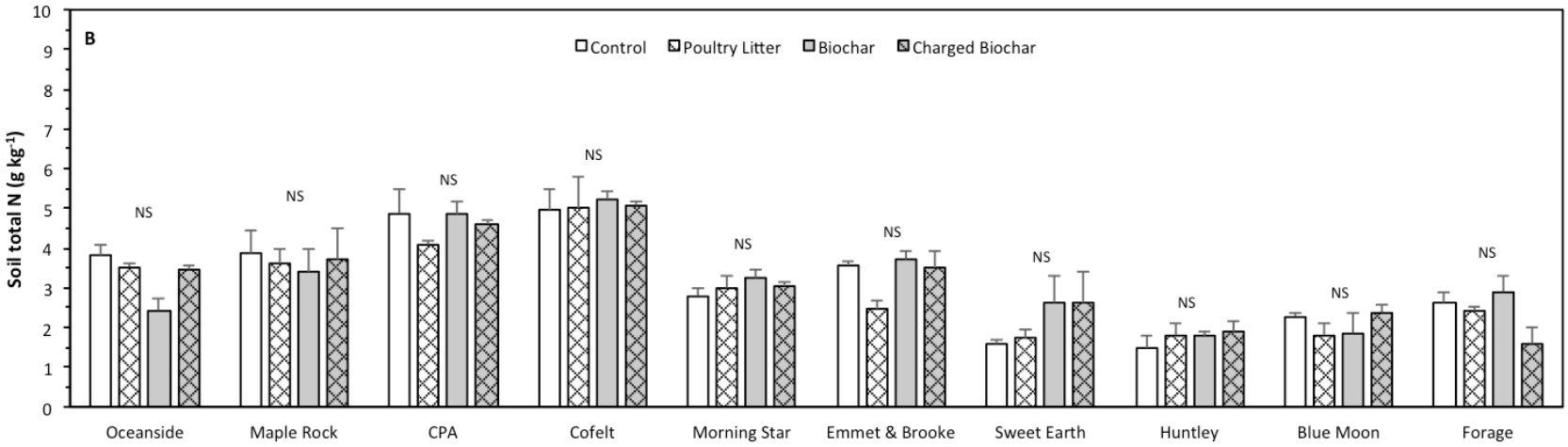
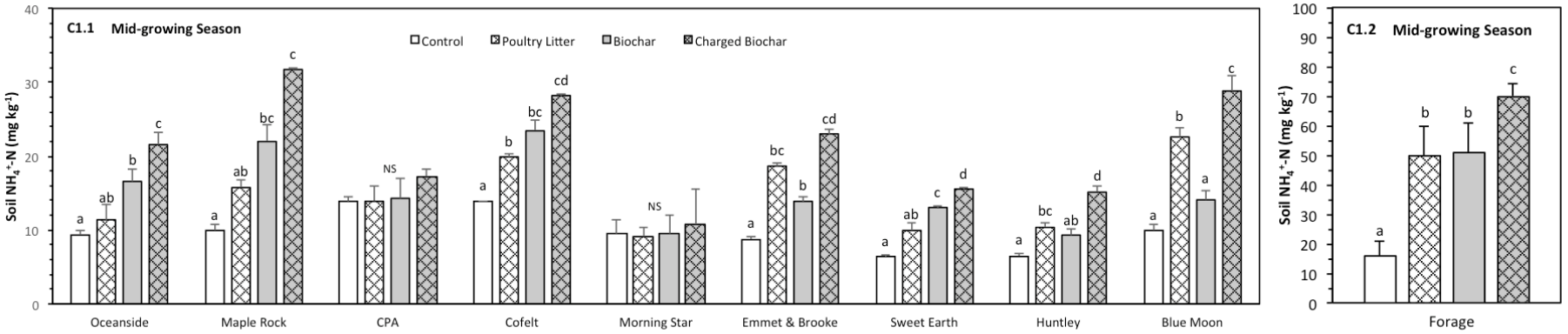
Appendix A. Soil total C content in response to application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



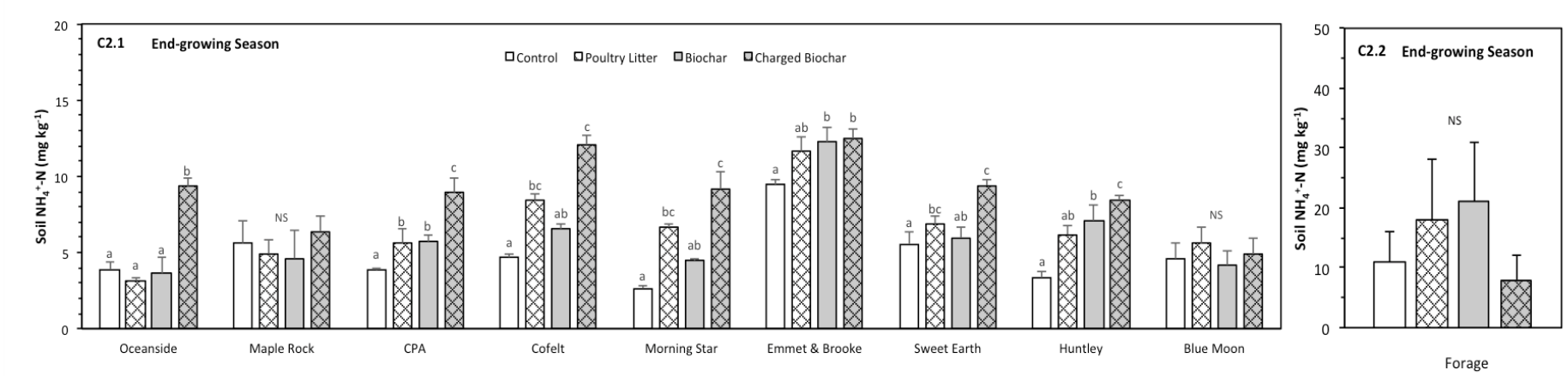
Appendix B. Soil total N content in response to application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



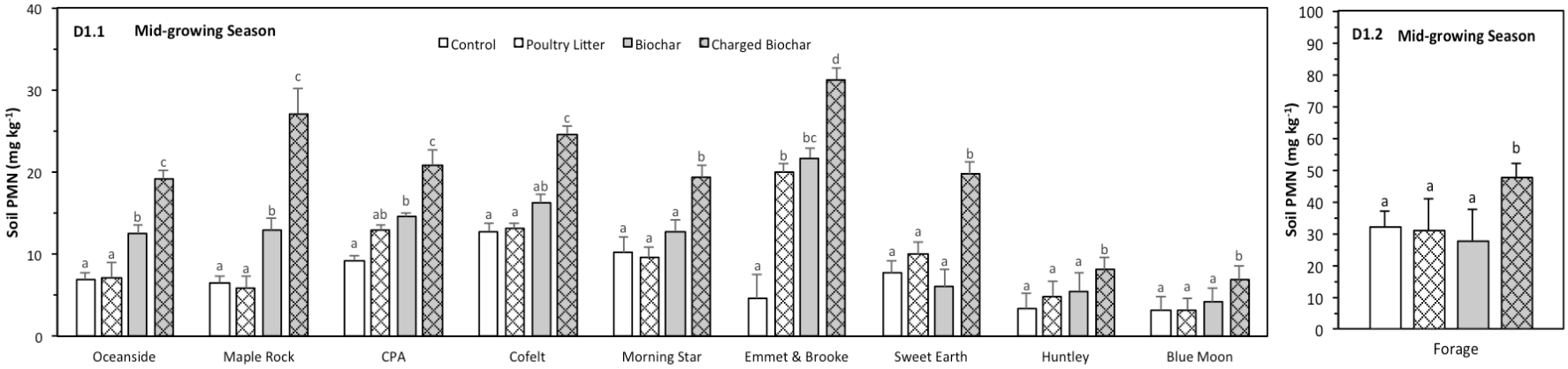
Appendix C1. Soil extractable NH_4^+-N at mid-growing season in response to application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



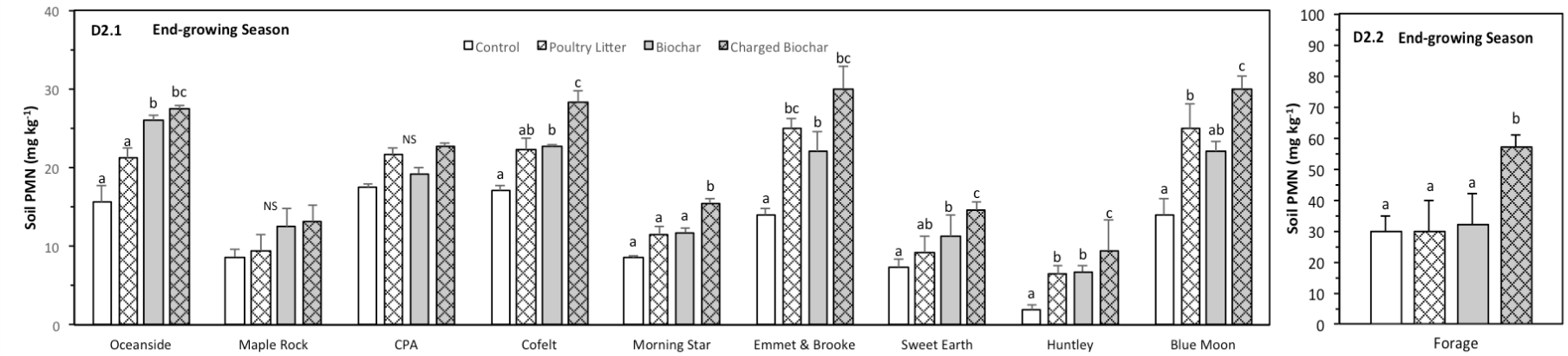
Appendix C2. Soil extractable NH_4^+-N at end-growing season in response to application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



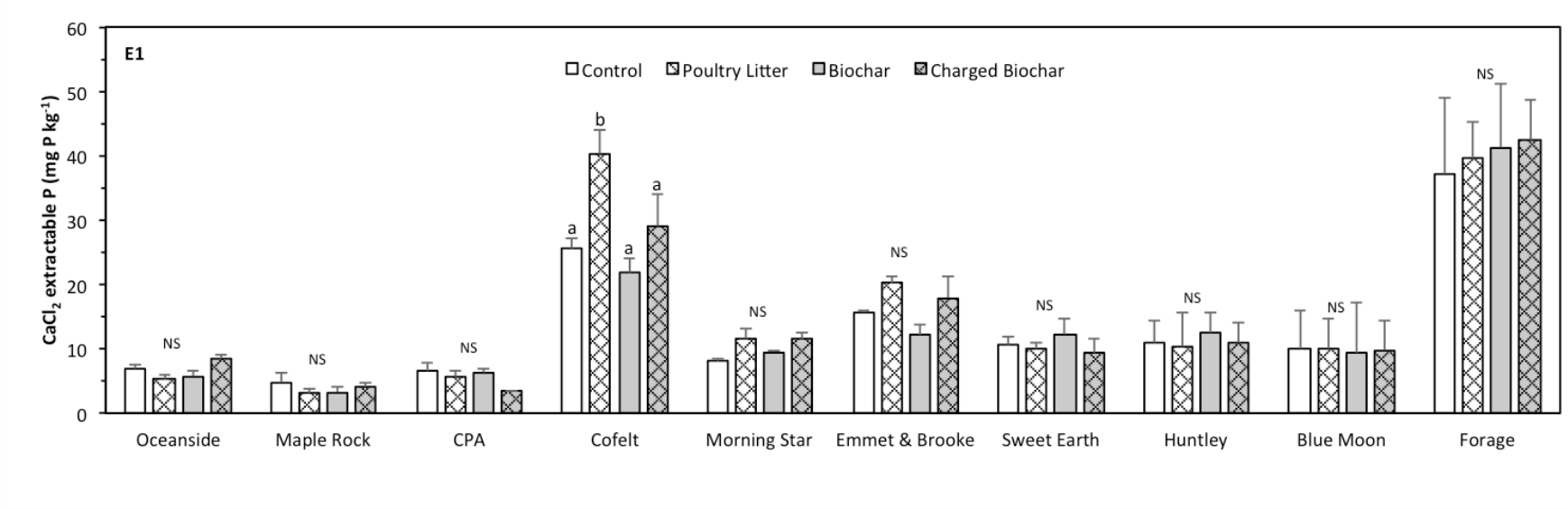
Appendix D1. Soil potentially mineralizable nitrogen (PMN) at mid-growing season in response to application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



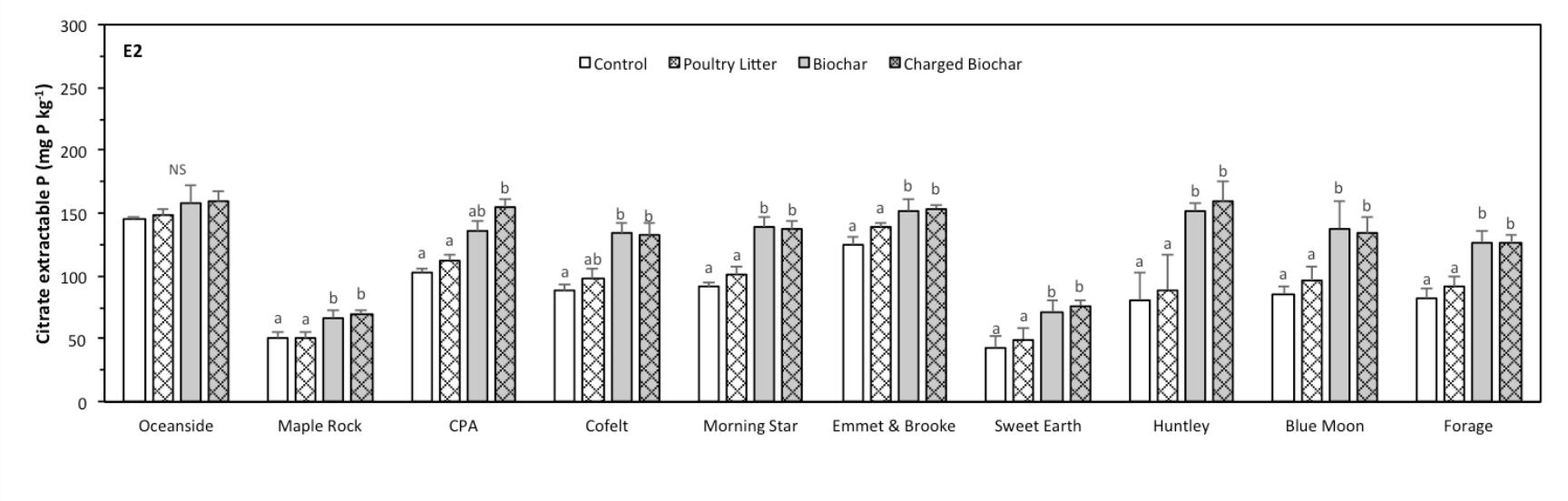
Appendix D2. Soil PMN at end-growing season in response to application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



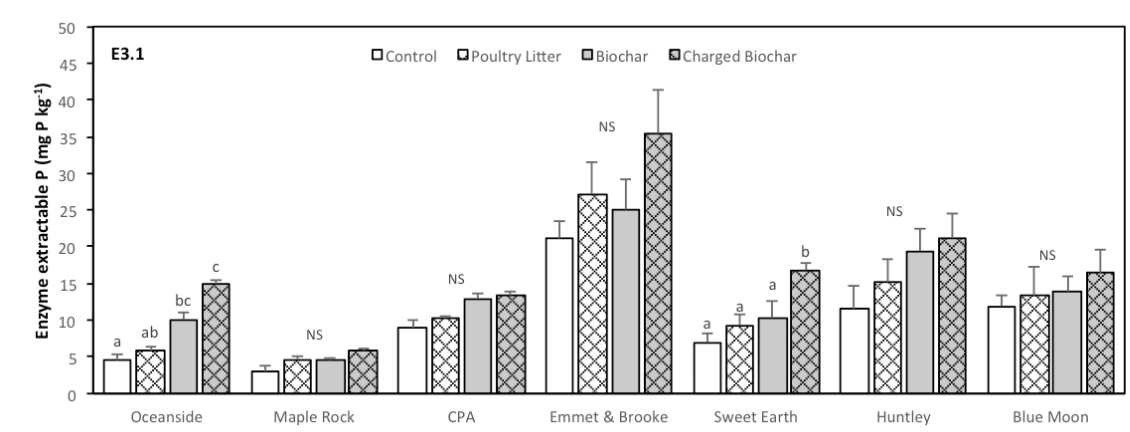
Appendix E1. Soil $CaCl_2$ extractable P concentration (mg kg^{-1}) four months after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.

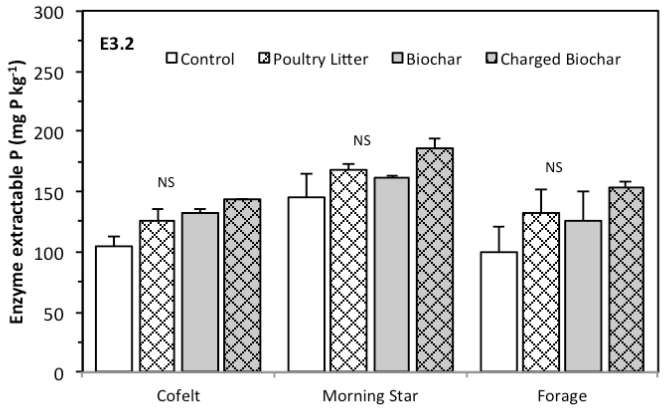


Appendix E2. Soil citrate extractable P concentration (mg kg^{-1}) four months after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.

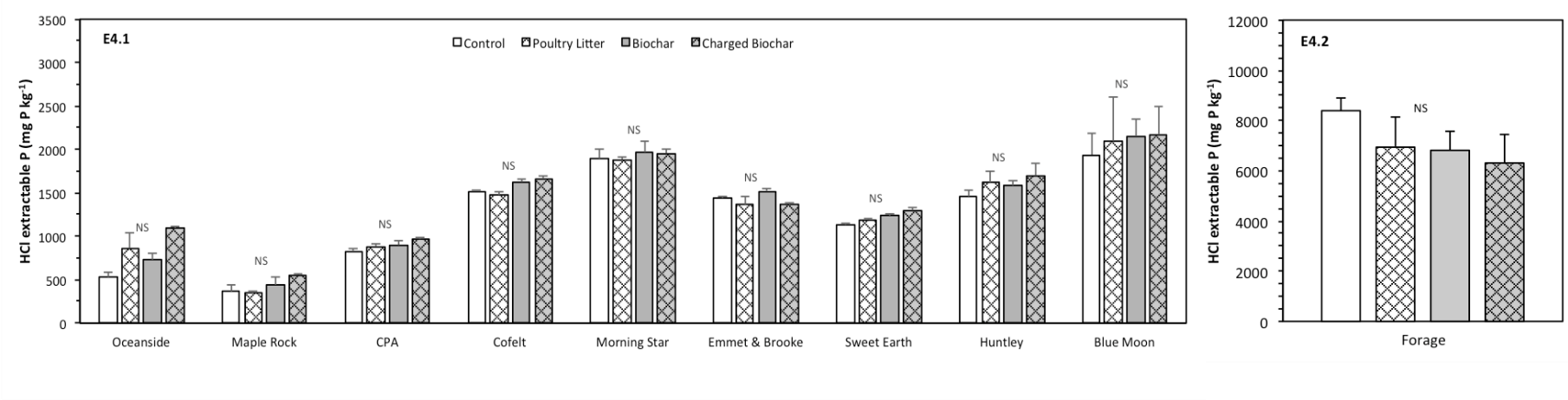


Appendix E3. Soil enzyme extractable P concentration (mg kg^{-1}) four months after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.

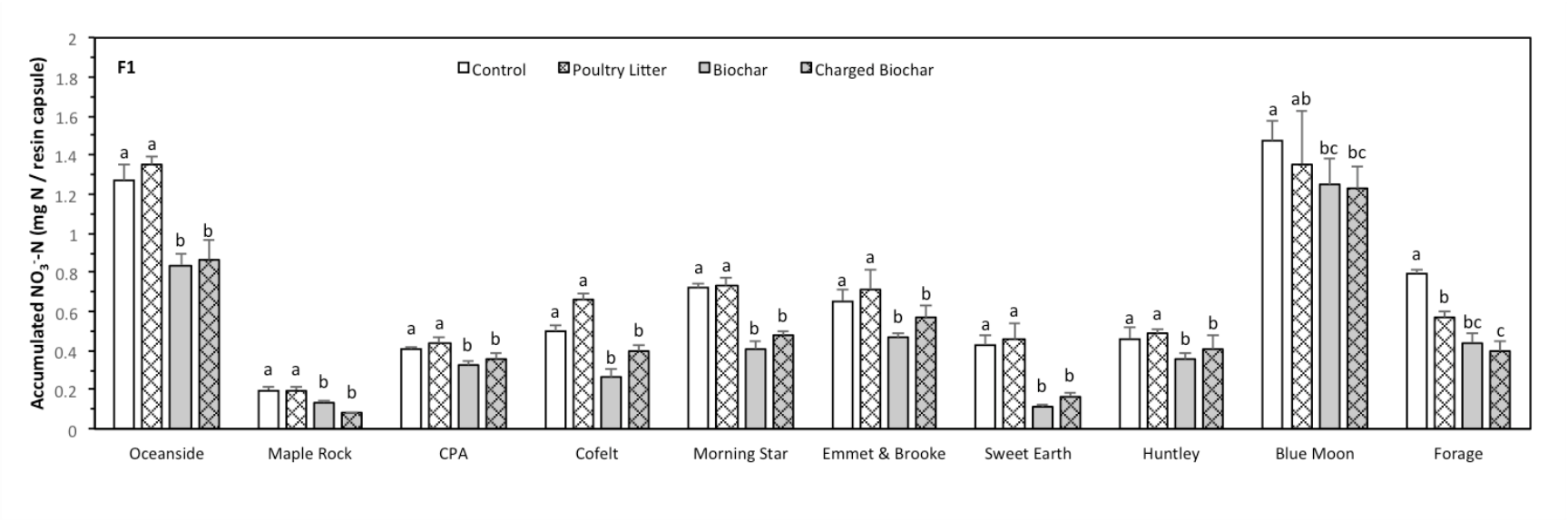




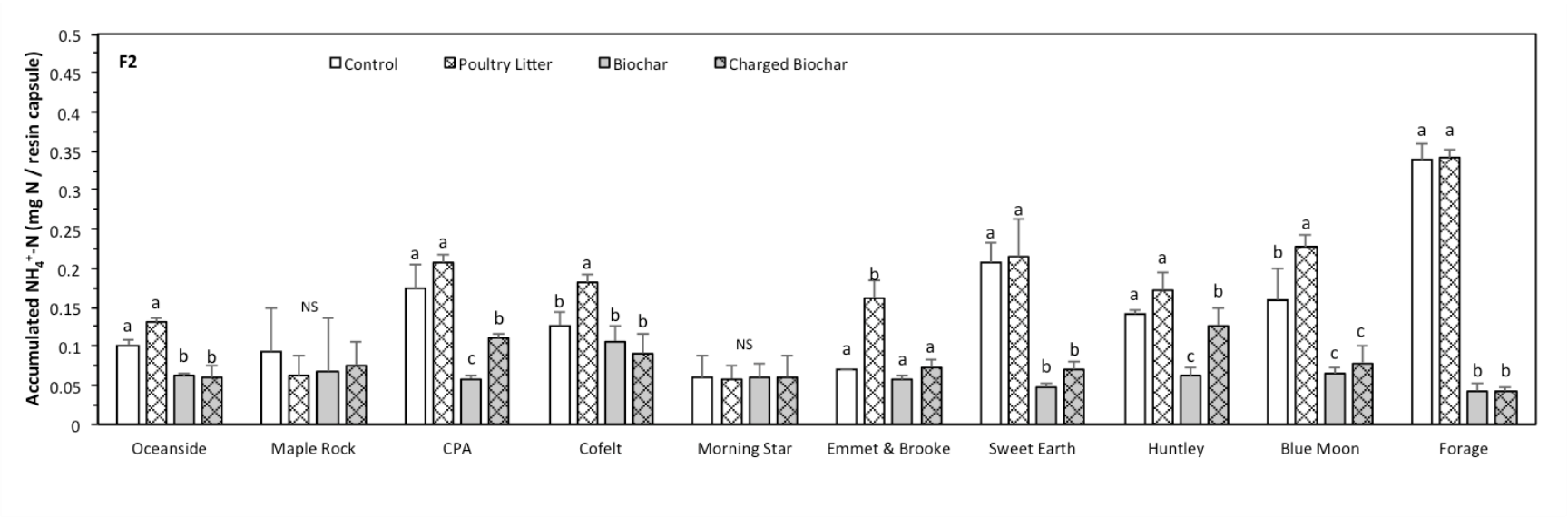
Appendix E4. Soil HCl extractable P concentration (mg kg^{-1}) four months after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



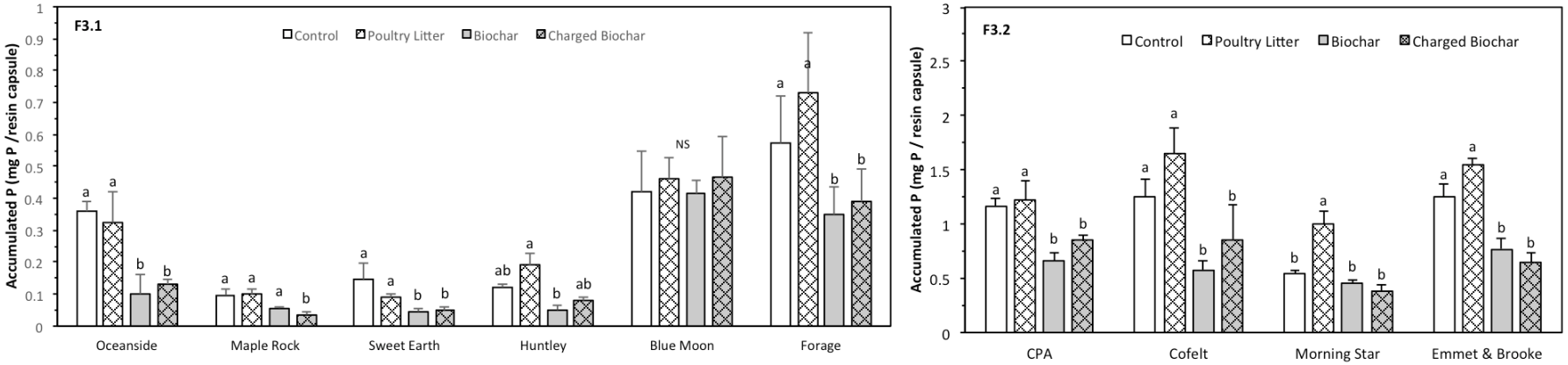
Appendix F1. Soil accumulated NO_3^--N of resin capsules below the rooting zone during the growing season following after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



Appendix F2. Soil accumulated NH_4^+-N of resin capsules below the rooting zone during the growing season following after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



Appendix F3. Soil accumulated P of resin capsules below the rooting zone during the growing season following after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.



Appendix G. Soil microbial biomass N concentration (mg kg^{-1}) measured at the end-growing season after application of biochar, poultry litter, or charged biochar treatments at 10 organic farms on the San Juan Islands.