

BIOCHAR INCREASES SOIL C SEQUESTRATION BUT WARMING TEMPERATURES
MAY INCREASE SOIL TEMPERATURE SENSITIVITY AND N₂O FLUX

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I AT MĀNOA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

NATURAL RESOURCES AND ENVIRONMENTAL MANAGEMENT

MAY 2016

By

Lauren M. Deem

Thesis Committee:

Susan E. Crow, Chairperson

Jonathan Deenik

C. Ryan Penton

John Yanagida

Keywords: agriculture, biochar, carbon, climate change, nitrogen, soil

ACKNOWLEDGEMENTS

Countless people have made this project successful. First and foremost, I'd like to thank my advisor, Susan Crow for her guidance over the last few years in lab work, experimental design and manuscript writing as well as providing me opportunities to travel and present my research both national and internationally. I am also appreciative to Jonathan Deenik for his knowledge of Hawaii soils and agriculture. I would also like to acknowledge the assistance and expertise of my other committee members, John Yanagida and Ryan Penton.

I am grateful to Julian Yu and Ryan Penton at Arizona State University for their work in analyzing the microbial communities of these soils, input into the experimental design, and allowing me spend a week in their lab. I am also appreciative of Mark Johnson for letting me spend time with the EPA in Corvallis and analyze my biochar samples. I would also like to thank David Beilman at UH for allowing me to utilize his lab space and equipment.

I'm indebted to everyone in the Crow Soil Ecology and Biogeochemistry Lab, but especially Jon Wells for teaching me how to operate all of the equipment and not grumbling too much at my phone calls when something went wrong. I am also grateful to Michelle Lazaro and Josh Silva for their combined soil expertise and late night rides home from the lab. I would also like to acknowledge the lab technicians, Oliva Schubert and John McMillian, for their assistance, as well as Nancy Parker for her GIS map-making skills. Lastly, to Alexandra Hedgpeth, for the occasional lab assistance and frequent coffee breaks.

This project would also not be possible without the assistance of Rogelio Corrales, Susan Migita, and everyone else at the Waimanalo and Poamoho Research Stations for ensuring the success of the field experiment. I know how much everyone enjoyed harvesting the napiergrass. I would also like to thank Jabez Meulemans for his part in this project as well as everyone I bribed into assisting me in the field, including Alexandra Hedgpeth, Nancy Parker, Josh Silva, and Jon Wells.

Lastly, I'd like to thank my family for their emotional and financial support throughout this crazy Hawaii adventure.

The funding for this project was provided by the United States Department of Agriculture National Institute of Food and Agriculture (USDA-NIFA 2012-67020-30234). The biochar for this project was provided by Diacarbon, Inc.

ABSTRACT

Global atmospheric carbon dioxide (CO₂) and nitrous oxide (N₂O) concentrations have increased rapidly in recent decades and in the United States agriculture accounts for 10% of greenhouse gas emissions (GHG). One potential method to combat GHG emissions is the application of biochar to agricultural soils. Biochar is organic matter that has undergone pyrolysis (i.e. combustion under low to no oxygen conditions), which results in a recalcitrant, carbonaceous material. In addition to direct CO₂ mitigation through pyrolysis, biochar can further reduce GHG emission and increase soil carbon (C) sequestration, soil quality and crop yields. To examine the impact of biochar in Hawaii soils, two important agricultural soils (Oxisol and Mollisol) with contrasting fertility under two different cropping (zero-tillage napiergrass and conventional sweet corn) with and without biochar were analyzed both post biochar amendment and after 1 year (two crop harvests for both napiergrass and sweet corn) for a suite of soil properties and microbial community composition. Additionally, individual bags of biochar were buried within the field and removed at year one to assess how the physical and chemical properties of biochar changed over time. Overall, biochar increased soil C by 47% compared to the control and influenced soil microbial community abundance but had little impact on other soil properties and crop yields. The biochar itself began to breakdown within the soil and become coated in clay particles.

While this represents a static view of how biochar influences soils in agronomic systems, given how difficult it is to remove biochar once amended, it is also important to establish the impacts of increasing global temperatures within these systems. To assess the response to temperature, the soils collected after one year were incubated across an eight-point gradient to determine temperature sensitivity for both soil respiration and N₂O flux. At 26°C there were no treatment effects in soil respiration, but for N₂O the Mollisol had increased flux ($p < 0.01$). Using the full gradient, the temperature sensitivity of the soils was assessed; almost all treatments had an increase in soil respiration with temperature. However, biochar nearly doubled the temperature sensitivity of soils ($p = 0.017$). Most soils were temperature insensitive for N₂O flux, with the exception of the Mollisol napiergrass biochar. Given the importance of N₂O in the context of climate change, the gene encoding nitrous oxide reductase (*nosZ*) was enumerated in concert with the total microbial community (*16S rRNA* gene) using quantitative PCR. While total microbial abundance and the abundance of genes involved in denitrification did not change with biochar and actually decreased with temperature ($p = 0.0088$), the ratio of denitrifying bacteria to total bacteria nearly doubled in the 31°C compared to the 23°C ($p = 0.0144$). The soils were then provided with an addition of a labile C source, similar to the addition of organic inputs or root exudates to see how the temperature-adapted communities responded. The biochar amended soils at 31°C had respiration 47% greater compared to soils with biochar at 23°C while the microbial abundance increased by 65% in the 31°C, although it was not significant. Conversely, no differences were found in N₂O flux or in *nosZ* genes for the glucose amended soils. However, the denitrifying bacteria had greater abundance in the day 60 soils than compared to the glucose

amended soils. Conversely, the overall microbial abundance was increased in the glucose amended soils compared to the day 60 soils. These results suggest that in some cases, while biochar increases soil C sequestration, it may exacerbate effects of climate change by increasing the temperature sensitivity of soil respiration in both more stable and more labile C pools as well as increase the temperature sensitivity of soil N₂O flux in a bioenergy crop with a ratoon harvest in a Mollisol soil. This indicates a need for a better understanding of how biochar alters the soil environment and a risk assessment for the use of biochar as a climate change mitigation strategy.

TABLE OF CONTENTS

	Page
CHAPTER 1. Introduction	11
1.1 Climate Change	11
1.2 Climate Change and Agriculture	11
1.3 Hawaii and Agriculture	12
1.4 Food and Energy Security	11
1.5 Agriculture and Soil Quality.....	13
1.6 Biochar.....	14
1.7 Greenhouse Gases and Soils.....	17
1.8 Objectives and Hypotheses.....	19
CHAPTER 2. Changes in Soil and Biochar Properties Over Time and Temperature Ranges ..	22
2.1 Introduction.....	22
2.2 Methods.....	23
2.2.1 Study Sites and Field Experiment	23
2.2.2 Biochar.....	24
2.2.3 Cropping Systems.....	24
2.2.4 Field Experiment Design	25
2.2.5 Buried Biochar Bags.....	25
2.2.6 Climate Monitoring	26
2.2.7 Characterization of Soil and Biochar.....	26
2.2.8 Soil Incubation.....	27
2.2.9 Temperature Sensitivity.....	29
2.2.10 Labile Amendment	29
2.2.11 Statistical Analysis	30
2.3 Results and Discussions – Field Experiment	31
2.3.1 Soil chemical properties	31
2.3.2 Soil biological properties.....	33
2.3.3 Biochar.....	34
2.3.4 Soil Respiration	34

2.3.5 N ₂ O Flux.....	36
2.3.6 Conclusions	38
2.4 Results and Discussions –Temperature Gradient Incubation.....	38
2.4.1 Soil Respiration	38
2.4.2 N ₂ O Flux.....	40
2.4.5 Conclusions	41
2.5 Results and Discussions – Mollisol Napiergrass	42
2.5.1 Soil respiration and total microbial abundance	42
2.5.2 Soil N ₂ O and denitrifying bacteria abundance	43
2.5.3 Conclusions	45
2.6 Conclusions.....	45
CHAPTER 3. Project Conclusions.....	66
3.1 Project Summary and Implications.....	66
3.2 Future Considerations.....	68
Appendix	
Appendix A: Site Maps.....	70
Appendix B: Biochar Particle Size Distribution	71
Appendix C: Plot Layout Poamoho	72
Appendix D: Plot Layout Waimanalo.....	73
Appendix E: Poamoho Weather Station	74
Appendix F: Waimanalo Weather Station	75
Appendix G: Climate Data.....	76
Appendix H: Initial soil properties following biochar amendment.....	77
Appendix I: Proximate analysis on biochar bags	78
Appendix J: pH and electrical conductivity of the biochar bags.....	79
Appendix K: Statistical output for soil properties at year 21	80
Appendix L: The cumulative respiration curves for the Oxisol soil over 8 temperatures	84
Appendix M: The cumulative respiration curve for the Mollisol soil over 8 temperatures.....	85
Appendix N: The table of values for cumulative respiration for both soils per g soil	86
Appendix O: The table of values for cumulative respiration for both soils per g soil.C	87
Appendix P: The statistical output for soil respiration at 26°C.....	88
Appendix Q: The cumulative N ₂ O flux curve graphs for the Oxisol soil	89

Appendix R: The cumulative N ₂ O flux curve graphs for the Mollisol soil	90
Appendix S: The table of values for cumulative N ₂ O flux for both soils.	91
Appendix T: The statistical output for soil N ₂ O flux per g soil at 26°C	92
Appendix U: The statistical output for individual soil respiration with temperature.....	93
Appendix V: The statistical output for individual soil N ₂ O flux with temperature	101
Appendix W: The statistical output for temperature sensitivity of biochar soil respiration	105
Appendix X: The statistical output for the day 60 Mollisol napiergrass at 23 and 31°C.....	106
Appendix Y: The statistical output for the ratio nosZ to 16S	107
Appendix Z: The statistical output comparing Day 60 to labile microbial communities	108
REFERENCES	111

LIST OF TABLES

Table 2-1: The chosen incubation temperatures and justifications.....	46
Table 2-2: The thermocycler program and gene specific primer sequences.	47
Table 2-3: The soil properties prior to the second harvest for each crop..	48
Table 2-4: Crop yields for the second harvest.	49

LIST OF FIGURES

Figure 2.1: The Scanning Electron Microscope (SEM) image of the biochar.....	50
Figure 2.2: The schedule of field events for the Oxisol.....	51
Figure 2.3: The schedule of field events for the Mollisol.....	52
Figure 2.4: The nMDS of the bacterial community structure on the soils.....	53
Figure 2.5: The SEM images of the initial biochar and after 1 year in the soil.....	54
Figure 2.6: The SEM images of the initial biochar and after 1 year in the bags and soil.....	55
Figure 2.7: The cumulative soil CO ₂ flux (µg C-CO ₂ g soil ⁻¹) at 26°C .	56
Figure 2.8: The cumulative soil CO ₂ flux (g C-CO ₂ g soil C ⁻¹) at 26°C.....	57
Figure 2.9: The cumulative soil N ₂ O flux (µg N-N ₂ O g soil ⁻¹) at 26°C.....	58
Figure 2.10: The cumulative soil CO ₂ flux (µg C-CO ₂ g soil ⁻¹) over eight temperatures.	59
Figure 2.11: The soil respiration for examining two temperature shifts (19-29°C and 31-40°C)..	60
Figure 2.12: The change in soil respiration per increase of °C.....	61
Figure 2.13: The cumulative soil N ₂ O flux (µg CN-N ₂ O g soil ⁻¹) over eight temperatures.	62
Figure 2.14: The cumulative CO ₂ and microbial abundance for Mollisol napiergrass	63
Figure 2.15: The ratio (nosZ) abundance to (16S).....	64
Figure 2.16: The comparison of microbial abundances with day 60 and labile soils.	65

LIST OF ABBREVIATIONS

°C	Celsius
Al	Aluminium
C	Carbon
CH ₄	Methane
CO ₂	Carbon dioxide
FTIR	Fourier transformed infrared spectroscopy
GHG	Greenhouse Gas
H	Hydrogen
K	Potassium
Mg	Magnesium
N	Nitrogen
N ₂ O	Nitrous Oxide
Na	Sodium
NH ₄	Ammonium
NO ₃	Nitrate
O	Oxygen
P	Phosphorus
qPCR	Quantitative Polymerase Chain Reaction
SEM	Scanning Electron Microscope

CHAPTER 1. INTRODUCTION

1.1 Climate Change

The earth is currently experiencing climatic change due to natural processes and anthropogenic activities with global impacts (IPCC 2014). These changes affect physical systems biological systems and human/managed systems. Specifically, impacts of climate change are evident through global sea level rise, increasing ocean temperatures and acidity, rising surface temperatures and more extreme weather events (IPCC 2014). In part, these changes are due to an increase in greenhouse gas (GHG) concentration including carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄) as a result of fossil fuel combustion, land use change and other anthropogenic impacts. Ice core data shows that increases in CO₂ concentrations correlate with an increase in atmospheric temperature (Shakun et al. 2012). Additionally, ice core data has revealed that over the past 800,000 years atmospheric CO₂ concentrations fluctuated but did not eclipse 300 ppm (Fischer et al. 2015). As of March 2016, the National Oceanic & Atmospheric Administration on Mauna Loa recorded the concentration of CO₂ in the atmosphere at 404.83 ppm. In comparison, the preindustrial atmospheric CO₂ concentration was 280 ppm (US Department of Commerce 2016). Levels of N₂O have increased by 49 ppb (parts per billion by volume) from pre-industrial times to 319 ppb currently. This increase is large, given that N₂O has a global warming potential (GWP) 265-298 times that of CO₂ (US EPA 2016b).

In Hawaii, climate change and the increase of atmospheric CO₂ may have a number of potential impacts. Tropical storms with increased intensity and frequency may cause flooding and overload water infrastructure systems which can cause a degradation of drinking water, especially when coinciding with high tides (Rotzoll and Fletcher 2013). Additionally, infrastructure located in low-lying areas will be threatened due to sea level rise in conjunction with coastal erosion (Anderson et al. 2015). Invasive species and diseases may expand, further decreasing biodiversity (Loope and Giambelluca 1998; Warren et al. 2013). Furthermore, ecosystem services such as fisheries and tourism revenues may be negatively impacted under future climate scenarios (US EPA 2015c).

1.2 Climate Change and Agriculture

Climate change and food security are linked; agriculture contributes 9% of global greenhouse gas emissions via direct soil fluxes (Laird et al. 2010; US EPA 2016a) and indirect sources such as manure management and fossil fuels used in farm machinery, transport, and pesticide production (West and Marland 2002; Burney, Davis, and Lobell 2010). However, agriculture has not always been fossil fuel intensive. There was an extensive increase in the use of technology and associated fossil fuel consumption during the first “green revolution” between 1966 and 1985, where cereal crop production increased by 300% (Pingali 2012). The

intensification of agriculture during this time however prevented an estimated 17.9-26.7 million hectares of land from conversion into agriculture (Stevenson et al. 2013), limiting the GHG associated with land use conversion to agriculture (Deng, Liu, and Shangguan 2014). Despite this, the first green revolution and its technological advances greatly contributed to current problems associated with GHG and agricultural intensification. With the increasing global demand for food and fuel, the next green revolution must focus on mitigating GHG emissions from agricultural practices while still maintaining or increasing soil fertility and crop production for both consumption and bioenergy (Kerr 2012; Godfray and Garnett 2014). Climate change and agriculture are inherently intertwined, it is important to find a balance between GHG emissions and food and energy security.

1.3 Hawaii and Agriculture

Hawaii has been under cultivation since its settlement with staple crops that included taro, sweet potato and sugarcane, among others (Clark 1986). The Hawaiian people divided the land into sections called ahupua‘a, which began in the mountains and ended along the coast. Each of these ahupua‘a had a person responsible for balanced resource allocation (Clark 1986). The arrival of European settlers and the eventual annexation of Hawaii into the United States in 1898 resulted in a very different agricultural system, one dominated by large-scale sugarcane plantations where land ownership was very concentrated (Goldberg 1996). However, the era of large scale sugarcane plantations in the Hawaii islands is ending. As of early 2016, the last sugar company in Hawaii, The Hawaiian Commercial and Sugar Company (HC&S) on Maui is ending sugar production after 145 years. Instead, agriculture in Hawaii is shifting to small-holder farms that use diversified agriculture with a focus on locally sourced foods or niche markets such as non-GMO or organic foods. There has also been a shift to bioenergy crops like napiergrass and energy cane. Research is underway to identify feedstocks with the highest energy potential for bioenergy and cellulosic liquid biofuels including jet fuel. This is an important step in working to meet Hawaii’s clean energy initiative, which aims to reduce dependence on oil imports by achieving 100% clean energy by 2045 (www.hawaiicleanenergyinitiative.org).

1.4 Food and Energy Security

Rising global temperatures will influence patterns of food and energy production worldwide. While some regions may experience an increase in productivity with climate change, the global trend is a decline in food production with subsequent spikes in food price (Porter et al. 2014). In 1996, the World Food Summit defined food security as “when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life” (WHO 2014). Between the years of 2010 -2012, it is estimated that 870 million people did not have access to enough food to cover basic nutritional needs; of this 870 million, 852 million were in developing countries (Food and Agricultural Organization 2013). As the demand for food increases with population and an increase in per capita consumption there is a subsequent need

for fuel to facilitate agriculture. As conventional fuel sources become more scarce and costly, the need for biofuels increases (Valentine et al. 2012). An inherent competition ensues however, as both food and fuel crops require arable land. Research is ongoing to reduce or avoid this competition, e.g., the development of water stress tolerant bioenergy crops such as perennial grasses that can be grown on marginal lands (Valentine et al. 2012). In addition, these issues are not isolated as there are other factors that impact food and energy security such as natural disasters, political instability, armed conflicts, and limited access. However, there are options to increase food and energy security on a localized scale by increasing and maintaining soil fertility and crop yields (Tschardt et al. 2012).

1.5 Agriculture and Soil Quality

Given increasing food demands in conjunction with an increasing global population, it is necessary to intensify agriculture. Current estimates project that by 2050, the global population will be over 9.3 billion people (Powlson et al. 2014). As of 2009, there was 4.9 billion hectares of land under cultivation in addition to 3.7 billion hectares for raising animals (Beddington et al. 2011). In 2012, 37.7% of the global land area was devoted to agriculture and pasture; in the United States, this number rises to 44.7% (Food and Agriculture Organization 2015). There are a number of issues with conventional agriculture including tillage, fertilizers, and land use change (Gomiero, Pimentel, and Paoletti 2011; Don, Schumacher, and Freibauer 2011). This is especially true in the tropics where low nutrient, acidic soils that harbor low biodiversity are subject to erosion (Cardoso and Kuyper 2006).

The intensive use of land for agriculture often results in a decline in soil quality with repeated harvest, residue removal, and subsequent conventional preparation tillage. Soil quality declines as a result of the removal of plant nutrients and a lowering of organic matter within the soil (Laird et al. 2010). Loss of soil C causes a decrease in soil structure, cation exchange capacity, and the ability of the soil to hold nutrients and water resulting in overall declines in soil productivity. As soils sequester approximately 80% of terrestrial organic C, this decrease in soil carbon is particularly alarming (McHenry 2011). Carbon that is no longer sequestered within soils will be respired, further contributing to climate change.

Sustainable intensification of agriculture should increase productivity while concurrently increasing soil quality and other ecosystem services provided by agricultural land (Bommarco, Kleijn, and Potts 2013). This would improve food security, energy security, soil quality and aid in mitigating climate change without converting more natural land to agriculture. This sustainable intensification needs to be in conjunction with a reduction of the demand for foods such as animal-based products that are comparatively more resource intensive than plant-based products as well as an overall reduction in food waste (Garnett et al. 2013). In addition to behavioral modifications, other methods to increase sustainable intensification include utilizing crops with a higher tolerance for stressors such as heat or pests (Dempewolf et al. 2014), leaving

crop residues, the use of perennial crops instead of annual crops, improved application of fertilizers (Powlson et al. 2011), the use of no-tillage management and a crop-fallow rotation (Page et al. 2013), the addition of organic matter (Abdollahi et al. 2014) and the use of biochar as a soil amendment (Xu et al. 2012; Wang et al. 2012). Biochar is of particular interest because it may both improve soil quality and reduce the increase of atmospheric CO₂ by directly and indirectly increasing carbon (C) storage in soils (Lehmann, Gaunt, and Rondon 2006) as well as the potential to decrease soil CO₂, N₂O, and CH₄ emissions (Case et al. 2014; Cayuela et al. 2014; Karhu et al. 2011).

1.6 Biochar

Biochar is currently promoted as a way to initiate a “doubly green revolution” (Barrow 2012) by potentially addressing soil organic matter GHG emissions and food insecurity concurrently (Jones et al. 2012; Mukherjee and Lal 2013; Sohi et al. 2010; Lehmann, Gaunt, and Rondon 2006). Biochar is a type of black carbon produced from a carbonaceous material through the application of heat or chemicals (Spokas et al. 2012; Lehmann 2007b; Novak et al. 2009). Black carbon in soils can be a result of anthropogenic activities like fire pits or natural occurrences like volcanic activity or forest fires (Spokas et al. 2012). Biochar is differentiated from black carbon in that it is created with the intent to be used as a soil ameliorant (Barrow 2012). Specifically, biochar is a stable substrate created from organic material that has been combusted under low or no oxygen conditions through the process of pyrolysis (Karhu et al. 2011; Atkinson, Fitzgerald, and Hipps 2010).

Biochars are heterogeneous in their properties due to the wide variety of feedstocks that can be used and pyrolysis technologies. Some common feedstocks include switchgrass, hardwoods, peanut hulls, corn hulls, pecan shells, bark, rice, sugar cane, leaves, paper sludge, cow manure, poultry manure, poultry litter, sewage sludge, and aquaculture waste (Barrow 2012; Manyà 2012; Spokas et al. 2012; Xu et al. 2012; Atkinson, Fitzgerald, and Hipps 2010). Once the feedstock is established, there are many different types of pyrolysis, including torrefaction, slow pyrolysis, fast pyrolysis, flash pyrolysis, and microwave assisted pyrolysis (Spokas et al. 2012; Manyà 2012). Other methods to create biochar include flash carbonization, hydrothermal carbonization and gasification (Spokas et al. 2012; Manyà 2012). The combination of many feedstocks and several pyrolysis technologies make for a plethora of biochars all varying in physico-chemical properties.

The ability to tailor biochar, either through feedstock or pyrolysis manipulation, offers considerable opportunity for the use of biochar as a soil ameliorant or crop enhancer. For instance, if the goal is to increase soil C sequestration then a high temperature biochar is the most useful whereas if the goal is to improve soil quality then low-temperature biochars have a greater impact (Ippolito, Laird, and Busscher 2012). Biochars made at high pyrolysis temperatures increase soil pH while poultry litter biochars increase available P and sodium (Na) (Novak et al.

2009). As more characterization studies, field trials, and greenhouse trials are undertaken, trends on how different biochars and their specific properties impact soils and crop growth can be ascertained. With this information, a soil with a deficiency or problem can be matched with a biochar that was created from a specific feedstock and under certain pyrolysis conditions to amend that precise problem (Novak et al. 2009).

This ability of biochar to amend soil quality issues, in conjunction with sequestering C has contributed to a surge in biochar interest. Prior to 2000, a Google Scholar search of “biochar” returned 595 papers. Between 2000 and 2010 there were 4,340 papers and within the past 6 years there were 15,400 papers published, an almost 2500% increase from pre-2000 levels. Despite this spike in published papers, the concept of incorporating biochar into soil for agriculture is not new. Areas in the Amazon have soils rich in black carbon that date back to between 450 BC and AD 950 (Barrow 2012). It is unclear whether the Amazonian dark earths, also known as Terra Preta, were anthropic (unintentionally formed by humans) or anthropogenic (intentionally formed by humans), but the source material it is most likely a mixture of ash from fires, midden waste, and slash and burn practices (Barrow 2012; Spokas et al. 2012). Terra Preta soil is characterized by increased amounts of C, nitrogen (N), phosphorus (P), potassium (K), nutrient and water holding capacity with a lower acidity, compared to the surrounding soil (Glaser et al. 2001). Terra Preta sites are several times higher in soil C, compared to the surrounding soil, and harbor specific soil biota that are thought to be responsible for the ability of the soil to maintain its higher quality (Barrow 2012).

Specifically, biochar is being targeted in tropical soils. Sustainable agriculture in the tropics is difficult because of the rapid degradation of soil organic matter in some soils as a result of limited stabilizing minerals in a hot and rainy climate (Glaser et al. 2001). In addition, the lack of stabilizing minerals means that fertilizer application is only effective for a short time post amendment (Glaser et al. 2001). The stable nature of biochar (Kuzyakov, Bogomolova, and Glaser 2014) could make it a more effective long-term soil ameliorate. In particular, one study found a single biochar application resulted in increased crop yields four years post amendment (Major et al. 2010).

Impacts of Biochar on Soil Chemical and Physical Properties

Biochar is a stable substrate that may increase soil pH, nutrient retention, cation exchange capacity (CEC), crop biomass and many other variables important to soil quality and agriculture (Xu et al. 2012; Schnell et al. 2012) The large surface area of biochar and its porous nature partly explain increased retention of nutrients and water, and increased microbial diversity due to more spatially derived niches (Barrow 2012; Xu et al. 2012; Atkinson, Fitzgerald, and Hipps 2010). Biochar also impacts the physical aspects of soil, including the bulk density, particle size distribution, porosity, structure and texture (Atkinson, Fitzgerald, and Hipps 2010; Manyà 2012; Xu et al. 2012). The application of biochar can reduce ammonia volatilization and increase the

immobilization of inorganic N (McHenry 2011). It can decrease emissions of GHG, including CO₂ (Case et al. 2014), CH₄ (Karhu et al. 2011), and N₂O (Wang et al. 2012), although other studies have also found a lack of significant differences for GHGs (Xiong, Xing, and Zhu 2007). However, there are very few studies on soil properties in long term, field scale trials, so more research needs to be done to investigate these changes and elucidate the mechanisms (Atkinson, Fitzgerald, and Hips 2010). In addition, while biochar impacts on soil have been documented, less is known about how biochar changes in the soil environment (McHenry 2011). Another important aspect of understanding how biochar impacts the soil is how it affects soil microbial communities and biogeochemical cycles (Xu et al. 2012).

Impacts of Biochar on Soil Biota

Biochar is considered recalcitrant due to its resistance to microbial decay (Lehmann et al. 2011). However, the high porosity can provide additional niches for microorganisms (Pietikäinen, Kiikkilä, and Fritze 2000; Barrow 2012). Depending on the biochar and soil type, biochar may also reduce changes within the microbial community structure and function (Anderson et al. 2011) or have no effect on species richness or diversity (Rutigliano et al. 2014). Biochar application has also been found to increase the amount of bacteria with decreased fungi abundance (Chen et al. 2013a) or by increasing k-strategist microbial biomass and increasing species richness (Liang et al. 2010; O'Neill et al. 2009). It may also increase plant root colonization by ectomycorrhizal fungi and arbuscular mycorrhizal fungi (Warnock et al. 2007). There may also be a microbial community shift to one that prefers aromatic C (Bamminger et al. 2014). The variety of different biochars, with varying chemical and physical properties, in conjunction with varying soil environments likely causes the wide range of microbial responses to biochar-amended soils.

The biochar induced changes in soil microbial community structure and function may result in altered C and N cycling and a subsequent change in soil respiration and N₂O flux. Biochar can influence how C is stabilized within soils (Chen et al. 2013a) and change the mineralization of native soil C (Liang et al. 2010). Biochar also may alter the N cycle within soils by increasing the relative abundance of microbial communities involved in the reduction of N₂O to N₂ and the fixation of N₂ to NH₄⁺ (Anderson et al. 2011). It is important to consider these changes in GHG emissions from soils when considering biochar as a way to mitigate climate change.

Biochar and Soil Temperatures Sensitivity

As global temperatures are projected to continue to rise, it is important to understand how soils will respond to this increase in temperature, especially given the large stocks of C that soils store (Lal 2008). Soil C may be respired back into the atmosphere via biochemical reactions mediated by microbial breakdown of organic matter or root respiration (Schlesinger and Andrews 2000). The rate of these reactions are dictated by temperature and substrate quality. The

Arrhenius function ($k = a \exp(-E_a/RT)$) postulates that as temperatures increase, the Q_{10} (the factor by which a rate increases given every 10° rise in temperature) of a reaction decreases. However, more stable materials require higher activation energy (E_a) in order for the reaction to occur, meaning the temperature sensitivity of the reaction increases (Davidson and Janssens 2006). This is “intrinsic temperature sensitivity” based only upon temperature and substrate quality, compared to “apparent temperature sensitivity” which accounts for the physical or chemical protection of organic matter which inhibits the decomposition enzymes from fitting properly (Davidson and Janssens 2006).

It is the ability of biochar to physically and chemically protect organic matter that may reduce the apparent temperature sensitivity of soils. A review of temperature and decomposition incubation studies support that more stable materials respond more quickly to an increase in temperature compared to more labile materials (Conant et al. 2011). Temperature increases may cause a shift in microbial carbon-use efficiency where they respire more C rather than incorporating it into biomass (Allison, Wallenstein, and Bradford 2010), although the mechanisms behind microbial partitioning of C are poorly understood (Frey et al. 2013). However, how biochar will respond to higher temperatures in terms of physical and chemical breakdown as well as influence the soil GHG flux and the soil microbial community remains to be determined.

1.7 Greenhouse Gases and Soils

Carbon Cycle

Soils are the third largest global carbon sink, accounting for approximately 2500Pg (Lal 2008), making soils a prime area for increasing C storage. The primary C inputs into soils are detritus from leaves and roots as well as root exudates (Davidson and Janssens 2006). Carbon dioxide (CO_2) from the atmosphere is converted into sugars by plants through the process of photosynthesis. Eventually some of these sugars become incorporated into the soil through root exudates or plant litter which are then used by soil organisms and respired, releasing the C back into the atmosphere (Brady and Weil 2008). Not all plant matter decays at the same rate; simple sugars and proteins decompose quickly while lignin and phenolic compounds decompose very slowly (Talbot and Treseder 2011; Jagadamma et al. 2014). Influxes of easily decomposable organic matter can result in an increase in microbial activity that can sometimes cause even resistant soil organic matter (SOM) to decay, in a process called priming (Qiao et al. 2014). Resistant SOM can resist decay through conversion into humus or physical protection in soil pores too small for microbial access (Brady and Weil 2008).

The application of porous biochar can provide more protected surface area thereby increasing stable soil-C by inhibiting the decomposition and subsequent respiration of C back to the atmosphere. There are studies that show biochar may decrease CO_2 emissions from soils. A *Miscanthus x giganteus* biochar in a 39 day incubation reduced cumulative CO_2 by 43% in the no

litter treatment and by 27% in the litter treatment (Bamminger et al. 2014). Others have also found biochar amended soil had significantly lower cumulative CO₂. The results indicated that the more aged the biochar, the lower the emissions, possibly due to a lack of nutrients that “younger” biochars may provide that can increase the biomass and activity of soil microorganisms (Zhao, Coles, and Wu 2015). A field experiment on a *Miscanthus* field (sandy loam soil) measured GHG fluxes over 2 years and found that a hardwood biochar reduced soil CO₂ emissions by 37% compared to the control soils. Soils collected 10 month post biochar amendment were incubated for 4 months with biochar resulting in a 53% decrease in CO₂ emissions, compared to a control (Case et al. 2014).

However, the mechanism by which biochar reduces CO₂ emissions are poorly understood. Biochar may shift the microbial community by providing additional microbial habitats (Pietikäinen, Kiikkilä, and Fritze 2000) that k-strategists find preferable (O’Neill et al. 2009). The application of biochar has been shown to decrease the activity of soil enzyme β-glycosidase (Chen et al. 2013b), associated with the breakdown of soil organic matter (Dick et al. 2013). Biochar can also promote the formation of aggregates (Liang et al. 2008), or sorb soil C (Lehmann et al. 2011) which protects organic matter. This protection, coupled with providing habitat for microorganisms, could result in an increase in C use efficiency due to co-location (Lehmann et al. 2011). There is some evidence that biochar can promote an increase of fungi over bacteria (Bamminger et al. 2014). Fungi have a greater C use efficiency, shunt more C towards their biomass than bacteria, and may obtain C from their plant hosts via hyphal bridges rather than obtaining C from the soil (Jin 2010).

Nitrogen Cycle

The global nitrogen cycle is an interconnected system in which nitrogen is converted into different forms via biological and physical processes. Unlike carbon, the diatomic N (N₂) in the atmosphere is unavailable to most organisms. The nitrogen needs to be “fixed” either through lightning or biological N fixation (BNF) into a form that is usable to plants, specifically ammonium (NH₄⁺) or nitrate (NO₃⁻) (Vitousek et al. 1997). Certain crops such as legumes have associated symbiotic bacteria that can fix atmospheric N and transfer the usable N to the plant in exchange for C (Vitousek et al. 2002). Through the process of nitrification, NH₄⁺ is oxidized by the bacteria *Nitrosomonas* to nitrite, which is then oxidized to nitrate by *Nitrobacter* (Henault et al. 2012). However, this only accounts for a small pool of available N globally. In terrestrial ecosystems N is often a growth limiting factor (Gruber and Galloway 2008). Arguably, one of the most important discoveries in the past century has been the ability to convert diatomic atmospheric nitrogen into the plant-available form of ammonia. It was this breakthrough, the Haber-Bosch process, that has allowed the world’s population growth to accelerate (Smil 1999) by doubling the amount of available N (Fowler et al. 2013). Both forms of plant-available N, regardless of whether they are products of BNF or the Haber-Bosch process and nitrification, can then be transferred back to N₂ by denitrifying bacteria via the reductive pathway of NO₃⁻ → NO₂⁻

→ NO → N₂O → N₂ (Aulakh, Doran, and Mosier 1992). The terminal step in the reduction of N₂O to N₂, is facilitated by the enzyme *nitrous oxide reductase*, which is encoded by the *nosZ* gene (Harter et al. 2014).

Unfortunately, the over application of synthetic fertilizers on agricultural lands can result in a transfer of highly mobile and available N to the surrounding ecosystems with negative consequences including water acidification and eutrophication (Erisman et al. 2013). In addition, one of the products of denitrification that may be produced in oxygen limited environments with adequate available organic material is nitrous oxide (Bremner 1997). Nitrous oxide may also be produced via nitrification at low pH due to an inhibition of NO₂ oxidation (Venterea and Rolston 2000). Nitrous oxide is a greenhouse gas, meaning it has the potential to be destructive to the ozone layer and contribute to climate change (Yanai, Toyota, and Okazaki 2007).

The application of biochar to agricultural soils has been shown to decrease soil N₂O emissions. A review of 30 studies found that in both laboratory and field studies there was a 54% reduction of soil N₂O emissions with biochar application (Cayuela et al. 2014). Biochar produced at a pyrolysis temperature >600°C has the potential for the increased adsorption of NO₃⁻ or at least prolonging its residence time in soil (Clough et al. 2013). Additionally, NH₄⁺ may become entrapped within the biochar pores (Clough et al. 2013). Biochar may also act as an electron shuttle to aid the transfer of electrons to soil denitrifying bacteria in the reduction of plant available N forms back into to N₂ (Cayuela et al. 2013). The addition of biochar with its high concentration of salts can increase the reduction of N₂O to N₂ by increasing the activity of denitrifying bacteria (Yanai, Toyota, and Okazaki 2007). While the mechanisms behind biochar reduction of N₂O emissions are not entirely clear, in most studies there is a reduction.

1.8 Objectives and Predictions

The increasing use of biochar in agriculture to target specific deficiencies in soil or crop quantity and increase soil C sequestration means it is critical to ascertain a deeper understanding of how biochar becomes incorporated into soil processes over time. To address this, a field experiment was designed to examine the biological, physical, and chemical changes in soils and crop yield in two soils of differing fertility following an anaerobic digester sludge biochar application. Two cropping systems were chosen (a) a napiergrass cropping system with no tillage and a ratoon harvest and a (b) sweet corn cropping system with conventional tillage and harvest. After it is established how biochar impacts soil properties and crop yields for the two different soils and cropping systems, it is important to consider how these relationships may change as global temperatures increase, especially with respect to soil respiration, N₂O flux, and microbial community composition and function. Yet, agricultural systems are dynamic with a constant influx of labile material. It is also important to consider how soils and their associated microbial communities will respond to inputs such as fertilizer and crop residues once the systems have acclimated to higher temperatures.

The overarching hypothesis is that biochar will increase soil C, improve soil properties; reduce the temperature sensitivity of soil respiration and N₂O flux and increase the total microbial abundance as well as the abundance of the denitrifying community both after the 60 day incubation as well as post amendment with a labile substrate.

Objective 1: Determine how contrasting soils respond to biochar amendment under two different crop and management systems and how biochar changes in the soil environment after one year.

Predictions:

- 1) The application of biochar will increase the concentration of soil C.
- 2) After one year, the napiergrass soils will have a greater soil C compared to sweet corn.
- 3) Biochar will increase exchangeable cations.
- 4) The soil microbial communities will vary.
- 5) After one year, the biochar will begin to breakdown.
- 6) Soil respiration will be lower in the Oxisol soil compared to the Mollisol soil.
 - a. Biochar will decrease soil respiration.
 - b. Biochar will reduce the overall soil C quality.
- 7) The N₂O flux will be lower in Oxisol compared to the Mollisol.
- 8) The application of biochar will reduce N₂O flux.

Objective 2: Determine how soils collected 1 year post amendment differ in soil respiration and N₂O flux over a broad temperature gradient.

Predictions:

- 1) Soil respiration and N₂O flux for both soils and crops will increase with temperature as biological reaction rates and/or total microbial abundance increase.
 - a. Biochar will reduce the apparent temperature sensitivity of soil respiration.
 - b. Biochar will reduce the apparent temperature sensitivity of soil N₂O flux.

To further resolve the microbial drivers of observed differences in gas flux and temperature sensitivity due to critical aspects of soil, crop management, and/or biochar amendment, the results of the first two experiments guided the selection of a subset of samples for a third, more intensive investigation.

Objective 3: Determine how labile inputs alter soil respiration, total microbial community abundance, N₂O flux, and the *nosZ* harboring denitrifying community abundance after acclimation to elevated temperature for the napiergrass in the Mollisol.

Predictions:

- 1) Differential responses of specific members of the denitrifying microbial community versus total bacterial community to the addition of a labile substrate following acclimation to elevated temperature will help resolve the primary drivers of the observed temperature sensitivity in soil respiration and N₂O flux in the biochar-amended Mollisol under zero-tillage management of napiergrass.
 - a. Biochar will increase microbial abundance.
 - b. The abundance of the overall microbial community and the denitrifying community will both equally increase with temperature.

CHAPTER 2. CHANGES IN SOIL AND BIOCHAR PROPERTIES OVER TIME AND TEMPERATURE RANGES

2.1 INTRODUCTION

Global atmospheric carbon dioxide (CO₂) and nitrous oxide (N₂O) concentrations are increasing rapidly, with agriculture accounting for 9% of greenhouse gas emission (GHG) in the United States in 2013 (US EPA 2016a). The emissions of CO₂ are due to land use change (West et al. 2010), tillage (Paustian et al. 2000) and fossil fuel usage in pesticide production, fertilizer production and transportation (West and Marland 2002) whereas N₂O emissions are a result of increased N fertilizer usage (Ussiri and Lal 2013) and manure management (Burney, Davis, and Lobell 2010). One method being utilized to combat these emissions is the application of biochar to agricultural soils.

Biochar is organic matter that has undergone pyrolysis (i.e. combustion under low to no oxygen conditions) (Woolf et al. 2010) which results in a recalcitrant and carbonaceous material (Lehmann, Gaunt, and Rondon 2006). When applied to agricultural soils, biochar can increase soil C sequestration (Crombie et al. 2015), improve soil physical properties (Mukherjee and Lal 2013) and chemical properties such as increasing pH and cation exchange capacity (Barrow 2012). Additionally, soils amended with biochar have shown decreased soil respiration (Case et al. 2014) and decreased N₂O flux (Zhang et al. 2010; Liu et al. 2012).

Biochar may decrease soil respiration via the protection of soil organic matter by sorbing soil C (Lehmann et al. 2011), decreasing soil enzymes associated with decomposition of C (Dick et al. 2013), or promoting the formation of soil aggregates (Liang et al. 2008). It can also influence soil microbial community composition to one more efficient at utilizing C (Lehmann et al. 2011; Jin 2010) or one that degrades more stable, C (Bamminger et al. 2014; O'Neill et al. 2009). The observed decrease in N₂O may be a result of adsorption or physical entrapment of NO₃⁻ and NH₄⁺ (Clough et al. 2013). Biochar may also effect denitrification either by transporting electrons to aid in the reduction of plant available N to N₂ (Cayuela et al. 2013) or by directly increasing the activity of denitrifying bacteria (Yanai, Toyota, and Okazaki 2007).

However, the mechanisms behind how biochar functions within soils and how it may alter biological processes are poorly understood. It is important to establish not only how biochar interacts with different soils, cropping systems, and microbial communities but also how these relationships may change over time in the context of global climate change. Given that biochar is being considered as one climate change mitigation strategy and the near impossibility of removing biochar once it has been amended, the determination of how biochar will respond in soils under increasing temperatures is imperative.

Temperature responses of soils is an area of intensive research (Davidson and Janssens 2006; Thiessen et al. 2013; Karhu et al. 2014a). In general, the more labile a substance, the less

sensitive it is to changes in temperature whereas more stable substrates will degrade at a faster rate as temperatures increase (Davidson and Janssens 2006). Soil is a heterogeneous substrate and biochar amended soil is even more complex, but soil temperature sensitivity with biochar additions is largely unexplored (Fang, Singh, and Singh 2014). It is also important to consider how biochar addition may alter the microbial community in different soils and cropping systems exposed to temperature increases. Changes in soil properties like pH can shift microbial communities (Rousk et al. 2010), a switch to a no-tillage system can increase the abundance of bacteria and mycorrhizae which can increase stable soil C content (Mbutia et al. 2015), and temperature increases can increase the abundance of pathways for C-degradation and denitrification (Luo et al. 2014). The influence of temperature on soil respiration and N₂O may also have threshold effects where certain temperature increases have a larger impact than others. Additionally, soil microbial communities are also influenced by the presence of biochar (Rutigliano et al. 2014; Lehmann et al. 2011), adding another level of complexity.

To elucidate some of these variables, this study examines biochar effects on CO₂ and N₂O fluxes in two tropical soils with contrasting crop management systems over an eight-point temperature range. The objectives of the study were to, **1)** determine how contrasting soils will respond to biochar amendment under two different crop and management systems after one year as well as how will biochar change over 1 year in the soil environment, **2)** determine how soils collected 1 year post amendment differ in soil respiration and N₂O flux over a temperature gradient, and **3)** determine i) how the Mollisol napiergrass microbial community abundance will change after acclimating to higher temperatures ii) how labile inputs alter the soil respiration and total microbial community abundance and iii) how labile inputs alter N₂O flux and the *nosZ* harboring community abundance. The overarching hypothesis for the study was that biochar will increase soil C, improve soil properties; reduce the temperature sensitivity of soil respiration and N₂O flux and increase the total microbial abundance as well as the abundance of the denitrifying community both after the 60 day incubation as well as post amendment with a labile substrate.

2.2 METHODS

2.2.1 Study Sites and Field Experiment

The two study sites were located on the island of Oahu within the Hawaiian island chain (Appendix A). The low fertility Oxisol soil is a Wahiawa series silty clay (very-fine, kaolinitic, isohyperthermic Rhodic Haplustox) with basaltic parent material and a deep, well drained profile that resists compaction (USDA 2013a). The Oxisol soil was collected from Poamoho Research Station (21.54488 N, 158.08818 W) on Oahu. The site receives 127 cm of annual precipitation and has an average temperature of 22.5°C (“Web Soil Survey” 2016). The soil is classified as prime agriculture land if irrigated and covers approximately 21,000 acres of land (“Web Soil Survey” 2016). The acidic, highly weathered soil is a deep red color, with 1.33% C and 0.16% N. It has a low N mineralization potential (Deenik 2006) and low concentration of base cations,

with 650 mg/kg calcium (Ca), 84 mg/kg sodium (Na), 113 mg/kg magnesium (Mg), and 344 mg/kg potassium (K). It is also susceptible to manganese (Mn) toxicity and has a high concentration of iron and aluminum oxides which can bind with phosphorous (P) in the soil, reducing plant P availability. Previously, the field site had been used to grow conventional papaya.

The high fertility soil is a Mollisol soil of the Waialua series (very fine, mixed, superactive, isohyperthermic Pachic Haplustoll) (USDA 2013b), collected from the field site at Waimanalo Research Station (21.33377 N, 157.71620 W) on Oahu. The site has an average annual temperature of 23°C and receives 95 cm of rain per year (“Web Soil Survey” 2016). The Mollisol soil comprises only a small part of Oahu at 5,500 acres but is important for agriculture in part due to its high ability to exchange cations, with 4234 mg/kg Ca, 147mg/kg Na, 679 mg/kg Mg, and 392 mg/kg K. The soil is 1.48% C and 0.15 % N. It also has a moderate potential to mineralize N and a clay content of 55% (Deenik 2006). In the decade prior to this experiment, the plots at Waimanalo had been used most often to grow corn although jicama was also grown. Additionally, there had been at least two events of deep plowing to 30cm and disking.

2.2.2 Biochar

The biochar used in the experiment was produced by Diacarbon, a Canadian commercial biofuel company (www.diacarbon.com). The feedstock was 20% anaerobic digester sewage sludge and 80% spruce, pine and fir wood chips that underwent pyrolysis in a continuous flow reactor at about 600 °C. The biochar was 74% carbon, 1.03% nitrogen, and 1.93% hydrogen. For exchangeable base cations, Ca was 626.98±53.90 mg/kg, Na was 1401.96±138.17 mg/kg, Mg was 663.48±65.41 mg/kg and was K 4323.81±522.17 mg/kg. The biochar had a pH of 9.54 and an electrical conductivity of 444.5 µS/cm. As determined by standard proximate analysis, the biochar was 29.73% volatile matter, 15.31% ash and 56.72% fixed C as determined by difference. Additionally, the biochar had a range of particle sizes (Appendix B), with 18% <0.15mm, 28% 0.15-0.5mm, 26% 0.5-1mm, 21% 1-2mm, 6% 2-4mm and 1% >4mm. Scanning electron microscopy (SEM) images revealed heterogeneity in the forms of feedstock by showing amorphous structures for the anaerobic digester residues and a more tubular structure with visible xylem holes for the woodchips (Fig 2.1).

2.2.3 Cropping Systems

The bioenergy crop was napiergrass of the green bana variety (*Pennisetum purpureum*). Napiergrass is a perennial C4 grass that is native to Africa and often used as a forage crop for livestock (Strezov, Evans, and Hayman 2008) with an extensive root system that can increase soil C (Somerville et al. 2010). Napiergrass is an upright grass species, similar to sugarcane, with a potential use as a bioenergy crop due to its high consecutive yields, although napiergrass has high water requirements for maximum yields (Foley et al. 2007). In constant growing conditions, napiergrass may generate over 100 million gallons more ethanol annually than sugarcane (Foley

et al. 2007). In this experiment the napiergrass was harvested by ratoon every 6 months, a form of zero-tillage managements. For the ratoon harvest, the plants were cut off 10-15cm from the soil surface; the napiergrass regrew from the remaining stalk stubble. This practice reduced the need for frequent soil tillage and the associated soil aggregate disruption, thereby increasing the sustainability of the cropping system. Yields for napiergrass were determined on a dry weight equivalent basis.

The food crop was Hawaiian Supersweet #9 corn. It is a widely grown supersweet hybrid variety in the tropics developed by James Brewbaker at the University of Hawaii in the 1960s which was bred to have a high sugar content and a longer shelf life (Lertrat and Pulam 2007). The corn chosen is resistant to *Puccinia sorghi* rust and *Fausarium moniliforme* kernel rot (Brewbaker and Nagai 1992). To prepare for planting, soils were ripped 15-20cm deep and then rototilled. Seeds were planted 2-3 per hole and thinned to one plant after a few weeks. The corn was harvested on approximately a 72 day cycle with pest control sprays being used if necessary. Yields for sweet corn were determined on a marketable fresh ear weight basis.

2.2.4 Field Experiment Design

The experimental design consists of two crops, each with and without a biochar treatment, in two different soils. There were eight plots with napiergrass, eight plots with sweet corn, and two plots with no crops, which were hand weeded. The napiergrass plots and the corn plots were separated from each other due to differences in row spacing, number of rows, and irrigation. The site layout at Poamoho (Appendix C) and Waimanalo (Appendix D) were identical. Each plot was 4.6 x 6.1m. At each site four napiergrass plots, four corn plots, and one bare plot were randomly chosen to receive 45.36 kg of anaerobic digester biochar per plot (16.28 Mg/ha). All plots received 10.89kg (3.91Mg/ha) of fish bone meal (9.07%N; 2.38%P; 0.63%K; 1.49%Ca; 0.13%Mg). The napiergrass plots received biochar and fish bone meal fertilizer prior to the initial planting of the napiergrass cuttings. Fish bone meal was then reapplied via surface broadcasting after the year one harvest. Biochar and fish bone meal were applied to the plots prior to the initial planting. For subsequent plantings only fish bone meal was applied. All the plots at Poamoho received 13.6 kg per plot (4.88 Mg/ha) of lime at the initial planting. Additionally, both sweet corn plots received 0.61kg (218.9 kg/ha) potash prior to the second planting. The bare plots were kept bare through periodic hand weeding. The Waimanalo bare plots were reduced to 2.3 x 3m to reduce weeding time. The bare plots were located closest to the napiergrass plots; at both sites the bare plots were kept on the same schedule of fertilization, irrigation, and sampling as the napiergrass due to the more consistent growth and harvest schedule.

2.2.5 Buried Biochar Bags

Nylon mesh (mesh diameter 0.1mm) was used to construct bags measuring 12 x 24cm. Each bag was fitted with a metal tag and then weighed. The bags were then filled with 100g

oven-dried biochar. Edges were sealed using a Uline Poly Bag Sealer. At the Poamoho Research Station on May 23, 2014, two bags each were buried in each biochar bare plot, biochar napiergrass plot and biochar sweet corn plot. A hole 10cm in depth was dug near the irrigation lines and the bags were laid out side by side in the hole and then covered by soil. This was then repeated at Waimanalo Research Station on May 27, 2014. Lastly, eight bags were placed in a covered bucket and stored in the laboratory. The napiergrass and bare plots' bags remained undisturbed, mimicking the no-till conditions within the plots. The sweet corn bags were dug up prior to plowing to both protect the bags and mimic the soil disturbance due to conventional tillage management practices. The set of corn bags at Waimanalo were plowed over and subsequently replaced on July 22, 2014. The replacement bags were then plowed over again in January 2015 and were not replaced.

2.2.6 Climate Monitoring

At the implementation of the experiment, a Decagon Devices Em50 Digital Data Logger weather station was installed at each site to measure soil moisture and temperature at two different depths, air temperature, relative humidity, precipitation, and photosynthetically active radiation (PAR) (Appendix E and F). These site-specific data can then be compared to overall precipitation maps, annual temperature, solar radiation and evapotranspiration for the entire island of Oahu (Appendix G).

2.2.7 Characterization of Soil and Biochar

Soil Analyses

Soil samples were collected at the start of the experiment, post-amendment with biochar or fish bone meal, and prior to harvest at both Poamoho (Fig 2.2) and Waimanalo (Fig 2.3), to be measured for a suite of characterization parameters to determine initial conditions and change over time as the cropping cycles progressed. Soil properties following the initial amendment with biochar can be found in Appendix H. A subsample of soil was air-dried and ground using a ball mill grinder until the entire sample was smaller than 150 μ m. The soils were then weighed into tin capsules and analyzed for total C and N using oxidative combustion (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA). To determine base cation concentrations at pH 7, soil was shaken in a 1M ammonium acetate ($\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$) solution at a ratio of 1:20 for 30 min and then filtered through a Whatman 42 filter. The resulting solution was frozen and then thawed prior to analysis for Ca, Na, Mg, and K (QuikChem 8500 Series Automated Ion Analyzer, Lechat Instruments, Loveland, Colorado). Available P was determined by extraction in Mehlich III at a 1:10 ratio (Sparks 1996, 758). The samples were shaken for 5 minutes, filtered through Whatman 42 filter paper, frozen, and then thawed and analyzed using a QuikChem 8500 Series Automated Ion Analyzer (Lechat Instruments, Loveland, Colorado). Soil was mixed with DI water at a ratio of 1:1 and after 10 min the solution was swirled and the pH meter probe (Accumet Research AR20, Fisher Scientific, Waltham, MA, USA) inserted into the

slurry until the pH remained constant (Sparks 1996, 487). Amplicon libraries for the 16s rRNA V4 region was generated using the protocol described by Kozich et al. 2013. For library preparation methods, a single PCR was performed per sample on a 96-well plate. The PCR product size was checked on a 2% agarose gel and purified using the SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA, USA) and pooled. Amplicon libraries were sequenced on Illumina MiSeq instrument using the 250-base paired-ends kit at the genomic core facility, Arizona State University.

Biochar Analysis

Biochar samples both from the initial biochar sample and from the buried biochar bags at year 1 were characterized. For proximate analysis, volatile matter was determined by weighing approximately 0.5g of oven dried soil into crucibles and putting the covered crucibles into an oven at 950°C for 6 minutes. Samples were then removed, cooled, and weighed to determine the percent volatile matter. Next the samples were put into an oven at 750°C with no crucible lids for 6 hours, removed, cooled and weighed to determine the percent ash content. The percent of fixed carbon was determined by subtraction from 100. The proximate analysis for the buried bags at year one can be found in Appendix H, showing a general decrease in the proportion of fixed C and an increase in the proportion of volatile matter. The pH and the electrical conductivity of the biochar also were measured using an Amber Science, EC meter Model 4083 (Amber Science, Inc., Eugene Oregon, USA). The pH and EC for the buried biochar bags at year one can be found in Appendix I, showing a general decrease over time. Images of the biochar were collected using a scanning electron microscope (SEM). The biochar samples were individually mounted onto aluminum stubs using conductive carbon tape and were not sputter coated due to the conductivity of the biochar. The samples were then viewed using a Hitachi S-4800 Field Emission Scanning Electron Microscope (Hitachi, Ltd., Tokyo, Japan) at an accelerating voltage of 2.0kV. Images were collected on both the initial biochar sample, on the biochar from the buried biochar bags, and biochar picked out of soil samples from year 1.

2.2.8 Soil Incubation

Sample Collection and Preparation

For the incubation experiment, the soils were collected after two sweet corn rotations and two napiergrass ratoon harvests. The corn plots at Poamoho were sampled on October 9, 2014. The corn plots at Waimanalo were sampled on November 7, 2014. The napiergrass plots and bare plots were harvested at Poamoho on December 2, 2014 while Waimanalo napiergrass and bare plots were harvested on December 11, 2014.

To sample, each plot was broken into two equal parts and a point randomly chosen along the interior crop rows or irrigation lines in the bare plots. At each point, three samples were taken (0-10cm) and homogenized. A subsample of the homogenized soil was collected, stored on dry

ice, and shipped to Arizona State University East, EC Polytechnic Campus in Mesa, Arizona for microbial analysis. The remaining soil was stored in the field on dry ice then kept in a freezer until being air-dried.

Incubation, Gas Sampling, and Analysis

Prior to incubating the soils, the soils were brought to approximate field capacity by saturating overnight followed by a 48 hr drainage period. This soil moisture weight was maintained throughout the experiment via rewetting as necessary when sampling. After the soils and biochar were at field capacity, the samples were inoculated and placed into individual 700 ml Lock n' Lock containers which were then placed in one of the three controlled environment chambers (Model 60021-1, Caron Products & Services, Inc., Marietta, OH) set at different temperatures. In total, there are eight temperatures chosen to represent a range of temperatures on Oahu (Table 2.1).

Three temperatures (23, 26, and 31°C) were chosen as the most representative of either current temperatures (23°C) or the IPCC increased projection for the tropics to for additional analyses following the 60-day incubation. To begin, the weight of the soil and the container was recorded to aid in maintaining field capacity moisture content. The Lock n' Lock lids were fitted with a septa though holes drilled in the top. The soils were allowed to equilibrate in the incubation chambers with the lids partially on for 48 hr. The first sampling occurred with the chamber lids open using a needled syringe, representing time 0. The lid was then replaced and left in the chambers for 24 hr before the next sample was taken through the septa using the needled syringe. Once the chambers are established, gas sampling occurred frequently initially and then gradually decreasing the frequency of sampling as emissions declined.

The incubations were carried out in three different rounds to cover the full eight point temperature range due to a limited number of refrigerated chambers. The initial round of incubations were at temperatures 29 and 40 °C and the chambers were sampled on days 1, 3, 6, 10, 15, 21, 27, 35, 52, and 60. The second round of incubations were at temperatures 19, 33, and 35 °C and the chambers were sampled on days 1, 4, 7, 12, 17, 23, 30, 38, 54, and 60. For the final round of incubations, the temperatures chosen were 23, 26, and 31°C and the GHG sampling occurred on days 1, 3, 6, 9, 14, 21, 29, 39, 50, and 60. Gas samples were taken and immediately injected into an evacuated Exetainer® (Labco Limited, UK) fitted with a Doubled Wadded Teflon/Silicon septa (Labco Limited, UK) for temporary storage until the samples were analyzed on a Shimadzu GC-2014 Gas Chromatograph (Shimadzu Scientific Instruments, Inc). To determine the concentrations of CO₂ after methanization, the system utilizes flame ionization detection. An electrical conductivity detector is used for N₂O analysis.

2.2.9 Temperature Sensitivity

Linear regressions were fitted to each treatment over the eight temperature points to determine the change in soil respiration or N₂O flux per °C (slope of the line). If the relationship with temperature for a treatment was not linear, non-linear relationships were examined using JMP Pro 12.

2.2.10 Labile Amendment

Incubation, Gas Sampling and Analysis

After the 60 day incubation period for the third round of temperatures, a subset of treatments was identified for additional microbial analyses. Due to sample size limitations only the Mollisol soil from the napiergrass plots with and without biochar at 23°C and 31°C were selected. These soils were identified as having the most apparent treatment difference from the cumulative emission curves for N₂O. From these soils, a subset was collected and frozen while the remaining soil in the containers were returned to the controlled environment chambers with the lids ajar and allowed to equilibrate again for 48 hrs. After the stabilization period was over, soils were amended with a glucose-D solution equivalent to the quantity of C that had been respired over the 60 day incubation. The incubation chambers were then gas sampled using the same protocol as the 60 day incubation, capped, and returned to the environment chambers. Final and initial samples were then taken for the next 10 hr, every 2.5 hr for a total of five sampling events. The process was repeated on day two. At 48 hr, the samples were destructively sampled for further microbial analysis. The gas samples were analyzed for CO₂, and N₂O.

Total Microbial Community and nosZ Harboring Bacteria Abundance

Soil samples were stored at -80°C until used for genomic DNA extraction. Total DNA was extracted from 0.25g of soil using the PowerLyzer PowerSoil DNA Isolation Kit (MoBio laboratories, Carlsbad, CA, USA). To improve desorption of DNA from clay particles in soil and cell lysis, 200-µl of Tris buffer (0.5 M Tris-HCl, pH 9) and 200-µl of phosphate buffer (0.2M Na₂HPO₄ pH 8) was added to bead tubes loaded with soil and bead solution and mixed briefly by vortexing before adding 60-µl of C1 solution. The tubes were subsequently incubated in a water bath at 70°C for 10 min followed by incubating at -80°C for 5 min. Following the pre-treatment, DNA extraction was carried out following the manufacturer's protocol. Total extracts were quantified using the Qubit dsDNA high sensitivity assay kit (Life Technologies, Carlsbad, CA, USA) and stored at -20°C.

In order to quantify the abundance of phylogenetic and functional gene markers quantitative polymerase chain reaction was performed (qPCR) in a 20-µl reaction volume using 1-µl of template DNA (~10 ng/µl), 1-µl of the forward and reverse gene-specific primers, 10-µl of the 2X PowerUp SYBR Green Master mix (Life Technologies, Carlsbad, CA, USA) and 7-µl

of sterile PCR grade water. The qPCR plasmid standards were generated from *E. coli* K-12, *P. stutzeri* str. DCP-PS1 and *A. dehalogenans* str. 2CP-C for 16S rRNA gene, *nosZ* clade I and *nosZ* clade II, respectively, and inserted into the pCR4TOPO plasmid using the TOPO-Cloning Kit (Life Technologies, Carlsbad, CA, USA). Transformants were isolated on LB media amended with 50µg/ml kanamycin. Positive isolates were grown in liquid media and plasmids were extracted using the PureLink Quick Plasmid Miniprep Kit (Life Technologies, Carlsbad, CA, USA). Plasmid standards were quantified in quadruplicate and each sample in triplicate on the QuantStudio 3 qPCR system (ThermoFisher Scientific, Carlsbad, CA, USA). Thermocycle program and gene specific primer sequences are listed Table 2.2.

2.2.11 Statistical Analysis

Statistics were performed using JMP Pro 12 (SAS Institute Inc., Cary, NC). Normality and homoscedasticity were assessed and the data transformed when necessary. Differences were considered significant at $p < 0.05$. The field soils samples collected at year 1, the soil respiration 26°C and the N₂O flux at 26°C were assessed using a three-way ANOVA with a three way interaction, and non-significant interactions terms were dropped from the model. A linear regression was performed on the soil GHG measurements for each treatment over the eight different temperatures. An ANCOVA was used to compare significant linear regressions between treatments. Two-way ANOVAs were used to assess differences in the glucose amended soil respiration and N₂O flux and in the 16S *rRNA* and *nosZ* clade I microbial data. Statistical analysis for the Illumina MiSeq sequencing was carried out using PRIMER-6 software (Clarke and Gorley 1993), raw abundances were normalized by Hellinger (square-root of relative abundance) transformation and soil cation (Ca, Na, Mg and K) and pH data were normalized with a log transformation and included as environmental variables. To understand the direction and magnitude of differences among the bacterial communities, a Bray-curtis resemblance matrix was produced for non-metric multidimensional scaling (nMDS). Significant differences between site/soil type, period of sampling and biochar treatment were tested with PERMANOVA (Anderson 2001) (999 permutations) and ANOSIM (Clarke 2006). Similarity percentages analysis (SIMPER) (Warwick, Clarke, and Suharsono 1990) was used to identify significant OTUs driving the differences among treatments. PERMDISP (Anderson 2001) was used to test for significant differences in sample dispersion. BEST (Clarke 2006) was used to determine the best match between the community assemblage and the environmental variables associated with those samples. Alpha diversity indices (Margalef's richness, Pielou's evenness and Shannon diversity) were subjected to one-way ANOVA using R-studio (RStudio Team 2015), post-hoc Tukey's HSD was computed using R's agricolae package. All differences were considered significant at a P-value of < 0.05 .

2.3 RESULTS AND DISCUSSIONS – FIELD EXPERIMENT

2.3.1 Soil chemical properties

Biochar application affected soil %C and N. The application of biochar increased ($p < 0.0001$), soil C in the biochar soils ($2.04\% \pm 0.12$) compared to the control ($1.26 \pm 0.03\%$), a 47% difference (Table 2.3). Biochar was not the only treatment that affected soil C; there was a three-way interaction between soil, crop and biochar treatment ($p = 0.0362$). Within the biochar amended Mollisol soil, the napiergrass ($2.73 \pm 0.14\%$) increased soil %C compared to the sweet corn ($1.69 \pm 0.06\%$) with a difference of 47%. The Mollisol control napiergrass soils ($1.38 \pm 0.08\%$) had a 13% difference compared to the Mollisol control sweet corn soils ($1.21 \pm 0.05\%$). Biochar increased the soil %N (Table 2.3; $p = 0.0162$) compared to the control soils. There were also differences by soil ($p = 0.0122$) with the Oxisol soil having greater %N compared to the Mollisol. Details of the statistical tests for the soil properties can be found in Appendix K.

The application of biochar to soils increased soil C as predicted, supporting the results of many other studies (Lehmann 2007a; Mukherjee, Lal, and Zimmerman 2014; Lehmann, Gaunt, and Rondon 2006). High pyrolysis temperature, such as used here, results in a very stable product with a high carbon content (Kloss et al. 2012). This is consistent with many studies with estimates of mean residence time (MRT) between 44 and 610 years for biochar produced at pyrolysis temperatures between 450 and 550°C (Fang et al. 2014) although some place MRT as much longer with approximately 4000 years (Kuzyakov, Bogomolova, and Glaser 2014). The Terra Preta of the Amazon has been shown to have increased soil C compared to surrounding soils with the carbon dated between 500-6000 years old (Atkinson, Fitzgerald, and Hipps 2010).

The differences in %C between napiergrass and sweet corn were not as strong as predicted. Increases between napiergrass and sweet corn occurred in the control Mollisol soils and the Oxisol biochar soils, however significant differences in %C between crop system only occurred in the Mollisol biochar. Previous work in the Mollisol indicated that the use of a ratoon harvest with a perennial bioenergy crop results in an increase of soil C in a short time period (Sumiyoshi et al, unpublished). The lack of aggregate disruption with the no tillage (Six, Elliott, and Paustian 2000) works to protect the soil organic matter thereby increasing soil C (Ramnarine et al. 2015). Additionally, the extensive belowground root systems of the perennial grass can extend root growth over a meter in depth and have greater root biomass compared to corn crops (Anderson-Teixeira et al. 2013). This provides belowground C with allocation of C to root systems (Roper et al. 2013) which are important for soil C sequestration (Kell 2012). Biochar effectively stabilized organic C via pyrolysis especially in conjunction with a perennial no-tillage ratoon harvested bioenergy crop. Biochar also increased soil %N, which has been attributed to a reduction in N leaching (Güereña et al. 2013) or an increase in BNF (Rondon et al. 2007). The soil difference in %N may be due to the protection of organic matter within soil aggregates (Six et al. 2002) in the Oxisol (Fabrizzi et al. 2008).

Biochar had no effect on exchangeable Ca, Na, Mg, K or pH (Table 2.3). The exchangeable Ca had a two-way interaction between soil and crop ($p=0.0021$), with the Mollisol napiergrass and sweet corn having the greatest Ca, followed by Oxisol napiergrass, then Oxisol sweet corn with the least Ca. The Na was greater in the Mollisol compared to the Oxisol ($p<0.001$) as well as greater in the corn compared to the napiergrass ($p=0.0078$). Both Mg and K were greater in the Mollisol compared to the Oxisol ($p<0.0001$ and $p=0.0006$, respectively). For pH there was a two-way interaction between soil and crop ($p=0.0136$), with the Oxisol sweet corn being more acidic than the Oxisol napiergrass, Mollisol napiergrass, and Mollisol sweet corn.

Biochar was hypothesized to increase exchangeable nutrients, given the evidence that many biochars increase soil CEC (Liang et al. 2006; Manyà 2012). A four year study in the tropics found an increase in exchangeable Ca and Mg with biochar application (Major et al. 2010). However, biochar had no effect on exchangeable nutrients after 1 year post amendment, indicating that biochar derived-CEC developed over time. Other field studies have found that biochar had no effect on exchangeable Ca and Na and a difference in exchangeable K was not found until year three (Jones et al. 2012) or that biochar had no effect on K, Na and Ca after three years but a reapplication of biochar resulted in a significant increase in those nutrients (Quilliam et al. 2012). Over time, the positive surface charge of biochar decreases and the negative surface charge increases (Cheng and Lehmann 2009); it is possible the one year post amendment was not long enough for sufficient negative charges to form on the surface area of biochar to significantly increase soil CEC (Revell, Maguire, and Agblevor 2012). Additionally, the anaerobic digester biochar had a high pyrolysis temperature, which in general results in a lower initial negative surface charge of the biochar (Kloss et al. 2012). Also the increases in exchangeable nutrients can be attributed to an increase of soil pH with biochar addition (Jones et al. 2012; Mukherjee, Lal, and Zimmerman 2014; Major et al. 2010; Peng et al. 2011). Higher pyrolysis temperatures results in an alkaline biochar (Novak et al. 2009), which can increase the overall soil pH upon biochar incorporation into the soil. However, not all studies find a biochar liming effect (Güereña et al. 2013). The high buffering capacity of the two finely textured clay soils (Uchida and Hue 2000) as well as the application of lime to the more acidic Oxisol soil most likely resulted in the lack of a biochar liming effect. Together, the unchanged pH and exchangeable nutrients with biochar amendment may partly explain the lack of yield increases with biochar application. Additionally, biochar effects on yields have varied. A review of 16 studies found that biochar increased yields, on average, by 10% usually in acidic to neutral soils. On the other hand, in coarse to medium soil texture, biochar created from biosolids resulted in a yield decrease (Jeffery et al. 2011).

Contrary to expectations, biochar did not increase soil exchangeable nutrients, pH or crop yields in the first year following amendment. This makes the use of biochar as a soil amendment, at least in these two agricultural systems, less beneficial in the short-term than many studies report. Barring implementation of a carbon market for Hawaii that operates with prices in line

with the upper estimates of the social cost of carbon (Greenstone, Kopits, and Wolverton 2013; Ackerman and Stanton 2012), a one-time application of biochar would not result in increased profit for most of our experimental systems (Meulemans et al, unpublished). For farmers who are hoping to see a short-term improvement in the soil quality and yields, biochar may not be a desirable amendment especially given the cost associated with biochar production and procurement. Nonetheless, in line with previous field experimental results, other benefits of biochar amendment may be forthcoming over time (Major et al. 2010; Jones et al. 2012).

2.3.2 Soil biological properties

Total soil bacterial community composition (Figure 2.4) was different between the Oxisol and the Mollisol (PERMANOVA; $p=0.001$) and had differences in dispersion (PERMDISP; $p=0.001$). There was also a difference between sweet corn and napiergrass (PERMANOVA; $p=0.001$) although no differences in dispersion (PERMDISP; $p=0.0634$). Biochar also resulted in a difference in microbial community composition (PERMANOVA; $p=0.001$) although it did not impact dispersion (PERMDISP; $p=0.059$). More specifically, within the Oxisol napiergrass, biochar changed the microbial community (PERMANOVA; $p=0.001$) and changed the dispersion (PERMDISP; $p=0.001$). This was also true in the Oxisol sweet corn (PERMANOVA; $p=0.026$; PERMDISP; $p=0.01$). Biochar had no effect in the Mollisol napiergrass (PERMANOVA; $p=0.158$). In the Mollisol sweet corn, biochar resulted in a difference (PERMANOVA; $p=0.010$) but did not change dispersion (PERMDISP; $p=0.085$).

Differences in soil properties between the two sites such as soil fertility can result in different microbial communities (Yao et al. 2000). Differences in crops and tillage management can have different associated communities (Lupwayi, Rice, and Clayton 1998; Drijber et al. 2000). For instance, no tillage increases gram positive bacteria, mycorrhizae and actinomycetes (Mbuthia et al. 2015). Biochar can also impact the community by increasing bacterial families that reduce nitrate, increasing communities that can degrade stable soil C and reduce plant pathogens (Anderson et al. 2011).

Microbial community shifts may indicate a more sensitive measurement of change (Sradnick et al. 2013) compared to changes in soil properties. It also may be that given more time, the biochar may influence the soil properties as well as the microbial community composition. A shift in microbial communities with biochar also supports the idea of designer biochar; specific biochar may be applied in certain systems to ameliorate problems. For instance, a soil with limited N may benefit from a biochar that promotes biological nitrogen fixation. However, further research is needed to isolate and identify the characteristics of biochar and how they influence soil physical, chemical, and biological properties.

2.3.3 Biochar Aging

The SEM images showed differences between the initial sample, the biochar from the buried bags, and biochar removed from the soil environment at year 1. The initial biochar sample image showed intact plant cell wall xylem structures with a smooth face of the structure in Fig 2.5A. After a year in the soil however, the ends of biochar pieces became much rougher (Fig 2.5B), the exterior became coated along the outer surface in soil particles (Fig 2.5C) that were also found inside the plant cell walls (Fig 2.5D). There were also changes from the initial sample (Fig 2.6A) with smooth upper surfaces and unobstructed pits to the biochar removed from the bags, which showed sharp, broken edges along the surface (Fig 2.6B). This same breakage was apparent in the biochar removed from soil, but the surface had become coated in soil particles which clogged the xylem holes (Fig 2.6C).

As hypothesized, biochar was not completely inert and began to decompose in the soil environment. The fractured biochar pieces most likely were not chemically different from the parent biochar material, rather physically degraded due to weathering in the soil environment (Spokas et al. 2014). The SEM images also indicated the biochar pieces had accumulated particulate material, possibly alumino-silicates which may work to stabilize the biochar from further degradation (Spokas et al. 2014; Brodowski et al. 2005). Previous work has demonstrated that biochar-clay composites created by combining biochar with montmorillonite and kaolinite clay particles resulted in a stable product where soil particles formed a thin coating over the biochar surface (Yao et al. 2014), similar to observation we present in the SEM images.

The SEM images demonstrate that biochar had begun to break down over the year in the field both in the bags and to a greater extent in the soil environment. This showed that while biochar is stable, it is not completely inert. Additionally, soil particles had begun coating the biochar pieces both on the exterior and interior. This could reduce the available surface area on the biochar by blocking access to the interior structures, reducing the ability of cations to exchange on negatively charged sites, even if clay particles on the surface of biochar can still exchange nutrients. The stability of biochar in soils may be a balance between weathering and protection with soil stabilizing mechanisms and not necessarily just inherent chemical recalcitrance.

2.3.4 Soil Respiration

Biochar consistently lowered CO₂ emissions compared to the control for all treatments except the Mollisol napiergrass at 26°C; however high variability of the replicates led to no significant soil, crop, or biochar treatment effects on cumulative soil respiration per g of soil (p=0.1696) (Fig 2.7).. Soil respiration curves for the other temperatures can be found in Appendix L for the Oxisol and Appendix M for the Mollisol. The table of values are in Appendix N. As an indicator of C quality, measured as soil respiration at 26°C per g of soil C, biochar decreased labile carbon to 60% of the control. Biochar amended soils had significantly (p=0.001)

lower emissions (5.24 ± 0.88 gC-CO₂ g⁻¹ soil C) compared to the control soils (9.74 ± 1.23 gC-CO₂ g⁻¹ soil C) (Fig. 2.8). Values for respiration per g soil C can be found in Appendix O. Details of the statistical tests can be found in Appendix P.

Soil respiration at 26°C showed no treatment effects, which was consistent with the biochar effect hypothesis, but contrary to the predictions for soil and crop type. The addition of C-rich biochar, while it increased soil C, did not increase soil respiration as expected. While biochar can have a small amount of initial labile biochar-derived C that is rapidly lost following application (Smith, Collins, and Bailey 2010), the initial flush of labile biochar-C was most likely released soon after application in the field. In addition, the lack of respiration in the biochar and sand treatment was consistent with many studies that found low decomposition rates among a range of biochar types, which results in an average soil mean residence time of 1000 years (Kuzyakov et al. 2009). Other incubation studies have also found a lack of biochar effect on soil respiration. In a three-year incubation, less than 4.5% of added ¹⁴C labeled biochar was lost as ¹⁴CO₂ flux (Kuzyakov et al. 2009). Biochar did not increase CO₂ flux during wheat or rice cropping in an upland soil (Wang et al. 2012) or in a 30 day incubation at 20°C with biochar amended soils (0.5% by weight) (Galvez et al. 2012). Given the wood-based feedstock (Kuzyakov et al. 2009) and the high pyrolysis temperature of the anaerobic digester biochar, the biochar used in this study should have high stability and would therefore be resistant to both physical and chemical degradation. The highest day 60 cumulative respiration from the biochar amended sand was only 4.74 ± 8.50 μg C-CO₂ g⁻¹ soil.

Biochar tends to not alter soil microbial biomass or enzymatic activity (β-glucosidase, alkaline phosphatase, and leucine aminopeptidase) (Galvez et al. 2012) and is not correlated with soil enzyme activity when used in conjunction with organic and inorganic fertilizers (Shao and Zheng 2014). However, biochar may promote soil microbial communities which are more adept at degrading stable soil C (Anderson et al. 2011), possibly by promoting the growth of gram positive bacteria which can utilize biochar-C (Farrell et al. 2013) and thereby promote decomposition of biochar as well as native, stable soil C, at least in the short-term (Luo et al. 2011). Biochar produced at higher pyrolysis temperatures (>500°C) showed negative priming whereas lower temperature biochars promoted positive priming of native soil C (Zimmerman, Gao, and Ahn 2011; Fang, Singh, and Singh 2015). In our experiments, the reduction in respiration in the biochar amended soils suggests that the high pyrolysis temperature of the biochar inhibited any priming of native soil C. However, it is also possible that biochar priming effects may have occurred during the first year in the field trial and therefore was not apparent in the incubation study.

Overall, the application of biochar decreased the carbon bioavailability as predicted by reduced soil respiration per gram of soil C. Biochar-C has a very low mineralization rate, therefore biochar itself is most likely not a source of C for most soil microorganisms (Kuzyakov et al. 2009). However, for the small amount of biochar-C that does degrade, the high C/N ratio

requires that soil microbial communities scavenge for N which can reduce the amount of N available for plant growth and inhibit the decomposition of other soil organic matter from more labile sources (Brady and Weil 2008, 370).

The absence of a cropping system difference on soil respiration was unexpected. The napiergrass plots were ratoon harvested and experienced no tillage during the first year of the field experiment whereas the sweet corn plots were tilled with planting preparation approximately 72 days prior to soil sampling. Previous research shows that the use of no-tillage cultivation decreases soil respiration (Yonemura et al. 2013; Marquina et al. 2014). In a long-term study with switchgrass and no-tilled corn, the switchgrass soils were found to have higher aggregate stability (Stewart et al. 2015), indicating that the labile C pool may be protected within soil aggregates (Six et al. 2002) and therefore reduce soil respiration. However, the only %C differences between napiergrass and sweet corn were in the Mollisol napiergrass biochar plots. It is possible that effects of no-tillage with a perennial grass on soil C may be found on a longer-term scale. Additionally, %C is not necessarily indicative of the labile C pool. Changes in total %C are much less sensitive to alteration than the labile C pool (Blair, Lefroy, and Lisle 1995). A more sensitive measurement of soil C pool change such as hot water extractable carbon may have provided more insight into soil respiration differences as a result of cropping system (Ghani, Dexter, and Perrott 2003). Additionally, it may be that 1 year is not sufficient time to see a difference by cropping system in these two soils.

Also unexpected was the lack of respiration differences between the two soils, which was contrary to the hypothesis. The Oxisol is a 1:1 clay dominated soil whereas the Mollisol is a mixed mineralogy clay with both smectite and halloysite. The lack of swelling-type clays in the Oxisol should protect organic matter more so than the Mollisol (Deenik 2006). It is unclear why neither tillage management or soil type affected soil respiration. It may be that the overall disturbance associated with soil sampling, homogenization and the preparation of the soil for incubation might have overwhelmed any treatment differences. *In situ* measurements may be different. Additionally, the small sample size and large heterogeneity in the field may make detecting small differences difficult.

The application of biochar to soils resulted in a pool of C that was not respired. This indicates that biochar will increase soil C and the soil will retain that new pool of stable C. This makes biochar a viable long-term C sequestration option in these two agricultural systems in Hawaii, regardless of soil type, crop or management system.

2.3.5 N₂O Flux

The emissions of N₂O at 26°C were not affected by biochar amendment or crop, only soil type had a significant effect ($p < 0.0001$). The N₂O flux was 107% different between the soils, with the Mollisol ($0.056 \pm 0.011 \mu\text{g N-N}_2\text{O g}^{-1} \text{ soil}$) having greater emission compared to the Oxisol ($0.017 \pm 0.005 \mu\text{g N-N}_2\text{O g}^{-1} \text{ soil}$). Soil N₂O curves for the other temperature can be

found in Appendix Q for the Oxisol and Appendix R for the Mollisol while the table of values are in Appendix S. Details of the statistical tests can be found in Appendix T.

Biochar had no significant effect on soil N₂O fluxes, which was contrary to the fluxes reported in much of the literature; a number of studies have found a decrease of soil N₂O fluxes with biochar addition (Kammann et al. 2012; Cayuela et al. 2013; Van Zwieten et al. 2014). One study suggested the decrease in N₂O flux was due to an increase in soil pH, which can reduce the N₂O/N₂ ratio (Kammann et al. 2012). Thus, the lack of a pH effect with biochar therefore may begin to explain the lack of N₂O flux reduction with biochar in these systems. A study examining 15 different soils with biochar found that most had a decreased N₂O/N₂ ratio and reduced the total amount of N that was denitrified (Cayuela et al. 2013). Specifically, with biochar, fine textured soils were found to significantly reduce the N₂O/N₂ ratio whereas overall reduction of N₂O emissions was strongly correlated with initial soil NO₃⁻ concentrations (Cayuela et al. 2013). It may be that after one year for the napiergrass and months for the sweet corn without fertilization, the available NO₃⁻ concentrations may not be significantly different between treatments. Additionally the authors suggest that biochar may act as an electron shuttle by transferring electrons to aid in the reduction of N₂O to N₂ for the process of denitrification. In another study comparing several soil types, the largest decrease in N₂O flux was found in the soil with the highest *nosZ* gene abundance. However biochar treatment did not have a significantly different abundance of *nosZ* compared to the control, although biochar did reduce N₂O flux (Van Zwieten et al. 2014). It is possible that while the overall abundance of *nosZ* harboring bacteria did not change, gene expression may have been upregulated in biochar amended soils (Van Zwieten et al. 2014). Alternatively, the N₂O produced in these systems may not be solely due to denitrification but rather may be due to nitrification, which can occur under conditions with sufficient oxygen (Khalil, Mary, and Renault 2004).

The soil N₂O fluxes were not affected by cropping system, contrary to the hypothesis where the no-tillage would increase protection of soil organic matter. However, differences between no-tillage and conventional tillage and the effects on N₂O vary. A review showed that within the first decade, N₂O fluxes are higher in no-tillage systems but then become similar in dry climates while in humid climates the N₂O fluxes in the no-tillage systems are lower compared to conventional tillage (Six et al. 2004). In a subtropical soil, over a one year field trial there were no consistent effects of tillage on soil N₂O fluxes. Instead it is suggested that cropping system and soil type in conjunction with available N more strongly controlled N₂O fluxes (Bayer et al. 2015). Another study in the tropics found that no tillage practices resulted in an increase of N₂O flux in a corn cropping system due to anaerobic conditions which promotes the production of N₂O (Marquina et al. 2014). Soil N₂O fluxes are complex and vary by system, thus tillage management differences are not strong enough to solely dictate N₂O fluxes. However, soil type is a strong enough driver to predict N₂O fluxes as hypothesized. The Oxisol had significantly lower N₂O fluxes compared to the Mollisol. The Mollisol soil has mixed mineralogy, including smectite clays with shrink swell properties and a lower clay content compared to the kaolinitic

Oxisol soil. In addition, the Mollisol has less protected soil organic matter which results in an increase of N mineralization compared to the Oxisol soil (Deenik, 2006).

2.3.6 Conclusions

The field trial indicated that biochar has the potential to improve soil C sequestration, though biochar was not completely inert, so eventually this C will be respired back into the atmosphere. Biochar had no effect on exchangeable base cations, soil pH, or on crop yields for either the napiergrass or the sweet corn. There were shifts within the total microbial community composition with biochar for the Oxisol napiergrass, which indicated that more sensitive measurements of change did see differences with biochar application after 1 year. The application of biochar also did not alter soil GHG flux, so while it may not be reducing GHG flux, it is at least not contributing to further increases in CO₂ or N₂O in the atmosphere. It is possible that more changes in soil properties may be found in a longer-term study. However, this was only a point in time assessment of the impacts of biochar at in the short-term. Given that biochar is being proposed as a way to mitigate climate change, it is important to establish how biochar will alter soil GHG flux and microbial community structure-function as temperatures increase.

2.4 RESULTS AND DISCUSSIONS –TEMPERATURE GRADIENT INCUBATION

2.4.1 Soil Respiration

Within the Oxisol napiergrass, the control soils had a positive, linear relationship of CO₂ respiration with temperature ($p < 0.01$; Fig 2.10A) with a $4.05 \mu\text{g C-CO}_2 \text{ g}^{-1}$ soil increase per °C. The effects of temperature on biochar soil respiration in the napiergrass, however, were not significant. Within the sweet corn (Fig 2.10B), both biochar and the control soils had a significant increase in respiration with increasing temperatures ($p < 0.01$ and $p = 0.017$, respectively) with biochar respiration increasing by $5.64 \mu\text{g } ^\circ\text{C}^{-1}$ and the control respiration increasing by $3.48 \mu\text{g } ^\circ\text{C}^{-1}$. Between the biochar and the control however, there were no differences by treatment ($p = 0.1020$).

Respiration in the Mollisol napiergrass (Fig 2.10C) had positive, linear relationships with temperature for both biochar ($p < 0.01$) and control ($p < 0.01$) soils with increases of 8.49 and $5.81 \mu\text{g C-CO}_2 \text{ g}^{-1}$ soil increase per °C, respectively. However, no biochar treatment differences were found ($p = 0.3320$). Within the Mollisol sweet corn soils (Fig 2.10D), only the biochar amended had a significant relationship with temperature ($p < 0.01$), increasing by $7.51 \mu\text{g C-CO}_2 \text{ g}^{-1}$ soil increase per °C.

However, within the Oxisol napiergrass biochar, there was a relationship by temperature when divided into a low temperature range (19-29°C) and a high temperature range (31-40°C) (Fig. 2.11A). The lower temperature range increased by $9.91 \mu\text{g C-CO}_2 \text{ g}^{-1}$ soil increase per °C

while the high temperature range increased by 13.25 ($p=0.01$ and $p<0.001$, respectively). The Mollisol sweet corn control (Fig 2.11 B), when split into the same temperature ranges, exhibited a significant relationship ($p<0.0001$) only at the lower temperature range, with an increase of $12.96 \mu\text{g C-CO}_2 \text{ g}^{-1}$ soil increase per $^\circ\text{C}$.

The treatments with a significant relationship with temperature across the full temperature range from Fig 2.10 were analyzed for biochar treatment effect. Biochar almost doubled ($p=0.017$) the temperature sensitivity of the soil respiration (Fig 2.12) with an increase of 47% between the slopes of biochar (7.16 ± 0.73) and the control (4.44 ± 0.75).

As expected, soil respiration increased with increasing temperature. A meta-analysis of 50 ecosystem warming sites indicated that a 2°C increase resulted an initial respiration increase of 12%, (Wang et al. 2014). This increase is due to an acceleration of enzyme kinetics as well as an increase in the microbial turnover rate at higher temperatures (Hagerty et al. 2014). The higher temperatures work to lower the activation energy needed for chemical reactions to occur (Davidson and Janssens 2006). For most of the treatments, the effect of temperature was linear, indicating within the $19\text{-}40^\circ\text{C}$ there were no threshold effects.

However, the Oxisol napiergrass biochar and the Mollisol sweet corn control soils that did not show a constant linear relationship. However in the Oxisol napiergrass biochar, when separated out into two separate temperature ranges, the relationship between soil respiration and temperature became significant; this could indicate that there was a shift in the microbial community structure due to temperature (Rousk, Frey, and Bååth 2012) somewhere between 29 and 31°C . The *16S rRNA* derived bacterial community composition of the year one soils (Fig 2.4) showed that for the Oxisol napiergrass, the control soils were more widely dispersed whereas the biochar amended napiergrass soils were more clustered, indicating the community is more homogenous (PERMDISP; $p=0.001$). The decrease in microbial community dispersion could result in a decrease of functional redundancy in the community making it less resistant to stressors, such as an increase of temperature (Brady and Weil 2008, 324). Therefore, the decrease in respiration between 29 and 31°C may be due to a change to a community dominated by different bacteria. The Mollisol sweet corn control soils also did not have a constant linear increase in respiration over the eight temperature points. Splitting the temperature range again into two revealed only the lower temperature range had a significant relationship. This again could indicate a shift in the composition of the microbial community. The mechanisms behind lack of temperature effect on soil respiration between 31 and 40°C are less clear.

Contrary to the initial hypothesis, biochar increased respiration as temperatures increased. Biochar itself may be both physically and chemically breaking down or creating conditions favorable to microbial communities which are adept at degrading more stable C (Bamminger et al. 2014). Thus, indigenous stable soil C may be decomposing as well. Quasi-stable substrates such as biochar require a high activation energy for reactions to occur and are therefore more

sensitive to increases in temperature (Davidson and Janssens 2006), which may aid in the microbial degradation of biochar at higher temperatures. Biochar also has a high C/N ratio, soils with high C/N ratios were found to have increased temperature sensitivity of soil respiration (Karhu et al. 2014b).

In these two agricultural soils, increasing temperatures resulted in a steady increase of soil respiration. At least up until 40°C, there did not appear to be a threshold effect with temperature. This is promising in that there will not be exponential increases with soil respiration with temperatures increases. However, rather than reducing the temperature sensitivity of soil respiration with biochar, biochar increased the temperature sensitivity of soil respiration. With projected increases in temperature over time, the increased C sequestration benefit of biochar may be offset by an increase in soil respiration of either the biochar C or native soil C.

2.4.2 N₂O Flux

The N₂O flux in the Oxisol soil with napiergrass (Fig 2.13A) and with sweet corn (Fig 2.13B) showed no significant relationship with temperature increases with or without biochar amendment. Within the Mollisol napiergrass soil, only the napiergrass biochar (Fig 2.12C) had a significant linear relationship with N₂O flux and temperature ($p=0.0026$), with a 0.0066 in $\mu\text{g N-N}_2\text{O g}^{-1}$ soil per increase in °C. The Mollisol sweet corn had no temperature effect or biochar effect (Fig 2.13D).

Contrary to the hypothesis that N₂O flux would increase with increasing temperature, most treatments were temperature insensitive for N₂O flux. While there is evidence that N₂O flux from soils co-varies with temperature (Burzaco, Smith, and Vyn 2013), other studies suggest that the initial available N concentration played a stronger role than temperature in denitrification (Elefsiniotis and Li 2006). Discrepancies may arise due to different temperature sensitivities of reactions within the overall N cycle. One study found that N₂O fluxes were more sensitive to temperature ($Q_{10} = 2$), compared to N₂ ($Q_{10} = 1.4$) in unamended pasture soils using a temperature range of 19-35 °C (Phillips et al. 2015). Another found that lower temperatures (<21°C) conditions increased N₂O, possibly due to a deactivation of N₂O reductase (encoded by *nosZ*) which is more temperature sensitive compared to the enzymes associated with production of N₂O (Paudel et al. 2015). Additionally, even if certain processes within the N-cycle are affected by temperature, such as an increase in N mineralization, it may not necessarily lead to increased production of N₂O. One study found that over a ten year period of warming soil by 5°C, while rates of N mineralization increased, no difference in N₂O flux was found. However, an increase in plant biomass suggested that the mineralized N was being utilized by plants rather than being released back into the atmosphere (Melillo et al. 2002). It may be that while N₂O is being produced at an accelerated rate, the reduction of N₂O to N₂ is also accelerated, resulting in no net changes of N₂O flux.

There was one exception to the N₂O temperature insensitivity. The Mollisol napiergrass with biochar had a significant increase in N₂O production with temperature. This may be due to a combination of factors. The Mollisol has greater N mineralization compared to the Oxisol (Deenik 2006). A study with napiergrass found that the plants may receive 18-70% of their N from BNF (Morais et al. 2012) and the application of biochar can increase BNF in plants (Güereña et al. 2015), although the mechanisms are poorly understood (Mia et al. 2014). Biochar may also sorb ammonium and nitrate from the soil environment (Clough et al. 2013) which are otherwise bioavailable (Taghizadeh-Toosi et al. 2011). It is possible that as available N sources within the soil become depleted, the biochar may provide nitrates for denitrification. If N₂O response is more strongly controlled by available N concentrations than temperature, the increase in BNF as well as the sorption and later release of nitrate and ammonium may cause the increases of N₂O flux. It is also suggested that biochar can act as an “electron shuttle” by transferring electrons on the positively charged surfaces, which should aid in the reduction of N₂O (Cayuela et al. 2013). However, as biochar ages these biochar loses some of its positively charged exchange sites (Cheng, Lehmann, and Engelhard 2008). This could make it less effective at retaining nitrate and ammonium as well as electrons. Additionally, the SEM images from the biochar removed from the field showed that the surfaces both exterior and interior had begun to get coated in clay particles. This may reduce the surface area of the biochar available for bonding to cations.

The temperature insensitivity of the N₂O flux from these two agricultural systems means that even as temperatures increased, the N₂O flux did not act as a positive climate feedback. Unfortunately, biochar did not act to reduce N₂O flux as found in many studies. In the specific case of the Mollisol napiergrass however, biochar addition did increase N₂O flux with increasing temperatures. This again indicates that while biochar can initially sequester C, the impact biochar may have on climate change may become less beneficial as temperatures increases. It also reinforces the need for a careful understanding of how biochar will impact different ecosystems and processes differently.

2.4.5 Conclusions

The increase of GHG flux with increasing temperature in biochar amended soils suggest that as global temperatures increase, biochar may contribute to the exacerbation of climate change effects rather than act as a method of mitigation. However, these effects are likely variable among different soil types, climatic conditions and vegetation types and thus should be evaluated on an individual site-specific basis.

2.5 RESULTS AND DISCUSSIONS – MOLLISOL NAPIERGRASS

2.5.1 Soil respiration and total microbial abundance

Respiration results from the 60 day incubation for the Mollisol napiergrass at 23 and 31°C did not vary according to temperature or biochar treatment (Figure 2.14A). However, the total microbial abundance for those treatments was different, but only by temperature (Figure 2.14B; $p=0.0088$). The total abundance at 23°C ($4.60 \times 10^5 \pm 2.88 \times 10^4$ 16S rRNA gene copies ng^{-1} DNA) was higher than the 31°C total abundance ($3.66 \times 10^5 \pm 1.18 \times 10^4$ 16S rRNA gene copies ng^{-1} DNA), a difference of 23%.

Following amendment with glucose, after 48h there were no differences in soil respiration by temperature or biochar treatment, with an average respiration of 86.12 ± 8.60 $\mu\text{g C-CO}_2 \text{ g}^{-1}$ soil. However, the biochar amended soil at 31°C had greater respiration with a 47% difference compared to average of biochar and control soils at 23°C and the 31°C control soils (120.88 ± 24.29 and 74.53 ± 19.56 $\mu\text{g C-CO}_2 \text{ g soil}^{-1}$, respectively), although this difference was not significant. Similarly, the total microbial abundance was not different by biochar treatment or temperature with an average of $1.11 \times 10^6 \pm 1.91 \times 10^5$ 16S rRNA gene copies ng^{-1} DNA. However, although not significant, the biochar amended soil at 31°C had $1.76 \times 10^6 \pm 6.89 \times 10^5$ 16S rRNA gene copies ng^{-1} DNA whereas the average of the other three treatments was $8.96 \times 10^5 \pm 1.91 \times 10^5$ 16S rRNA gene copies ng^{-1} DNA, a difference of 65%. High variability between the field replicates contributed to the lack of significance in both soil respiration and total microbial abundance. However, between the day 60 and the labile soils, the soils amended with glucose had 2.8 times greater microbial abundance ($p=0.0009$) than the day 60 soils (Fig 2.16A)

There was no biochar effect on soil respiration for the Mollisol napiergrass at 23 and 31°C for the reasons established earlier. Unexpectedly, soil respiration did not significantly increase with increasing temperature, although most likely due to high variability between replicates. The temperature effect in the total microbial abundance however was hypothesized, with the lower temperatures having higher abundance. This suggests that increased soil respiration with temperature is due to an increase in metabolic and enzymatic reaction rates, and not due solely to increases in bacterial abundance (Hagerty et al. 2014). In this case, a larger community at 23°C is respiring the same amount as a smaller community at 31°C. The lack of biochar effect on microbial abundance was unexpected. However, one study has shown that after three years in an agricultural system, there was very little colonization of biochar by microbial communities, either on the surface or interior (Quilliam et al. 2013). This could indicate that on the short term, biochar does not immediately provide favorable conditions for colonization.

Following glucose amendment, the large pool of available C (Hopkins et al. 2014) resulted in an increase of total microbial abundance compared to the more C-depleted day 60 soils. There were no treatment differences with soil respiration, indicating that all soils had a similar response to the labile amendment. Specifically, biochar did not alter microbial

community abundance or reaction rates in soils with either more stable C or with labile C, although the biochar at 31°C may have become significantly higher on a longer time frame. However, it is possible this is only a transient effect. In a global temperature gradient experiment, the addition of sucrose to warmed soils indicate that the same amount of sucrose was then lost, the soils at a warmer temperature processed the substrate faster (Hopkins et al. 2014). However, the day 60 incubation data and the increase in temperature sensitivity of soil respiration with biochar amendment suggest the effect may be more long-term.

The comparison between the day 60 23 and 31°C soils and microbial abundance may be promising for climate change. While reaction rates appear to have increased, microbial abundance decreased with temperature. The net effect of decreased abundance but increased reaction rates may work to maintain balance in soil respiration rates, even with increasing temperatures. However, in the longer term, the microbial community may acclimate to higher temperatures, resulting in a rebound of microbial abundances as functional communities shift. The presence of biochar had no effect, so while biochar is not beneficial to mitigating climate change by reduced soil respiration, biochar is not actively contributing. However, the large, but insignificant increase in biochar amended soils with a labile input at the higher temperatures is important to consider when determining the effects of higher temperature on the temperature sensitivity of soil respiration. Given the constant influx of labile inputs from fertilizer, crop residues and root exudates, the continuous respiration increases over time will be compounded. Additionally, when considering the effect of biochar on the temperature sensitivity of the day 60 soils, it appears that biochar increases the temperature sensitivity of both labile and stable C.

2.5.2 Soil N₂O and denitrifying bacteria abundance

The day 60 soil N₂O flux for the Mollisol napiergrass at 23 and 31°C was not different by temperature or biochar treatment (Figure 2.14A) with an average flux of $0.095 \pm 0.019 \mu\text{g N-N}_2\text{O g}^{-1}$ soil. There was also no difference by temperature or biochar treatment for the *nosZ* harboring denitrifying bacteria with an average abundance of 1513.64 ± 144.08 copies ng⁻¹ DNA. While there was a 34% difference between 23°C (1255.55 ± 145.80) and 31°C (1771.73 ± 220.64), large variability between replicates resulted in a lack of significance. The proportion of denitrifying bacteria to the total microbial community abundance (Fig 2.15) increased at 31°C ($0.50 \pm 0.10\%$ of the total microbial community as assessed by 16S rRNA gene abundance) compared to 23°C ($0.30 \pm 0.00\%$ of the total microbial community), a difference of 50%. Biochar however had no effect on *nosZ*-harboring denitrifying bacteria abundance.

Soil N₂O flux exhibited no treatment differences 48h following the amendment with glucose, with an average flux of 0.03 ± 0.01 . Similarly, there were no treatment differences in *nosZ* gene abundance, with an average of 2513.13 ± 306.07 . Additionally, the ratio of *nosZ* to *16S rRNA* microbial abundance was not significant by biochar treatment or by temperature. However, comparing between day 60 and the labile amended soils, the increase in *nosZ* gene

abundance was also significant ($p=0.0245$), with a difference of 50% (Fig 2.16B). Additionally, together the day 60 and labile soils also were different by temperature as the 31°C soils had a difference of 37% compared to the 23°C (Fig 2.16C). The ratio of *nosZ* to *16S* also had a difference ($p=0.0199$) of 57% between day 60 and labile, with the day 60 soils having increased abundance compared to the labile (Fig 2.16D).

While not significant, the possible mechanisms for increased N₂O production with biochar were discussed earlier. Unexpectedly, the total abundance of *nosZ* harboring bacteria did not change with either temperature or biochar. There are however, other pathways to N₂O which could explain the increase in N₂O. Only *nosZ* clade I was examined, it may be possible *nosZ* clade II denitrifiers are contributing to N₂O production in these systems (Jones et al. 2013). Also, denitrification, which occurs under anoxic conditions, is not the only process that can result in N₂O production. Under conditions with sufficient oxygen, nitrification can also produce N₂O (Khalil, Mary, and Renault 2004). Biochar has been shown to increase soil aeration (Case et al. 2012), so nitrification may be more responsible for the flux increase in this system. Also, while there was not a change in the total abundance of *nosZ* harboring bacteria, the proportion of abundance did increase with higher temperatures. A study on temperature and the abundance and activity of denitrifying soil communities has also shown that not only does temperature increase the rates of denitrification, it also increased abundance of nitrate reducers (Braker, Schwarz, and Conrad 2010). This indicates higher temperatures may be shifting the overall microbial community composition to one more dominated by denitrifying bacteria. It is also possible that other communities associated with N₂O flux, such as nitrifiers, may also have increased activity (Zhang et al. 2014) and abundance with higher temperatures.

The lack of effect of glucose amendment on N₂O is not surprising; after the 60 day initial incubation, much of the available N has already been utilized and the glucose did not supply additional nitrogen. This indicates that within these cropping systems, mineralizable C is not a limiting factor to organic matter decomposition (Mitchell et al. 2013). The lack of a significant difference in abundance of denitrifying *nosZ* genes by temperature or biochar supports the lack of an increase with temperature or biochar in N₂O flux in the glucose amended soils. It may also be that fungal denitrification is responsible for the increase in N₂O flux (Higgins et al. 2016). Fungi degrade more stable organic matter, so the addition of a labile substrate will have little effect (Koranda et al. 2014). Unexpectedly, there was no biochar treatment effect in either N₂O flux or in *nosZ* gene abundance, which is contrary to the 60 day incubation data. However, other studies have found that biochar had little effect on *nosZ* gene abundance (Dicke et al. 2015).

To a lesser extent, the addition of glucose did increase the denitrifying bacteria community compared to the day 60 soils, again by providing an easily degradable C substrate. Biochar also had no effect on the abundance of denitrifying communities, although the impacts of biochar on other communities which can produce N₂O on not explored. When combining day 60 and labile soils *nosZ* gene abundance, the increase with higher temperature does become

higher, supporting that this system has temperature sensitive N₂O production. Interestingly however, the ratio of denitrifying bacteria to total bacteria decreases in the labile amended soils compared to the day 60 soils, suggesting that fungal denitrification may be occurring rather than bacterial.

The increase in N₂O production is not a result of a total increase in *nosZ* denitrifying bacteria, so it is important to further explore other pathways associated with N₂O production such as nitrification or through which do not harbor *nosZ* genes. Additionally, it is important to consider changes in the overall activity of the bacteria and not just total abundance. However, the expression of denitrifying genes can be confounded by short-term variability, therefore more intensive temporal sampling is necessary (Saleh-Lakha et al. 2005). Additionally is important to establish how biochar may interact with these the different communities associated with nitrification and denitrification. Biochar also prompted different responses in the day 60 soils compared to the labile amended soils. The effect of biochar was not as apparent in the labile amended N₂O soils as compared to the day 60 incubations. Additionally, while the labile amendment did increase the abundance of denitrifying bacteria, the ratio of denitrifying bacteria to the total community abundance decreased with labile amendment. This suggests that management decisions, such as maintaining the labile C pool within these systems, might work to mitigate or reduce the effects of biochar and temperature on increasing N₂O fluxes.

2.5.3 Conclusions

Biochar increases the temperature sensitivity of soil respiration for soils with both stable C and a large labile C pool. It may be possible to find a balance between stable and labile C to reduce the effects of biochar on temperature sensitivity or find soils and cropping systems where the effect of biochar on soil respiration at increases temperatures are lessened. This may also work to reduce the effects of biochar and temperature on N₂O flux, which was reduced in soils with a large labile C pool, although this may be a factor of reduced available N as well.

2.6 CONCLUSIONS

The assessment of biochar as a climate change mitigation strategy indicated it may act both as a positive and a negative feedback to climate change. It will be important to continue working towards a greater understanding of the specific aspects of biochar that prompt different responses in different ecosystems and establish sustainable management decisions to reduce the positive feedbacks to climate change. Additionally, it may be necessary to balance both the benefits and risks associated with biochar amendment and decide the best way to address climate change, either by increase soil C sequestration or reducing GHG fluxes from agricultural soils.

TABLES.

Table 2.1: The chosen incubation temperatures and justifications.

Temperature (°C)	Justification
19	The coldest average morning temperature in January/February for both sites
23	Average annual temp for Waimanalo (22°C) and Poamoho (24°C)
26	Midpoint between the average annual temperature and the warmest average temperature
29	The warmest average afternoon temperature in August/September for both sites
31	For the north Pacific, the IPCC projects an average increase of 2.3°C with values ranging from 1.5 to 3.7°C. An even increase of 2°C was chosen
33	Increase 2°C
35	Increase 2°C
40	Increase 5°C was chosen for an extreme temperature

Table 2.2: The thermocycler program and gene specific primer sequences.

Gene	Strain	Primers	Sequence	Thermocycle program
16s rRNA 466 bp	E.coli K-12	797F 341R	5'-GGA CTA CCA GGG TAT CTA ATC CTG TT-3' 5'-CCT ACG GGA GGC AGC AG-3'	95°C 3min 40cycles 95°C 45s 60°C 45s 72°C 1min 72°C 10min
nosZ clade I 267 bp	P. stutzeri DCP-PS1	nosZ I F nosZ I R	5'-CGC RAC GGC AAS AAG GTS MSS GT- 3' 5'-CAK RTG CAK SGC RTG GCA GAA-3'	95°C 3min 40cycles 95°C 24s 56°C 24s 58°C 24s 72°C 24min 72°C 7min
nosZ clade II 750 bp	A. dehalogenans 2CP-C	nosZ 2 F nosZ 2 R	5'-CTI GGI CCI YTK CAY AC-3' 5'-GCI GAR CAR AAI TCB GTR C-3'	95°C 3min 40cycles 95°C 24s 56°C 24s 58°C 24s 72°C 24min 72°C 7min

Table 2.3: The soil properties prior to the second harvest for each crop. This percent carbon (C), percent nitrogen (N), calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), and pH with standard error. Statistical difference ($p < 0.05$) by column indicated by different lowercase letters. Symbols denote statistical test: *Three way ANOVA, ^Two way ANOVA (soilxcrop), One way ANOVA with ^biochar effect, #soil effect, or <crop effect.

Soil	Crop	Treatment	*C (%)	^#N (%)	&Ca (mg kg ⁻¹)	<#Na (mg kg ⁻¹)	#Mg (mg kg ⁻¹)	#K (mg kg ⁻¹)	&pH
Oxisol	Napiergrass	Biochar	1.96±0.18 ^b	0.17±0.01	1598.78±164.76 ^b	101.50±15.71	238.19±31.35 ^b	548.28±29.91 ^b	6.75±0.17 ^a
		Control	1.20±0.27 ^e	0.15±0.03	1750.82±202.19 ^b	106.08±19.66	175.55±30.32 ^b	590.02±91.93 ^b	6.86±0.19 ^a
	Sweet Corn	Biochar	1.77±0.07 ^{bc}	0.17±0.01	1203.76±55.74 ^c	117.35±8.18	183.02±11.23 ^b	715.56±87.17 ^b	6.22±0.21 ^b
		Control	1.26±0.06 ^{de}	0.16±0.01	1212.68±45.99 ^c	110.40±21.07	177.23±27.32 ^b	644.34±51.10 ^b	6.11±0.20 ^b
Mollisol	Napiergrass	Biochar	2.73±0.14 ^a	0.16±0.00	3865.57±121.48 ^a	155.05±4.54	1420.36±36.85 ^a	933.23±119.87 ^a	6.78±0.05 ^a
		Control	1.38±0.08 ^{cde}	0.14±0.00	3925.94±50.79 ^a	155.33±4.09	1479.63±30.32 ^a	820.69±118.81 ^{ab}	6.82±0.07 ^a
	Sweet Corn	Biochar	1.69±0.06 ^{bcd}	0.15±0.00	4058.92±97.28 ^a	180.86±8.26	1489.85±22.00 ^a	1063.24±100.60 ^a	6.71±0.06 ^a
		Control	1.21±0.05 ^e	0.14±0.01	4030.82±221.83 ^a	183.16±13.18	1409.77±62.17 ^a	1206.47±295.90 ^a	6.79±0.25 ^a

^ # For %N, biochar increase %N compared to the control and the Oxisol had greater %N compared to the Mollisol
 < # For Na, the sweet corn has greater Na compared to the napiergrass and the Mollisol has more than the Oxisol

Table 2.4: Crop yields for the second harvest.

Soil	Treatment	Napiergrass	Sweet Corn
		Mg dry biomass ha ⁻¹	Marketable ear weight Mg ha ⁻¹
Oxisol	Biochar	40.77±3.53 ^b	13.88±1.34 ^a
	Control	34.37±2.15 ^b	15.36±0.71 ^a
Mollisol	Biochar	73.43±6.57 ^a	2.54±0.66 ^b
	Control	61.08±11.20 ^{ab}	2.25±0.51 ^b

FIGURES

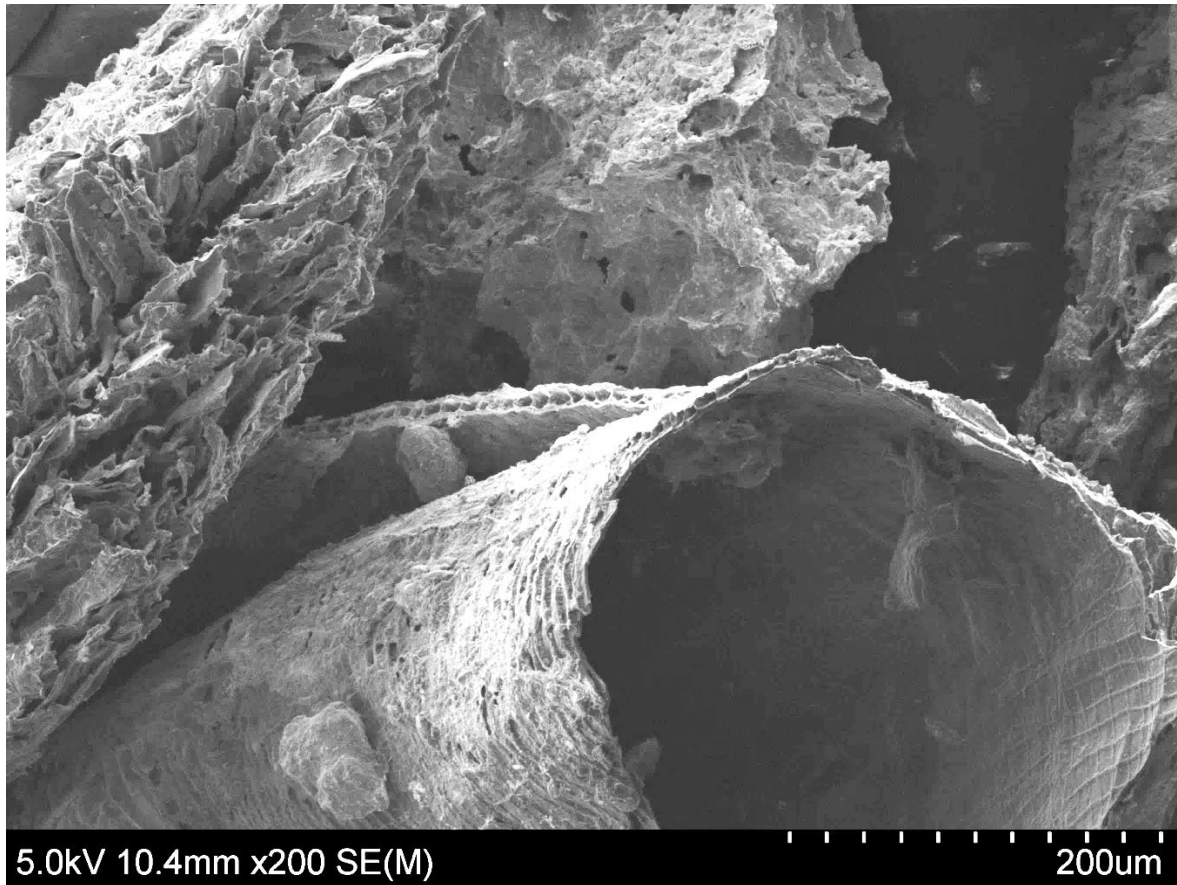


Figure 2.1: The Scanning Electron Microscope (SEM) image of the biochar showing differences between the two feedstocks after they have been pyrolyzed. On the left is the dairy sludge from the anaerobic digester with a more amorphous structure and on the right is woodchips with a more tubular, woody structure.

Poamoho

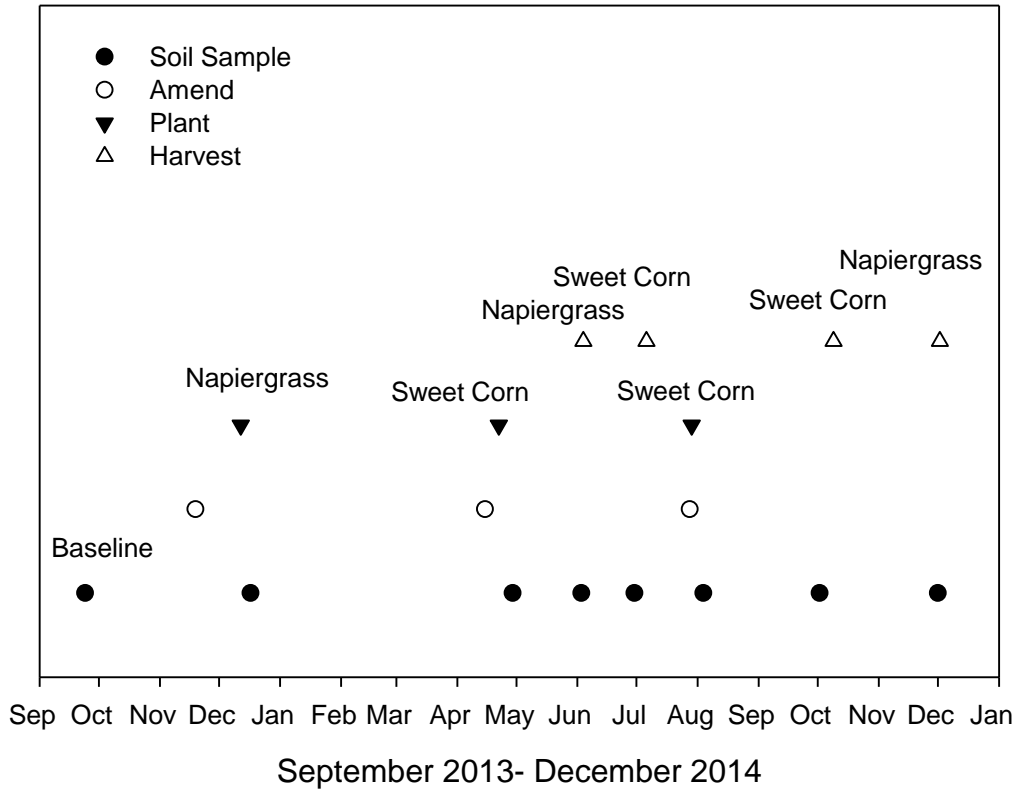


Figure 2.2: The schedule of soil sampling, amendment with biochar or fertilizer, planting of the crops and the harvests for the first year of the project for Poamoho Research Station and the Oxisol soil.

Waimanalo

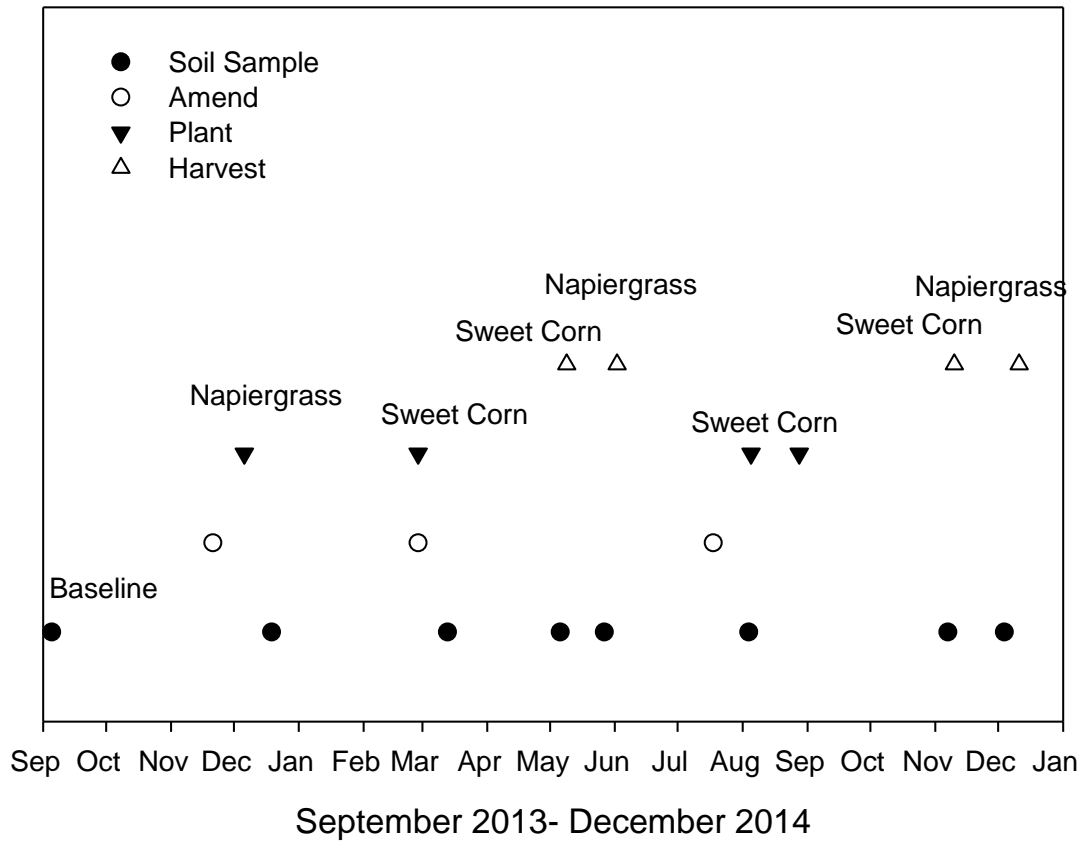


Figure 2.3: The schedule of soil sampling, amendment with biochar or fertilizer, planting of the crops and the harvests for the first year of the project for Waimanalo Research Station and the Mollisol soil.

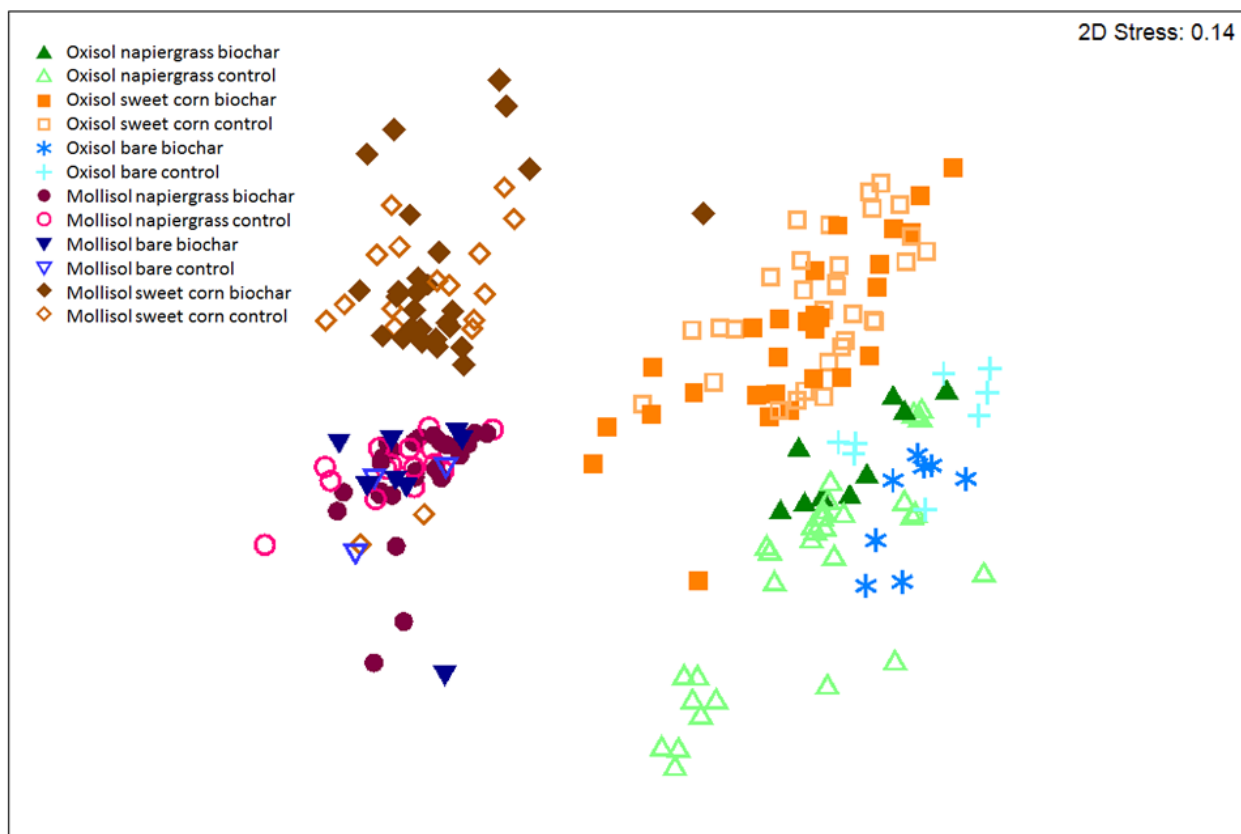


Figure 2.4: The nMDS of the bacterial community structure on the soils collected prior to the second harvest of the napiergrass and sweet corn for both the Mollisol and the Oxisol with and without biochar.

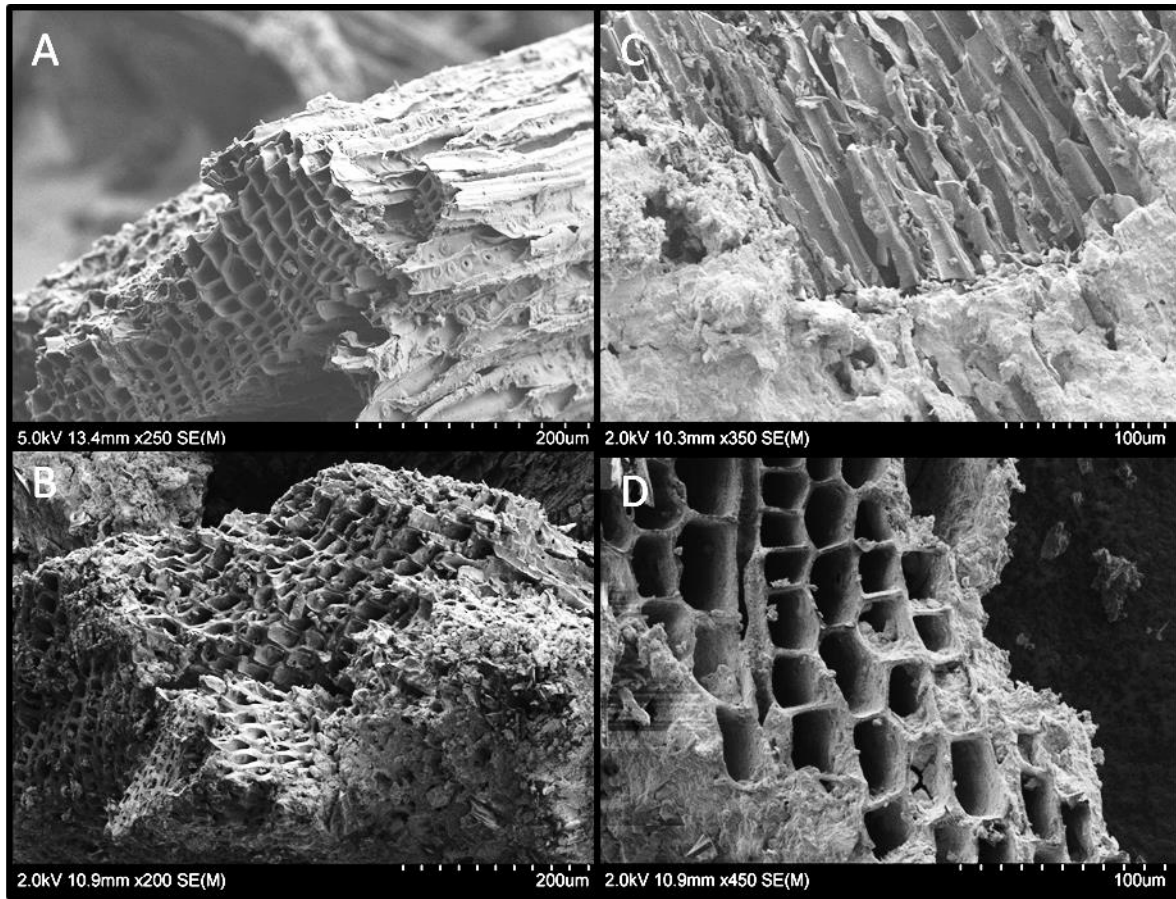


Figure 2.5: The SEM images of the (A) initial biochar sample and (B and D) biochar pieces removed from the year 1 soils from the Mollisol napiergrass plots as well as the (C) Oxisol sweet corn plots (C). The biochar in (A) show smooth plant cell walls which over time become much less smooth (B) and are coated in soil particles both along the outside of the biochar (C) and the inside (D).

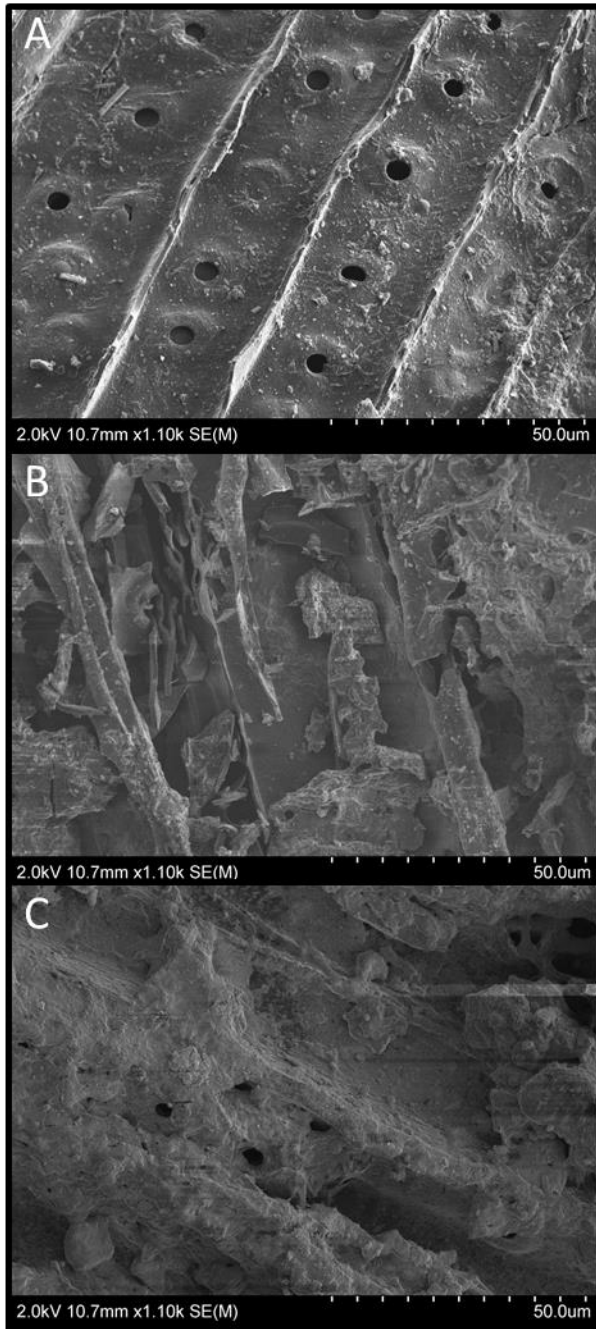


Figure 2.6: The SEM images of A) the initial biochar sample and biochar from the Oxisol napiergrass plots removed from the (B) bags and from the (C) soil. The initial samples show smooth surfaces with open xylem holes whereas the samples from the bags show fracturing of the surface. The samples from the field show both a breakdown of the smooth surface as well as xylem holes which have become coated and partially filled.

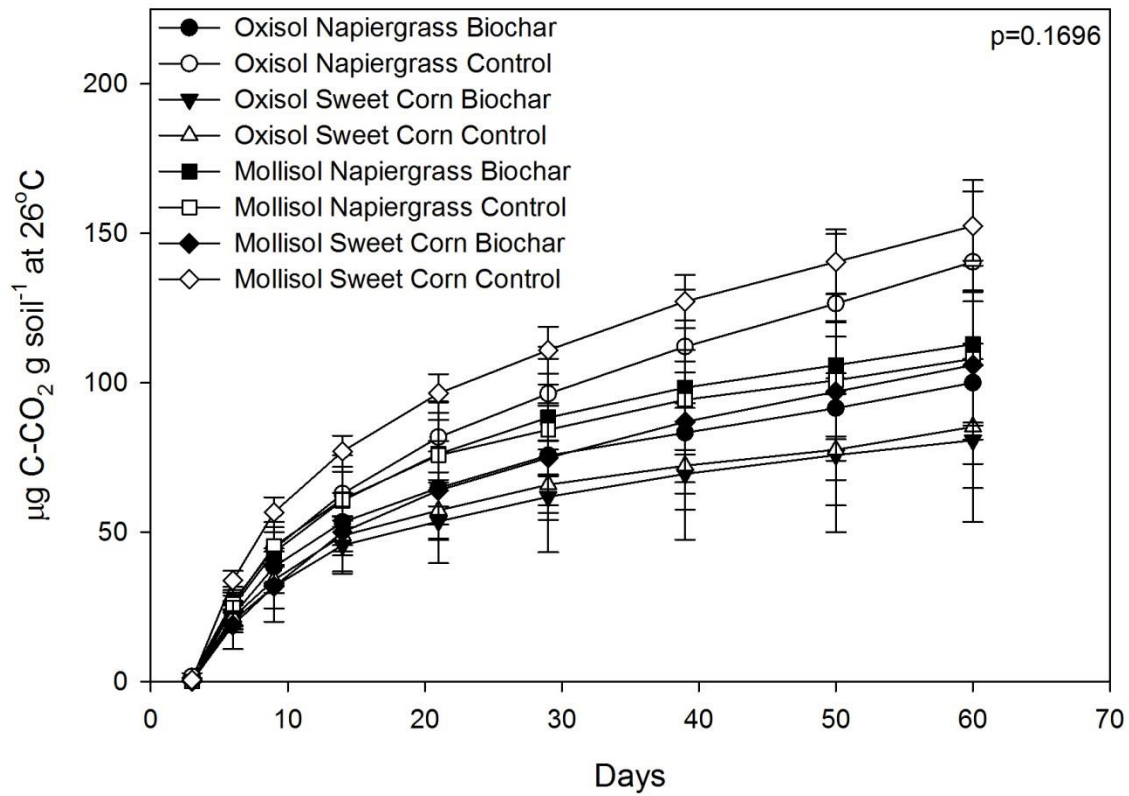


Figure 2.7: The cumulative soil CO₂ flux (µg C-CO₂ g soil⁻¹) at 26°C for both soils, crops, and biochar treatments at day 60 of the incubation. No differences by crop, soil, biochar or any interactions were found.

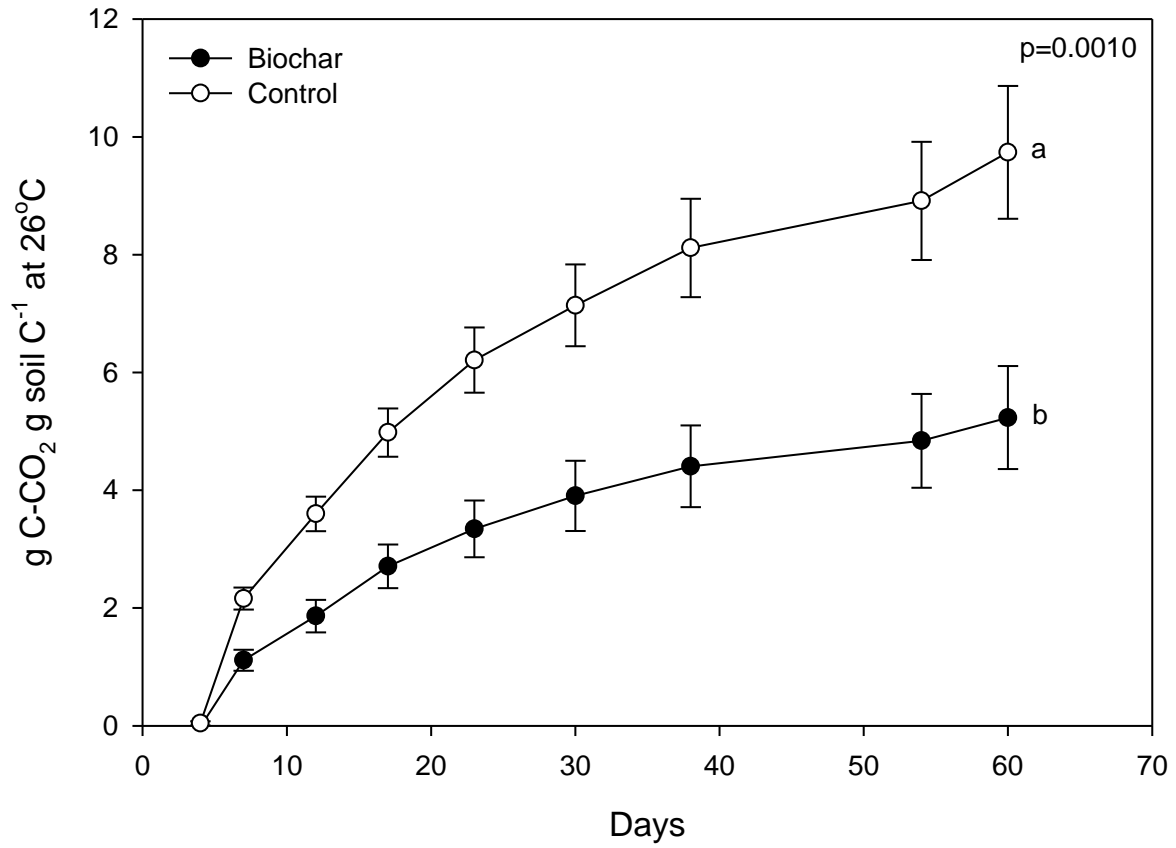


Figure 2.8: The cumulative soil CO₂ flux (g C-CO₂ g soil C⁻¹) at 26°C with and without biochar at day 60 of the incubation. Different letters denote statistical differences.

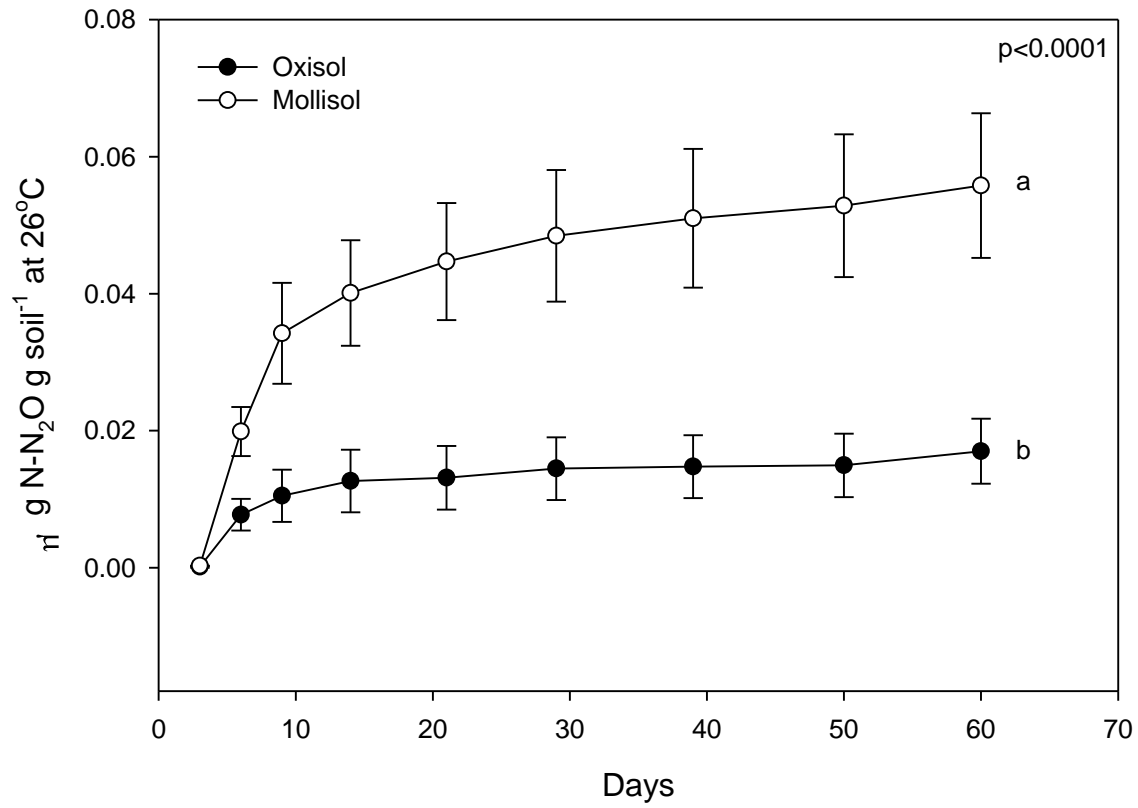


Figure 2.9: The cumulative soil N₂O flux (µg N-N₂O g soil⁻¹) at 26°C for Oxisol and Mollisol at day 60 of the incubation. Different letters denote statistical differences.

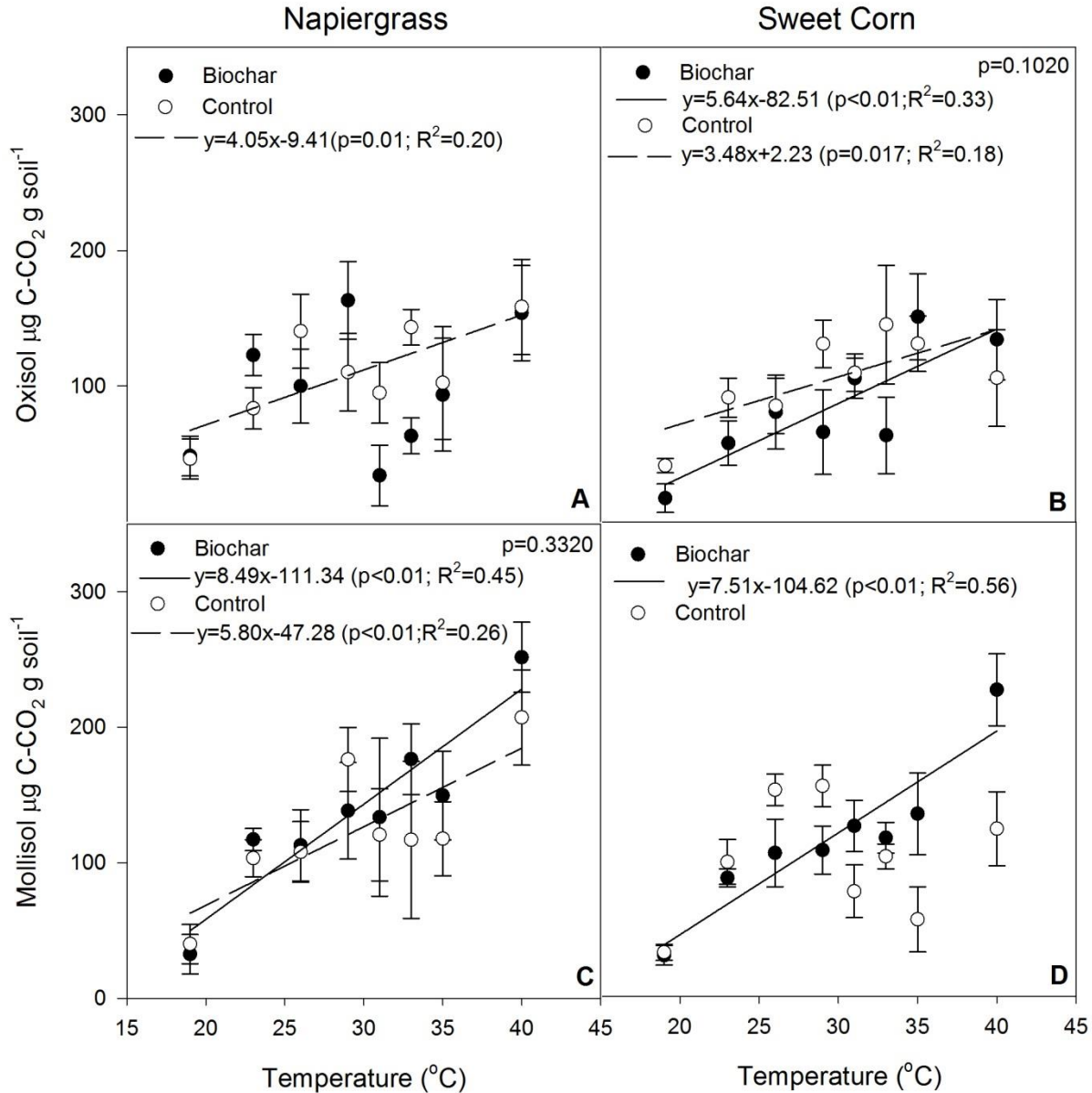


Figure 2.10: The cumulative soil respiration (µg C-CO₂ g soil⁻¹) at day 60 over the temperatures 19, 23, 26, 29, 31, 33, 35 and 40°C for A) Oxisol napiergrass, B) Oxisol sweet corn, C) Mollisol napiergrass, and D) Mollisol sweet corn. Regression lines indicate a significant relationship between soil respiration and temperature. If the pair of biochar and control soil both had a significant change with temperature an ANCOVA was used to determine if there were treatment differences.

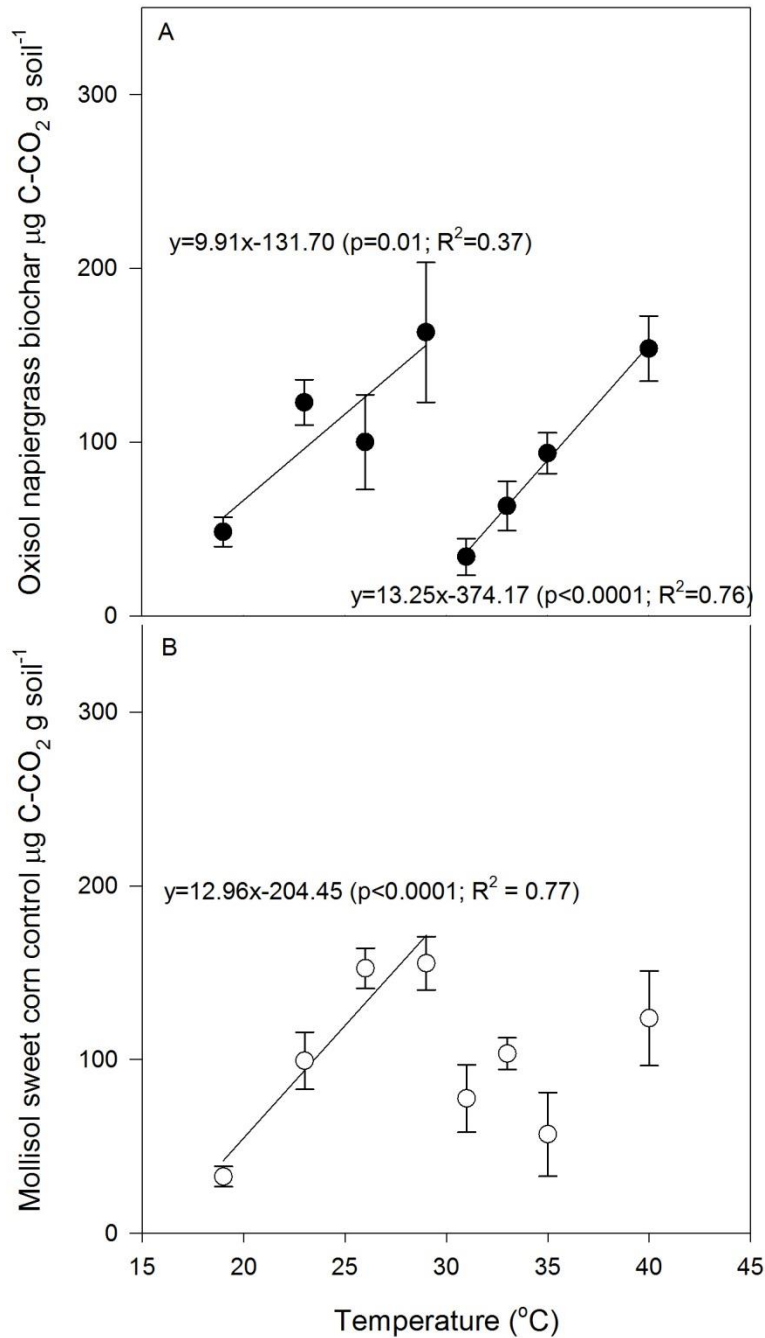


Figure 2.11: The soil respiration for A) the Oxisol napiergrass biochar and B) the Mollisol sweet corn control assessed using two different temperature shifts from 19-29 $^{\circ}\text{C}$ and 31-40 $^{\circ}\text{C}$. Lines indicate a significant change in respiration with temperature.

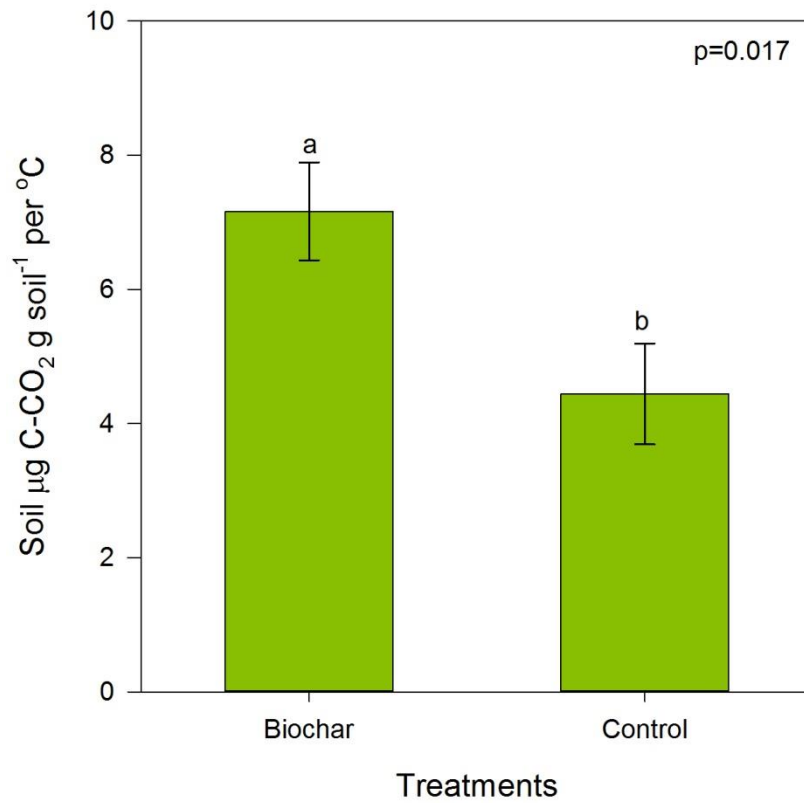


Figure 2.12: The change in soil respiration per increase of $^\circ\text{C}$ from the treatments with a significant relationship to temperature with and without biochar. Different letters denote statistical significance.

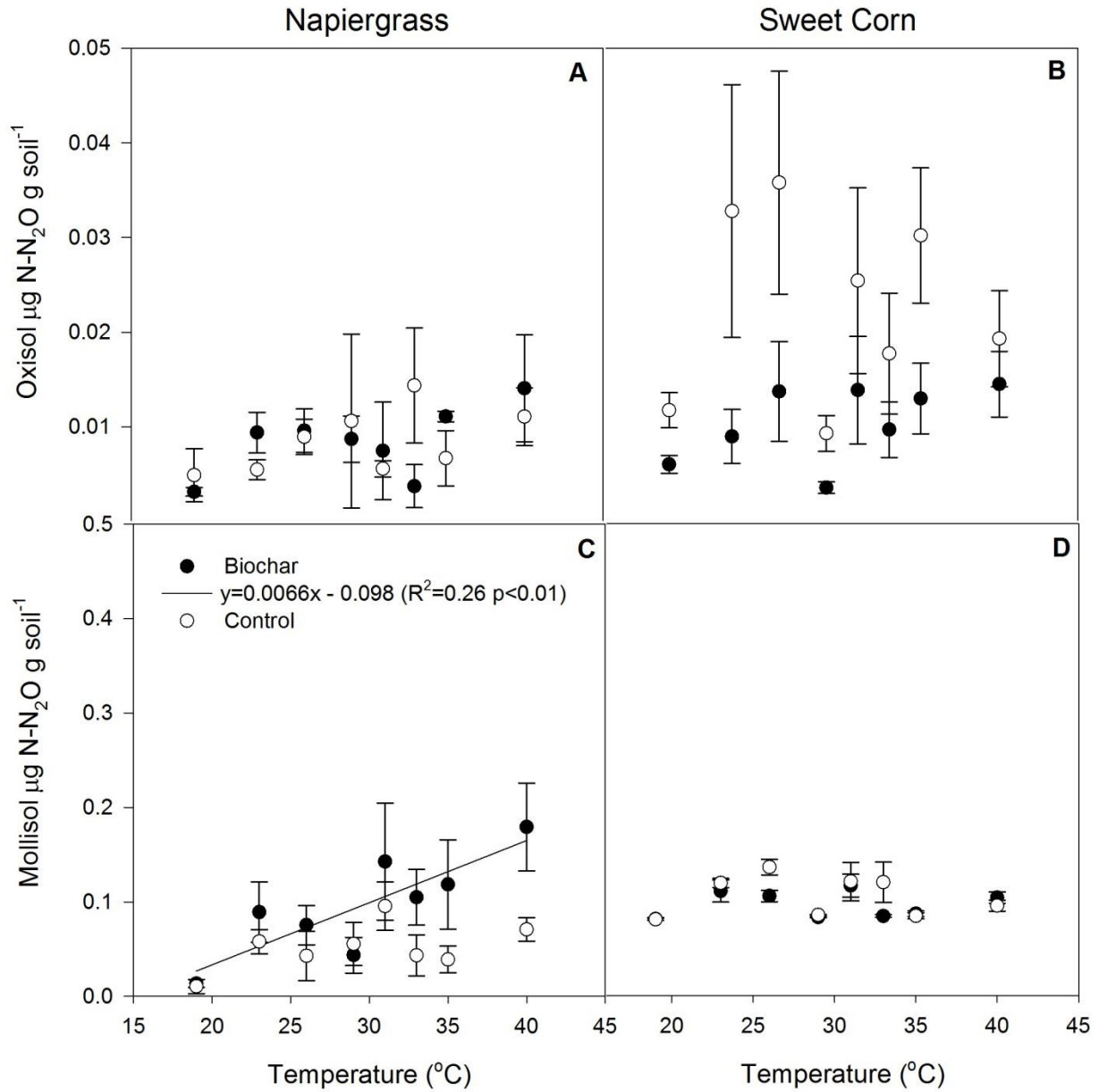


Figure 2.13: The increase of soil N₂O for the Oxisol soil in µg N-N₂O g soil⁻¹ at day 60 over the temperatures 19, 23, 26, 29, 31, 33, 35 and 40°C for A) Oxisol napiergrass, B) Oxisol sweet corn, C) Mollisol napiergrass, and D) Mollisol sweet corn. Regression lines indicate a significant relationship between soil respiration and temperature.

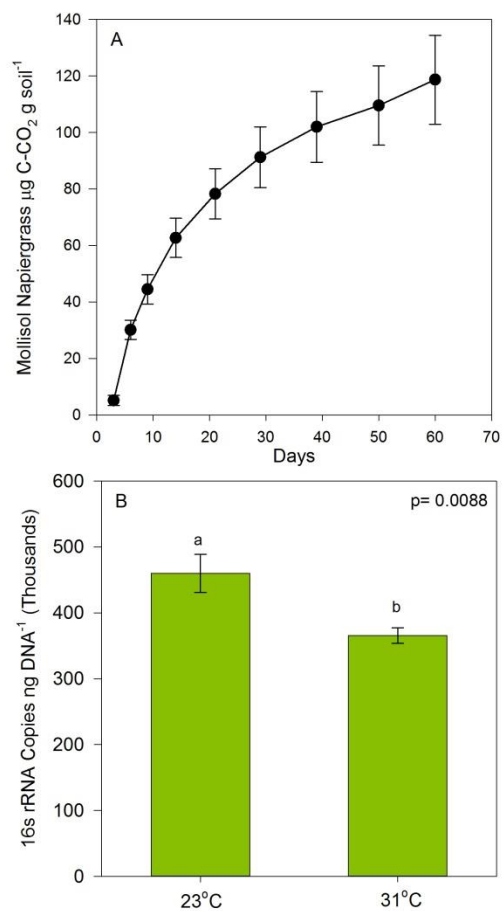


Figure 2.14: The A) cumulative emissions ($\mu\text{g C-CO}_2 \text{ g soil}^{-1}$) for the Mollisol soil for napiergrass with and without biochar at 23 and 31°C over the 60 day incubation and B) the total microbial abundance (16s rRNA gene copies ng DNA⁻¹) by temperature. Different letters denote significant differences.

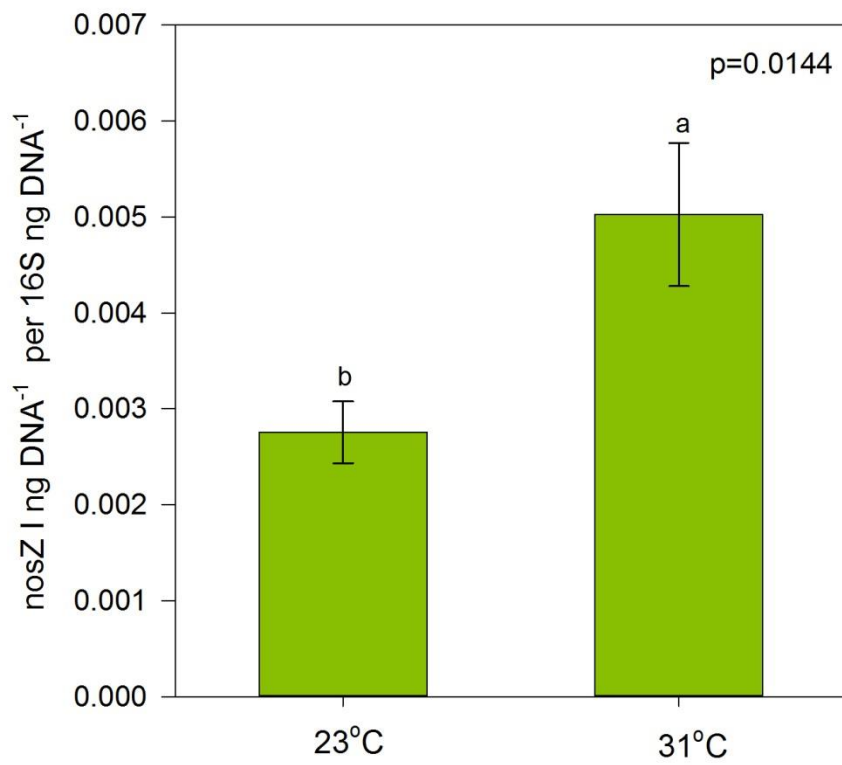


Figure 2.15: The ratio of denitrifying bacteria (*nosZ*) abundance to total microbial abundance (16S) for the day 60 incubation soils by temperature. Different letters denote significant differences.

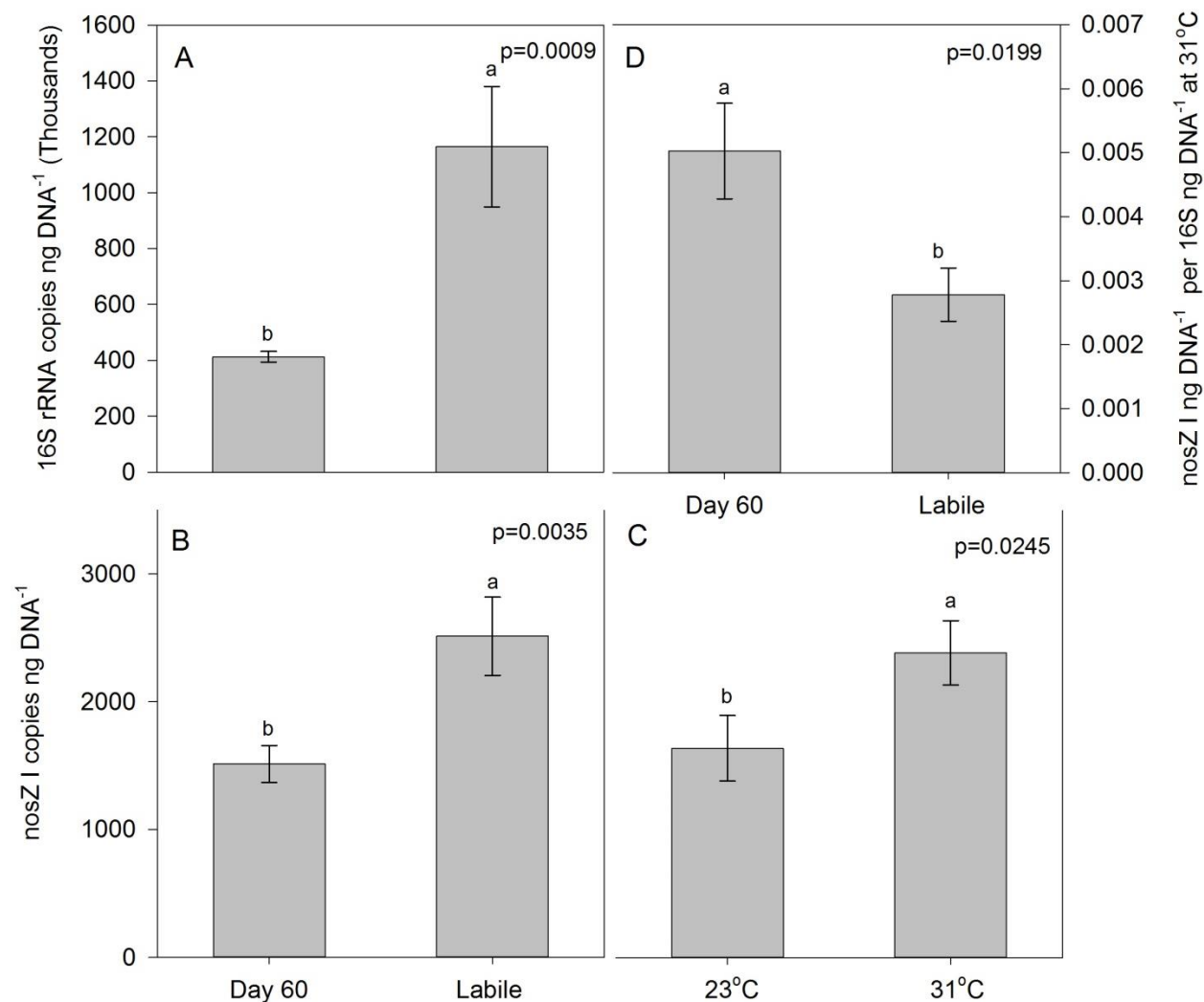


Figure 2.16: The A) total microbial abundance for the Mollisol napiergrass soils between Day 60 and the labile (glucose amended soils), B) the abundance of denitrifying bacteria (*nosZ*) between Day 60 and labile soils, C) the combined Day 60 and labile soils by temperature, and D) the ratio of denitrifying bacteria (*nosZ*) abundance to total microbial abundance (16S) for the day 60 and labile soils. Different lowercase letters denote statistical significance between treatments for each individual graph.

CHAPTER 3. PROJECT CONCLUSIONS

3.1 Project Summary and Implications

The increasing levels of atmospheric CO₂ and N₂O are shifting global processes and resulting in changes such as altered climate patterns and rising sea levels. These increases in CO₂ and N₂O can partially be attributed to agriculture, specifically through fossil fuel usage, land use conversion, soil disturbance and degradation, and increased N-fertilizer application. As global populations continue to increase and the subsequent increase in food and fuel demand, it becomes important to find ways to sustainably intensify agriculture for both food production and crops for bioenergy usage while still maintaining the quality of the soil and the many ecosystem services it provides. One solution being examined to help address these issues is the amendment of soil with biochar. Biochar is organic matter that has undergone pyrolysis (i.e. combustion under anoxic conditions), which results in a very recalcitrant material with a high C content. The application of biochar to soils is shown to improve soil quality and reduce GHG emissions from soils.

To determine the effect of biochar in Hawaii, a field experiment was undertaken that compared the response of two contrasting soils (a low fertility Oxisol soil and a high fertility Mollisol soil) under two different cropping systems (napiergrass with no-tillage management and a ratoon harvest and sweet corn with conventional tillage and harvests) both with and without biochar amendment. Additionally, biochar was buried within the biochar plots in mesh bags to determine the change of the biochar itself in the soil environment for both soils and both cropping systems.

As expected, biochar increased soil C by a difference of 47% compared to the control. However, other soil properties like exchangeable base cations and pH did not increase with biochar amendment. Biochar also had no effect on crop yields, most likely due to the lack of change in other soil properties. The diversity of the microbial community in the Oxisol napiergrass decreased with biochar amendment. The biochar itself became broken down over time and coated in soil particles. Biochar amendment had no effect on soil respiration at 26°C and decreased the quality of soil C. It also had no effect on soil N₂O flux at 26°C.

Biochar is a long-term soil C sequestration option within these two agricultural systems by adding a stable pool of C to soils that was not quickly respired back into the atmosphere and did not prime native soil C. It did not improve other soil properties and crop yields, making biochar as a soil conditioner not as beneficial as some studies have found. This demonstrates the importance of assessing both the biochar and the intended system properties prior to soil amendment with biochar.

While this study represents how biochar impacted soil properties under current climatic conditions, as global temperatures continue to rise it is important to establish how higher

temperatures will impact the soil, cropping system and biochar relationship. To help explain this, soils at year 1 from the field trial were incubated over an eight point temperature gradient.

Rising temperatures resulted in increased soil respiration for most treatments. In addition, biochar application nearly doubled the temperature sensitivity of soil respiration over the control. However, soil N₂O flux was temperature insensitive, with the exception of the Mollisol napiergrass with biochar soils, which had increased fluxes with increasing temperatures.

These data show that biochar will increase the temperature sensitivity of soils, so that eventually the sequestered C may be counterbalanced by increased soil respiration. Additionally, rather than decreasing soil N₂O flux as expected, biochar either had no effect, or in the case of the Mollisol napiergrass, increased soil N₂O flux. While no change is preferable to exacerbating GHG concentrations in the atmosphere, the effect of biochar of GHG flux was either negative or neutral, making it a less than ideal solution to climate change mitigation.

To help elucidate the mechanisms behind the increased N₂O flux in the Mollisol napiergrass at 23 and 31°C, the soils after the 60 day incubation were assessed for total microbial community abundance and the abundance of denitrifying bacteria. The total microbial community abundance decreased with temperature while soil respiration rates remained constant. The abundance of the denitrifying community exhibited no difference by temperature and neither the total community abundance nor the denitrifying community abundance had a biochar effect. This indicates that there may be a stabilizing effect as the microbial abundance decreases as respiration rate thermodynamically increases, resulting in a lack of net change in soil respiration.

However, this assessment occurred in soils with no inputs over the 60 days. To make the system more dynamic, following the 60 day incubation period for 23 and 31 °C, soils were amended with glucose, a labile C input to simulate the addition of organic matter or crop residues to agricultural systems. Within the labile amended soils, the respiration in the biochar amended soil at 31°C nearly doubled compared to 23°C -and total microbial biomass also increased in the 31°C biochar amended soils, although neither respiration or microbial abundance was significant. However, over time and given the constant inputs into agricultural systems, these insignificant differences can result in large C losses over time. Additionally, this shows that biochar can increase the temperature sensitivity of soil with more stable soil C pools as well as more labile soil C pools.

Conversely, the N₂O flux had no significant treatment effects by temperature or biochar and the community abundance of denitrifying bacteria remained the same regardless of treatment. However, comparing the day 60 soils to the glucose amended soils, while the glucose amended soils did increase in the overall abundance of soil denitrifying bacteria, the overall ratio of denitrifying bacteria to the total community abundance decreased with labile amendment. This suggests these communities can compete better in a more substrate-poor environment at higher

temperatures compared to the overall microbial communities. It is possible careful management decisions can reduce the effect of biochar on N₂O flux.

The three components of this project show mixed results for the use of biochar as a climate change mitigation strategy. While biochar did increase soil C sequestration, it in many cases it stimulated an increase in soil respiration and N₂O flux. Over time, these increases in GHG flux may negate the increased C sequestration. However, management decisions may be able to reduce some of these effects. In addition, the results of biochar have varied in other studies. This highlights the importance of carefully evaluating both the biochar and the system in which it is applied and balancing the risks and benefits that may come with biochar amendment into agricultural systems.

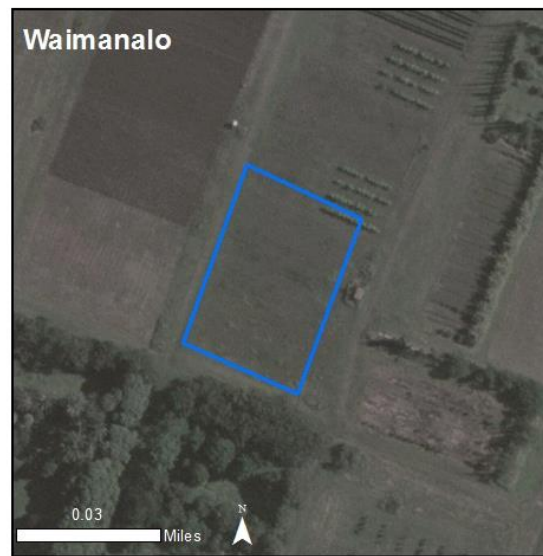
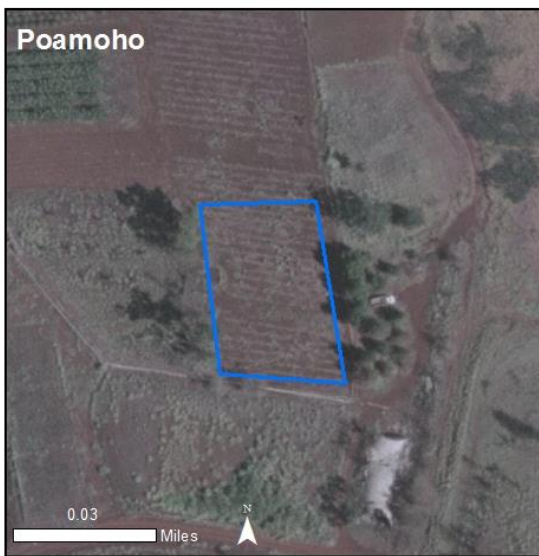
3.2 Future Considerations

As CO₂ and N₂O levels in the atmosphere increase, it is important to considering a variety of mitigation strategies. However, options need to be considered carefully to ensure that rather than working towards alleviating the problem, the mitigating strategies are not making the problem more difficult or costly to resolve. With all of the excitement surrounding the effects of biochar, it is important to consider the long-term ramifications of biochar application. Biochar has a long mean residence time within the soils and the particulate nature of biochar makes its removal unpractical if not unfeasible. Inarguably, biochar does increase the soil C by both taking organic matter and pyrolyzing it into a stable form of carbon and by sequestering it in soils which have a high capacity to store C. However, it is imperative to understand how biochar will respond different in different environments (e.g. Mollisol v. Oxisol) and different management systems (e.g. no-tillage with a ratoon harvest v. traditional harvest and tillage). Additionally, biochar itself is also highly variable, based on differences in feedstock, pyrolysis temperature and pyrolysis method. It is also important to think beyond the snapshot of what is happening in the soil; as global temperatures increase, agricultural systems and their respective microbial communities that have been amended with biochar may begin to respond very differently in terms of GHG emissions or even soil C sequestration potential. This is especially important within bioenergy agricultural systems. While it would seem advantageous to both use biochar to sequester C while also possibly improving bioenergy crops, the combination together may result in a less desirable outcome.

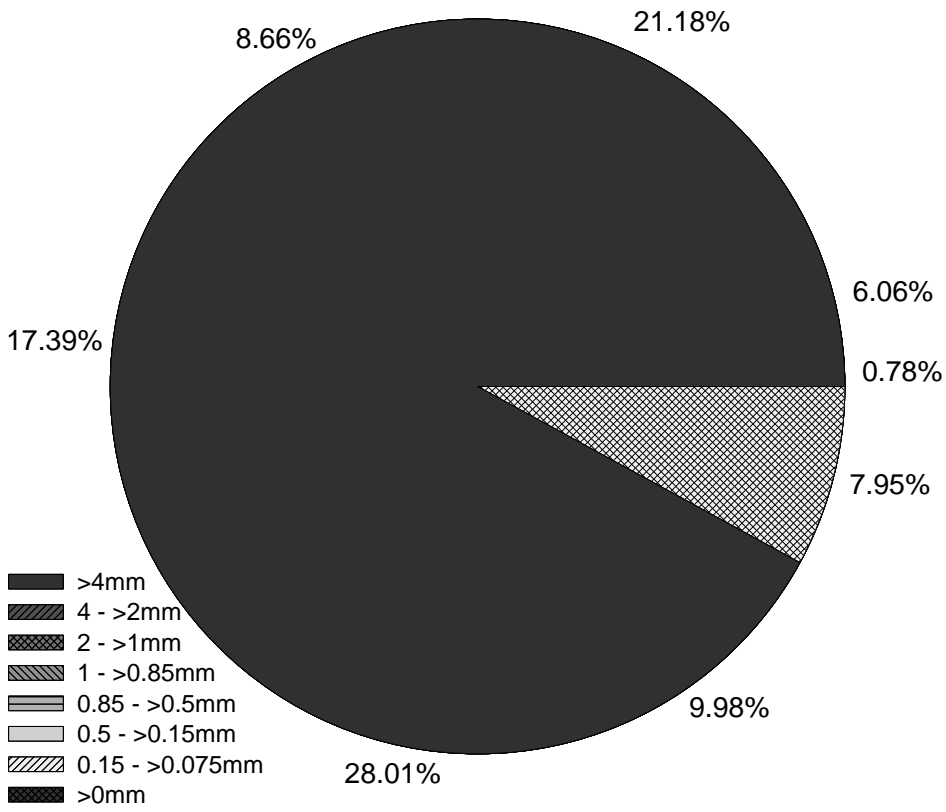
However, the results of this study do have limitations. The sample size of the study was small and there was large variability within the field trials. In addition, the GHG were based upon a laboratory incubation, which a convenient way to test on a small scale. However, it is also important to see if these results are replicated in a large-scale, soil warming *in situ* experiment especially with crop and root interactions, an aspect that was not addressed in this incubation study.

APPENDIX

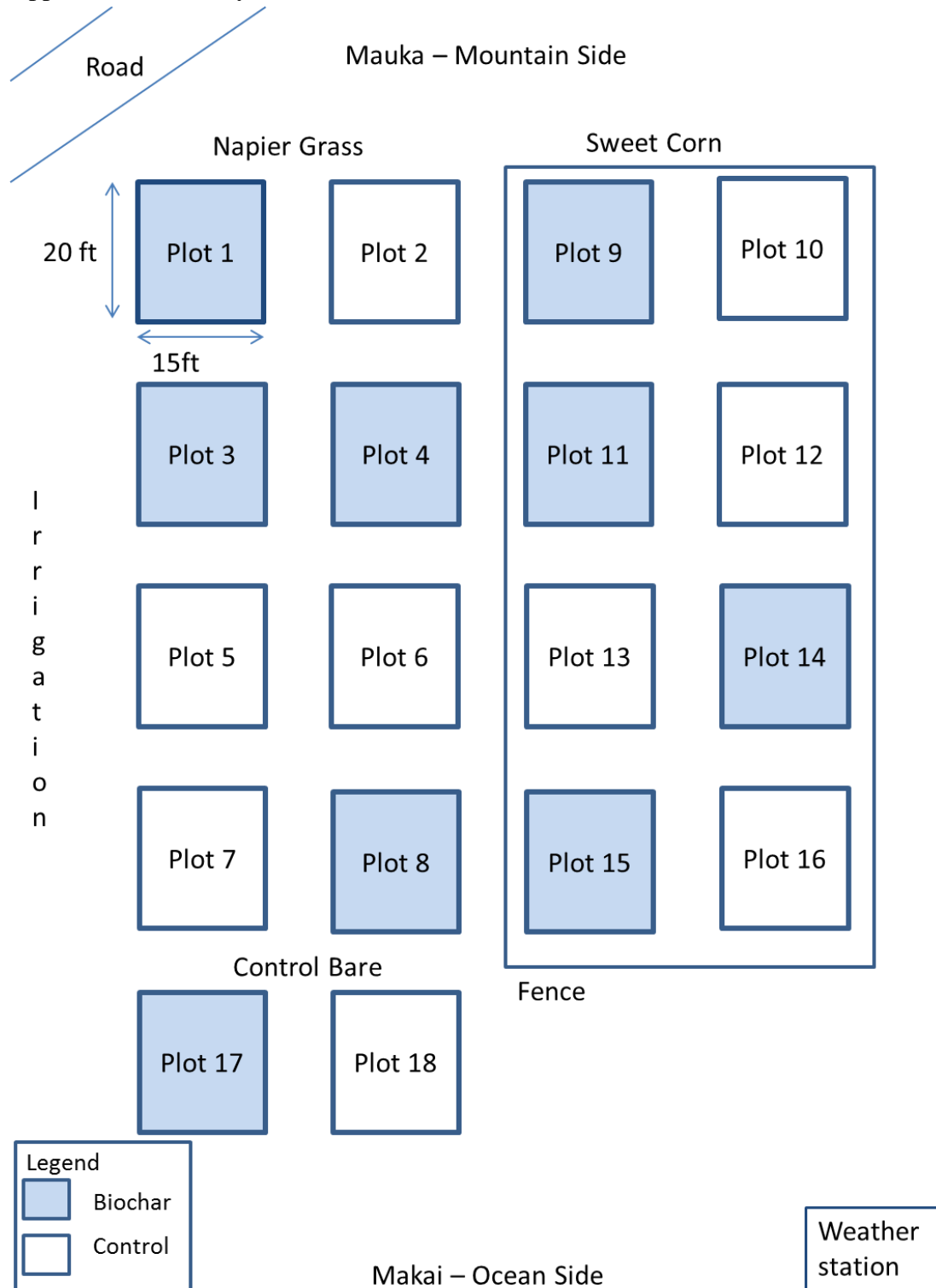
Appendix A: Site Maps



Appendix B: Biochar Particle Size Distribution

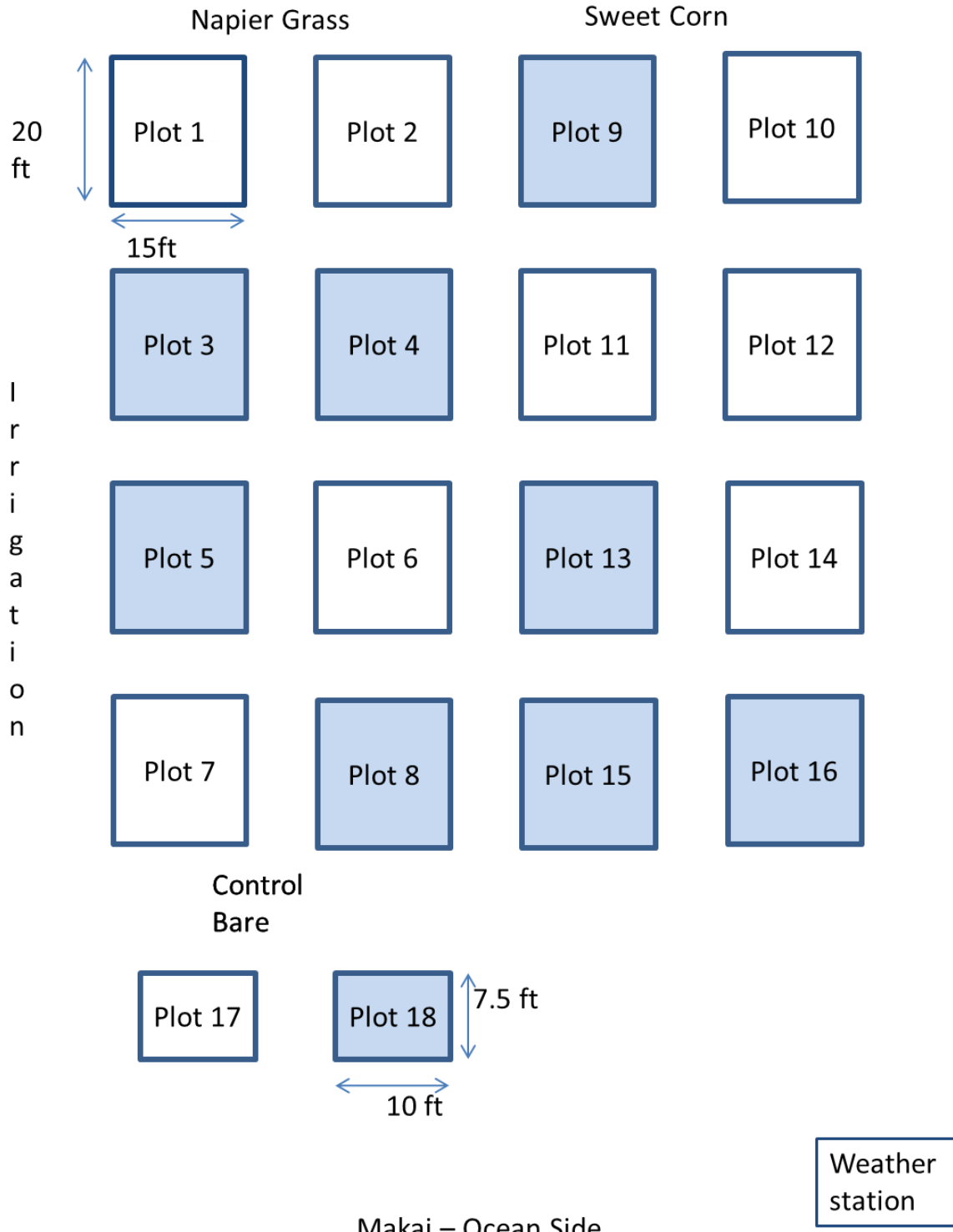


Appendix C: Plot Layout Poamoho

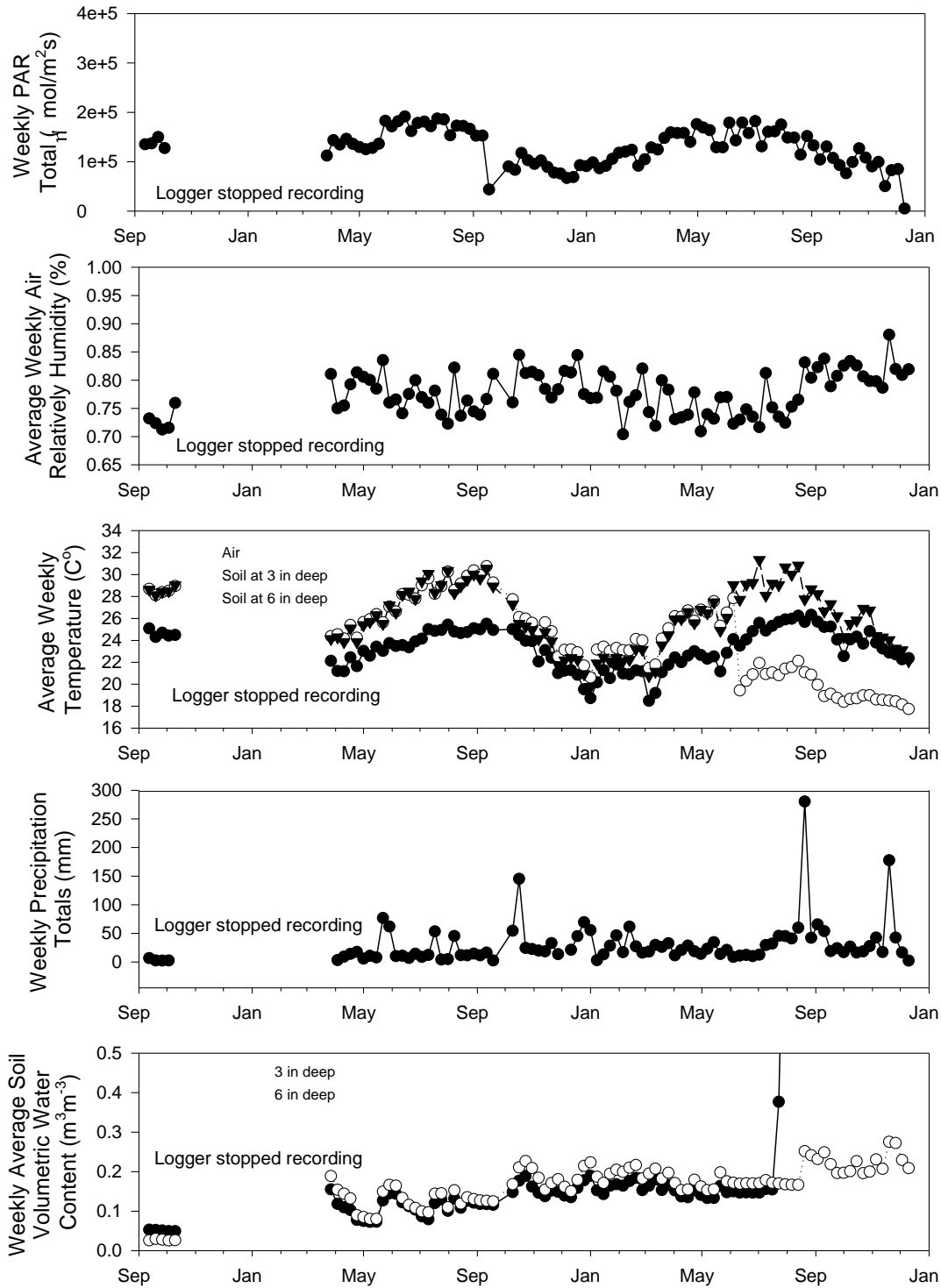


Appendix D: Plot Layout Waimanalo

Mauka – Mountain Side

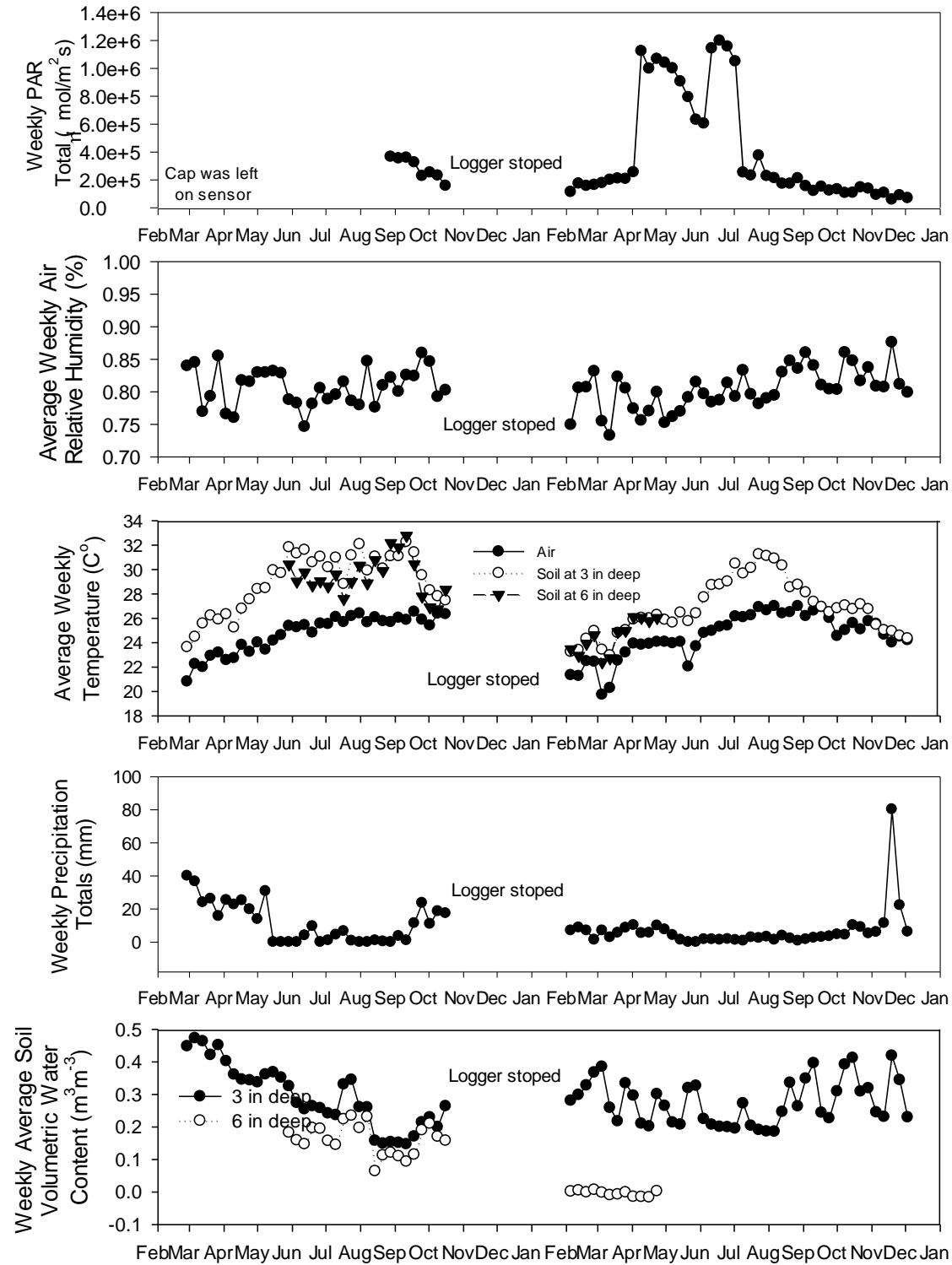


Appendix E: Poamoho Weather Station



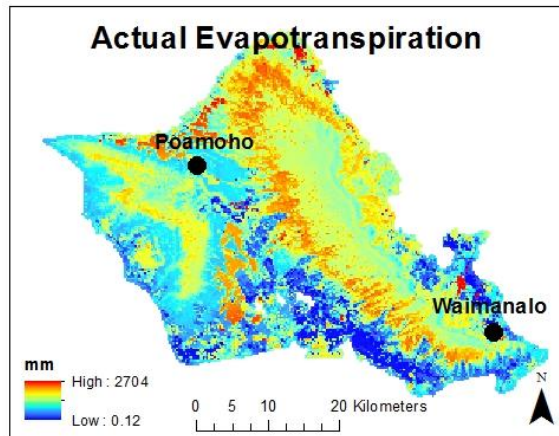
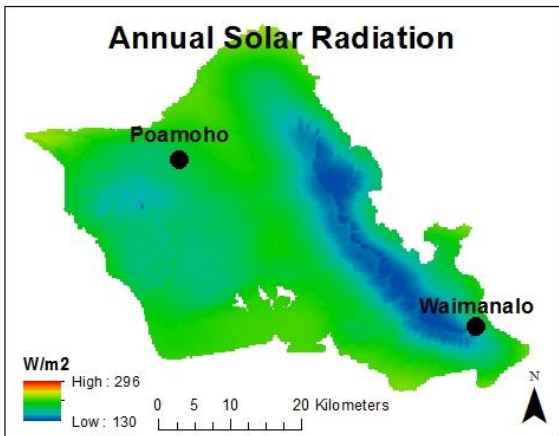
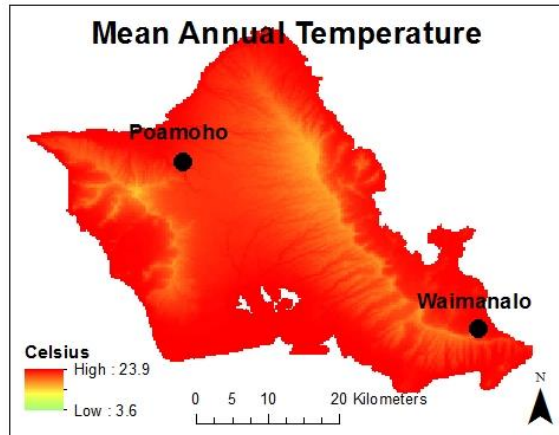
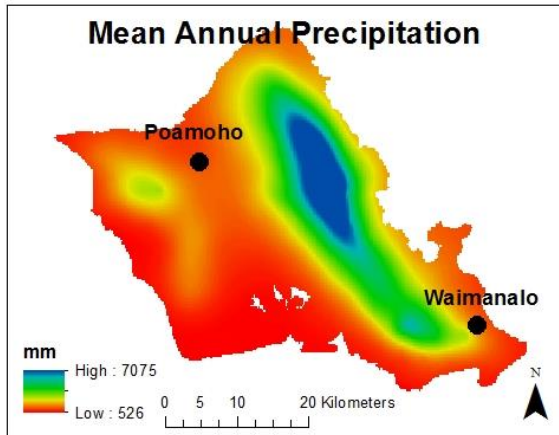
Poamoho September 2013- December 2015

Appendix F: Waimanalo Weather Station



Waimanalo February 2014 - December 2015

Appendix G: Climate Data



Data from: Giambelluca, T.W. et al. 2014. Evapotranspiration of Hawaii. Final report submitted to the U.S. Army Corps of Engineers—Honolulu District and the Commission on Water Resource Management, State of Hawaii.

Appendix H: The soil properties after the initial biochar, fertilizer, and lime application. This includes percent carbon (C), percent nitrogen (N), calcium (Ca), sodium (Na), magnesium (Mg), potassium (K) and pH with standard error. Statistical difference by column indicated by a different lowercase letters.

Soil	Crop	Treatment	C (%)	N (%)	Ca (mg kg ⁻¹)	Na (mg kg ⁻¹)	Mg (mg kg ⁻¹)	K (mg kg ⁻¹)	P (mg kg ⁻¹)	pH
Oxisol	Napiergrass	Biochar	1.76±0.05 ^b	0.17±0.01 ^a	1454.36±293.05 ^b	BD	127.18±7.03 ^b	352.14±31.94 ^c	152.54±34.85	6.84±0.17 ^c
		Control	1.26±0.04 ^d	0.16±0.01 ^{ab}	2125.09±639.01 ^b	BD	110.95±3.21 ^b	275.33±39.70 ^c	250.26±123.07	6.61±0.36 ^{ab}
	Sweet Corn	Biochar	1.95±0.13 ^{bc}	0.18±0.00 ^a	1822.73±97.52 ^b	113.84±8.25	107.97±2.46 ^b	439.76±37.74 ^b	125.26±21.33	6.40±0.25 ^{bc}
		Control	1.42±0.06 ^{cd}	0.19±0.01 ^{ab}	2077.84±146.39 ^b	82.80±22.48	111.76±4.29 ^b	400.57±12.26 ^b	136.72±22.01	6.36±0.10 ^c
Mollisol	Napiergrass	Biochar	2.23±0.11 ^a	0.17±0.00 ^{ab}	4420.20±63.42 ^a	126.48±3.13	688.94±15.43 ^a	488.32±26.02 ^{ab}	179.82±0.86	6.03±0.05 ^{ab}
		Control	1.42±0.06 ^{cd}	0.17±0.01 ^{ab}	4270.34±95.99 ^a	117.64±4.09	646.56±23.09 ^a	471.38±33.53 ^{ab}	167.24±9.85	6.07±0.05 ^{ab}
	Sweet Corn	Biochar	2.10±0.09 ^{bcd}	0.18±0.01 ^{ab}	4531.32±64.51 ^a	116.16±4.14	683.67±25.95 ^a	543.45±19.56 ^a	204.75±5.17	6.54±0.18 ^{abc}
		Control	1.46±0.02 ^{cd}	0.17±0.01 ^b	4517.56±171.14 ^a	102.22±2.74	665.10±6.79 ^a	566.69±59.90 ^a	192.63±12.56	6.28±0.22 ^{abc}

Appendix I. The proximate analysis for the biochar buried bag samples. The biochar initial sample is the biochar prior to amendment, the lab sample is one that had been kept in the laboratory at ambient conditions for one year, and the other samples had been buried in the field in the biochar plots for one year.

Soil	Type	Volatile matter (%)	Ash Content (%)	Fixed C (%)
Non-soil	Initial	18.24	14.31	67.45
	Lab	15.36±1.47	13.04±0.62	71.60±1.14
Oxisol	Napiergrass	20.87±2.03	15.91±2.28	63.23±2.13
	Bare	29.02±2.06	11.82±1.37	59.16±1.86
	Sweet Corn	24.71±1.74	14.78±0.57	60.51±1.21
Mollisol	Napiergrass	13.25±1.26	15.59±2.23	71.17±1.91
	Bare	24.06±3.43	18.99±1.56	56.95±4.48

Appendix J: The pH and electrical conductivity of the biochar both initially and after 1 year.

Location		pH	Electrical conductivity ($\mu\text{S/cm}$)
Non-soil	Initial	9.54 ± 0.06^a	444.50 ± 85.50^a
	Lab	9.37 ± 0.05^a	284.38 ± 35.04^b
Oxisol	Napiergrass	6.80 ± 0.04^c	40.53 ± 2.36^c
	Bare	6.40 ± 0.07^d	52.63 ± 2.13^c
	Sweet Corn	7.08 ± 0.04^{bc}	93.75 ± 5.56^c
Mollisol	Napiergrass	7.45 ± 0.09^b	35.28 ± 0.66^c
	Bare	6.19 ± 0.16^d	47.33 ± 2.47^c

Appendix K: The statistical output for soil properties at year 1.

%C

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	7.5285680	1.07551	28.8173
Error	24	0.8957188	0.03732	Prob > F
C. Total	31	8.4242867		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	1.6510938	0.034151	48.35	<.0001*
Site[Poamoho]	-0.103594	0.034151	-3.03	0.0057*
Crop[Corn]	-0.167344	0.034151	-4.90	<.0001*
Site[Poamoho]*Crop[Corn]	0.1373438	0.034151	4.02	0.0005*
Treatment[Biochar]	0.3857813	0.034151	11.30	<.0001*
Site[Poamoho]*Treatment[Biochar]	-0.068281	0.034151	-2.00	0.0570
Crop[Corn]*Treatment[Biochar]	-0.135781	0.034151	-3.98	0.0006*
Site[Poamoho]*Crop[Corn]*Treatment[Biochar]	0.0757813	0.034151	2.22	0.0362*

Level	Least Sq Mean
Waimanalo,Napier,Biochar	A 2.7250000
Poamoho,Napier,Biochar	B 1.9550000
Poamoho,Corn,Biochar	B C 1.7750000
Waimanalo,Corn,Biochar	B C D 1.6925000
Waimanalo,Napier,Control	C D E 1.3937500
Poamoho,Corn,Control	D E 1.2600000
Waimanalo,Corn,Control	E 1.2075000
Poamoho,Napier,Control	E 1.2000000

%N

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00289062	0.001445	6.8361
Error	29	0.00613125	0.000211	Prob > F
C. Total	31	0.00902187		0.0037*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.1565625	0.00257	60.91	<.0001*
Site[Poamoho]	0.006875	0.00257	2.67	0.0122*
Treatment[Biochar]	0.0065625	0.00257	2.55	0.0162*

Level	Least Sq Mean
Oxisol A	0.16343750
Mollisol B	0.14968750

Level	Least Sq Mean
Biochar A	0.16312500
Control B	0.15000000

Exchangeable Ca

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	52118483	17372828	263.4605
Error	28	1846346	65940.915	Prob > F
C. Total	31	53964829		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	2705.9084	45.39442	59.61	<.0001*
Site[Poamoho]	-1264.402	45.39442	-27.85	<.0001*
Crop[Corn]	-79.36656	45.39442	-1.75	0.0914
Site[Poamoho]*Crop[Corn]	-153.9228	45.39442	-3.39	0.0021*

Level	Least Sq Mean
Waimanalo,Corn A	4044.8663
Waimanalo,Napier A	3895.7538
Poamoho,Napier B	1674.7963
Poamoho,Corn C	1208.2175

Exchangeable Na

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	29265.792	14632.9	31.4722
Error	29	13483.452	464.9	Prob > F
C. Total	31	42749.244		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	140.39094	3.811769	36.83	<.0001*
Site[Poamoho]	-28.20813	3.811769	-7.40	<.0001*
Crop[Corn]	10.902188	3.811769	2.86	0.0078*

Level	Least Sq Mean
Waimanalo A	168.59906
Poamoho B	112.18281

Level	Least Sq Mean
Corn A	151.29313
Napier B	129.48875

Exchangeable Mg

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	12628434	12628434	2641.075
Error	30	143447	4781.5503	Prob > F
C. Total	31	12771881		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	821.69953	12.22389	67.22	<.0001*
Site[Poamoho]	-628.2027	12.22389	-51.39	<.0001*

Level	Least Sq Mean
Waimanalo A	1449.9022
Poamoho B	193.4969

Exchangeable K

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1163468.3	1163468	16.2820
Error	30	2143719.9	71457	Prob > F
C. Total	31	3307188.3		0.0003*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	815.22969	47.25507	17.25	<.0001*
Site[Poamoho]	-190.6787	47.25507	-4.04	0.0003*

Level	Least Sq Mean
Waimanalo A	1005.9084
Poamoho B	624.5509

pH

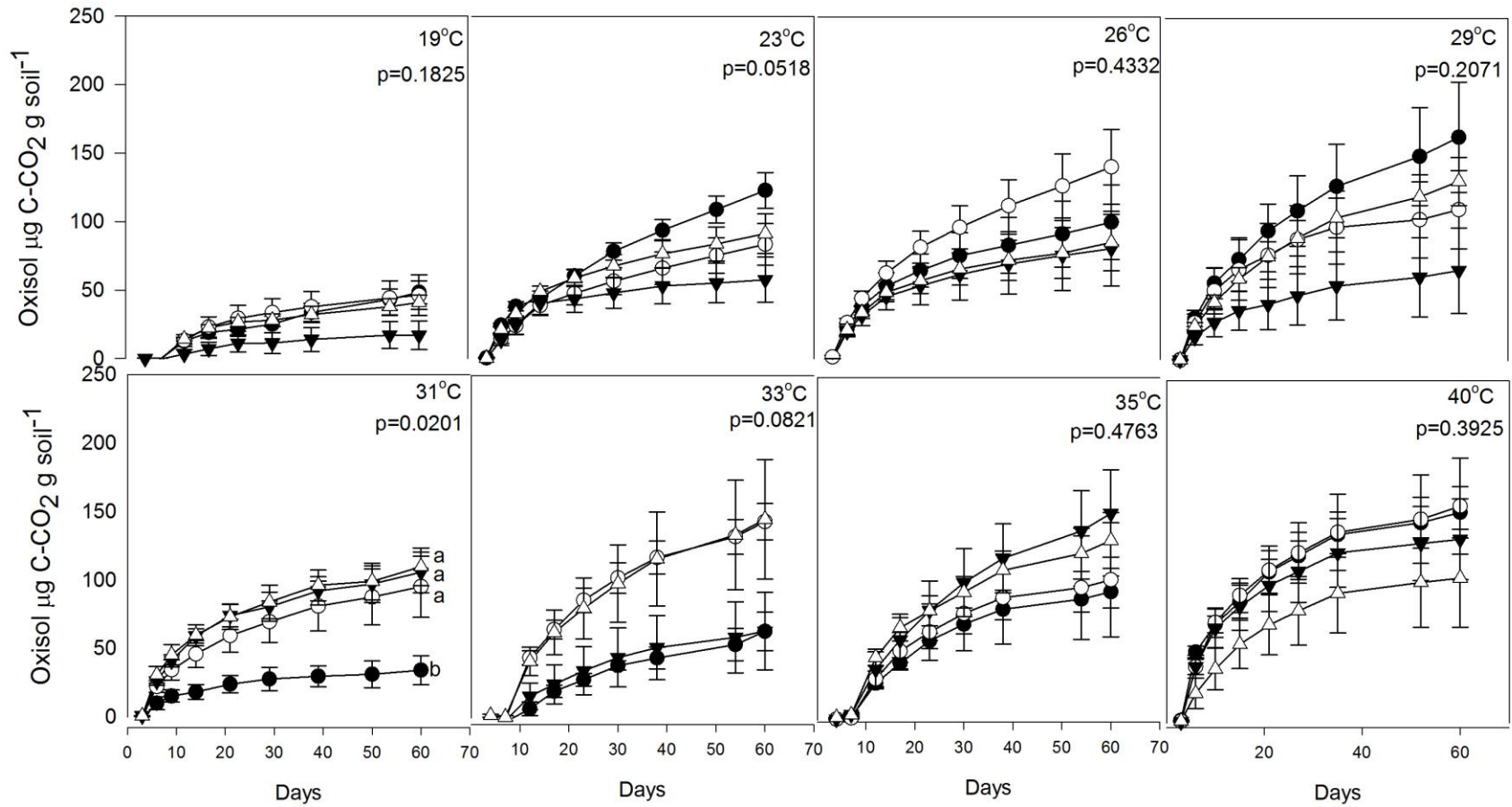
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	2.2956813	0.765227	7.7817
Error	28	2.7534188	0.098336	Prob > F
C. Total	31	5.0491000		0.0006*

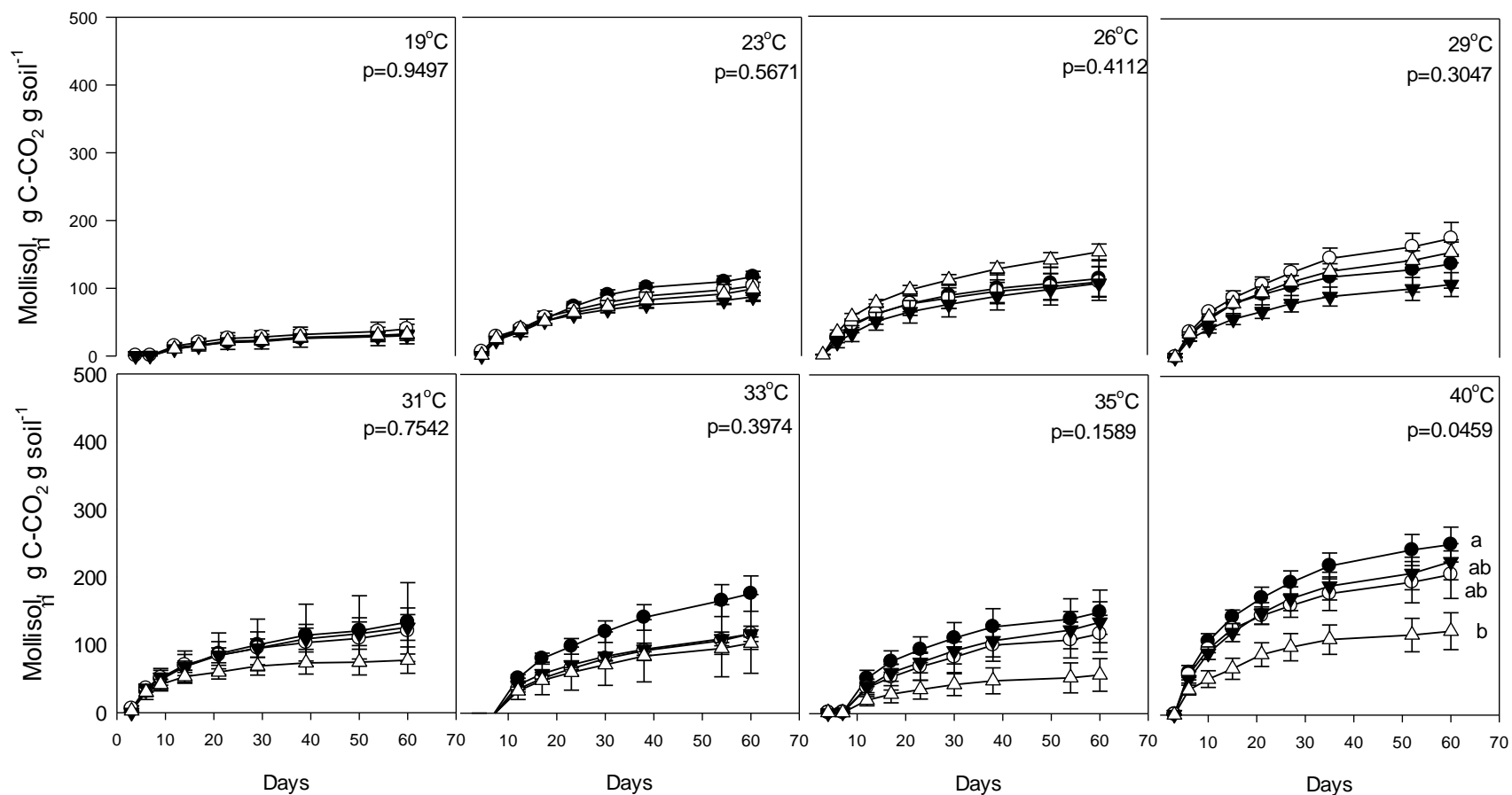
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	6.63	0.055435	119.60	<.0001*
Site[Poamoho]	-0.145313	0.055435	-2.62	0.0140*
Crop[Corn]	-0.17125	0.055435	-3.09	0.0045*
Site[Poamoho]*Crop[Corn]	-0.145938	0.055435	-2.63	0.0136*

Level	Least Sq Mean
Poamoho,Napier A	6.8018750
Waimanalo,Napier A	6.8006250
Waimanalo,Corn A	6.7500000
Poamoho,Corn B	6.1675000



Appendix L: The cumulative emissions ($\mu\text{g C-CO}_2 \text{ g soil}^{-1}$) for the Oxisol soil for the treatments napiergrass biochar, napiergrass control, sweet corn biochar and sweet corn control at A) 19°C, B) 23°C, C) 26°C, D) 29°C, E) 31°C, F) 33°C, G) 35°C, and H) 40°C. Statistics are a two way ANOVA with an interaction between crop and biochar treatment. Statistics are for each individual graph and different lowercase letters denote significant differences at $\alpha=0.05$.



Appendix M: The cumulative emissions ($\mu\text{g C-CO}_2 \text{ g soil}^{-1}$) for the Mollisol soil for the treatments napiergrass biochar, napiergrass control, sweet corn biochar and sweet corn control at A) 19°C, B) 23°C, C) 26°C, D) 29°C, E) 31°C, F) 33°C, G) 35°C, and H) 40°C. Statistics are a two way ANOVA with an interaction between crop and biochar treatment. Statistics are for each individual graph and different lowercase letters denote significant differences at $\alpha=0.05$.

Appendix N: The cumulative emissions at day 60 of the incubation in $\mu\text{g C-CO}_2 \text{ g soil}^{-1} \pm$ standard error for both the Oxisol soil and the Mollisol soil for napiergrass, sweet corn and bare plots with and without biochar in addition to the 1% biochar amended sand.

Soil	Crop	Treatment	19°C	23°C	26°C	29°C	31°C	33°C	35°C	40°C
$\mu\text{g C-CO}_2 \text{ g soil C}^{-1} \pm$ standard error										
Oxisol	Napiergrass	Biochar	48.39±8.46	122.90±13.06	100.02±27.23	163.20±40.29	34.04±10.51	63.23±14.08	93.64±11.69	153.86±18.83
		Control	46.29±14.78	83.58±15.26	140.42±27.39	110.28±28.56	95.02±22.38	143.37±13.18	102.40±41.57	158.43±35.13
	Sweet Corn	Biochar	17.10±10.48	57.72±16.41	80.69±27.30	65.81±31.27	105.57±14.88	63.52±28.26	150.88±31.87	134.17±29.58
		Control	41.08±5.26	91.39±14.42	85.23±20.54	130.91±17.56	109.59±13.85	145.15±43.72	131.02±20.45	105.82±35.76
	Bare Soil	Biochar	6.46±11.01	52.52±20.33	67.66±11.29	58.39±7.31	114.70±25.70	44.88±5.61	72.33±10.99	113.05±16.60
		Control	-11.95±1.27	-1.33±4.22	53.02±25.20	1.35±6.26	47.26±27.38	75.75±13.44	40.17±7.71	117.76±24.33
Mollisol	Napiergrass	Biochar	32.57±14.49	117.21±8.19	112.93±26.31	138.42±35.62	133.63±58.24	176.45±26.10	149.67±32.74	251.76±25.97
		Control	40.03±14.42	103.43±13.67	108.01±22.35	176.22±23.58	120.64±34.03	116.99±58.03	117.77±27.30	207.27±35.10
	Sweet Corn	Biochar	30.39±7.23	87.49±6.69	105.91±24.95	108.00±17.73	125.84±18.96	117.10±11.35	134.81±30.18	226.30±26.62
		Control	32.57±5.86	99.24±16.51	152.47±11.57	155.36±15.34	77.54±19.43	103.35±9.12	56.93±24.03	123.73±27.25
	Bare Soil	Biochar	18.98±9.86	97.43±5.37	131.27±11.47	81.26±9.67	96.34±16.80	120.93±16.89	64.37±21.27	156.26±5.49
		Control	21.22±7.56	56.95±13.68	91.97±14.52	113.56±34.68	126.27±6.79	172.33±70.59	146.55±10.31	204.26±6.74
	Sand	Biochar	-13.26±5.39	-13.95±2.64	-16.03±3.46	-3.94±2.58	-4.24±1.42	4.74±8.50	-6.94±6.69	-7.77±5.00

Appendix O: The cumulative emissions at day 60 of the incubation in g C-CO₂ g soil C⁻¹ ± standard error for both the Oxisol soil and the Mollisol soil for napiergrass, sweet corn and bare plots with and without biochar in addition to the 1% biochar amended sand.

Soil	Crop	Treatment	19°C	23°C	26°C	29°C	31°C	33°C	35°C	40°C	
g C-CO₂ g soil C⁻¹ ± standard error											
Oxisol	Napiergrass	Biochar	2.51±0.41	6.58±1.13	5.64±2.01	8.80±2.43	1.91±0.72	12.40±2.81	4.90±0.67	8.06±1.16	
		Control	3.81±1.19	6.90±1.12	11.66±2.13	9.37±2.66	7.99±1.97	28.63±2.59	8.57±3.45	13.18±2.84	
	Sweet Corn	Biochar	0.96±0.61	3.31±0.95	4.71±1.78	3.74±1.82	5.96±0.77	12.42±5.72	8.35±1.51	7.65±1.70	
		Control	3.29±0.46	7.35±1.30	6.97±1.81	10.59±1.68	8.83±1.33	28.80±8.78	10.40±1.55	8.65±3.11	
	Bare Soil	Biochar	0.33±0.57	2.71±1.05	3.49±0.58	3.01±0.38	5.91±1.32	8.67±1.18	3.73±0.57	5.83±0.86	
		Control	-0.85±0.09	-0.09±0.30	3.76±1.79	0.10±0.44	3.35±1.94	15.45±2.84	2.85±0.55	8.35±1.73	
	Baseline	N/A	3.66±1.10	5.65±0.68	4.05±1.05	-0.19±0.08	7.01±1.51	6.12±2.00	6.85±2.06	3.56±1.38	
	Mollisol	Napiergrass	Biochar	1.13±0.48	4.35±0.42	4.31±1.20	5.10±1.36	5.28±2.47	6.61±1.28	5.69±1.42	9.38±1.26
			Control	3.05±1.19	7.52±1.15	7.58±1.31	13.02±2.33	8.44±2.34	8.65±4.64	8.46±2.07	14.94±2.61
		Sweet Corn	Biochar	1.78±0.41	5.16±0.27	6.28±1.45	6.51±1.24	7.46±1.13	6.96±0.72	7.83±1.52	13.57±1.92
Control			2.67±0.37	8.18±1.14	12.74±1.11	12.83±0.80	6.53±1.78	8.68±1.06	4.93±2.31	10.54±2.78	
Bare Soil		Biochar	1.31±0.68	6.72±0.37	9.05±0.79	5.60±0.67	6.64±1.16	8.34±1.16	4.44±1.47	10.78±0.38	
		Control	1.17±0.42	3.13±0.75	5.05±0.80	6.24±1.91	6.94±0.37	9.47±3.88	8.05±0.57	11.22±0.37	
Baseline		N/A	1.58±0.42	4.13±0.21	5.74±1.04	-0.41±0.07	3.04±1.36	5.79±1.46	3.89±2.12	10.13±2.68	

Appendix P: The statistical output for soil respiration at 26°C.

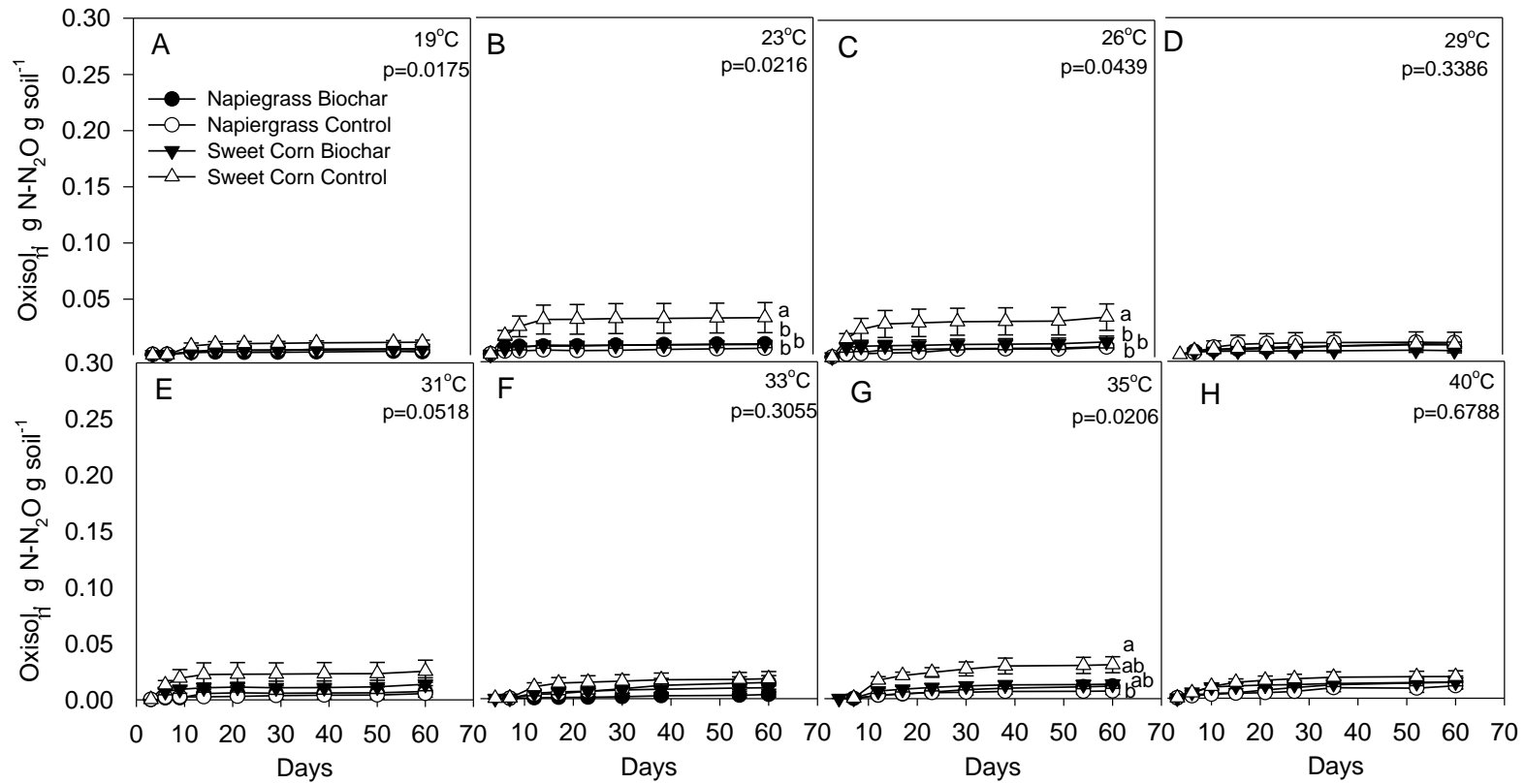
Soil respiration per g soil at 26°C (three way ANOVA interaction model)

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	21892.752	3127.54	1.6496
Error	24	45503.056	1895.96	Prob > F
C. Total	31	67395.809		0.1696

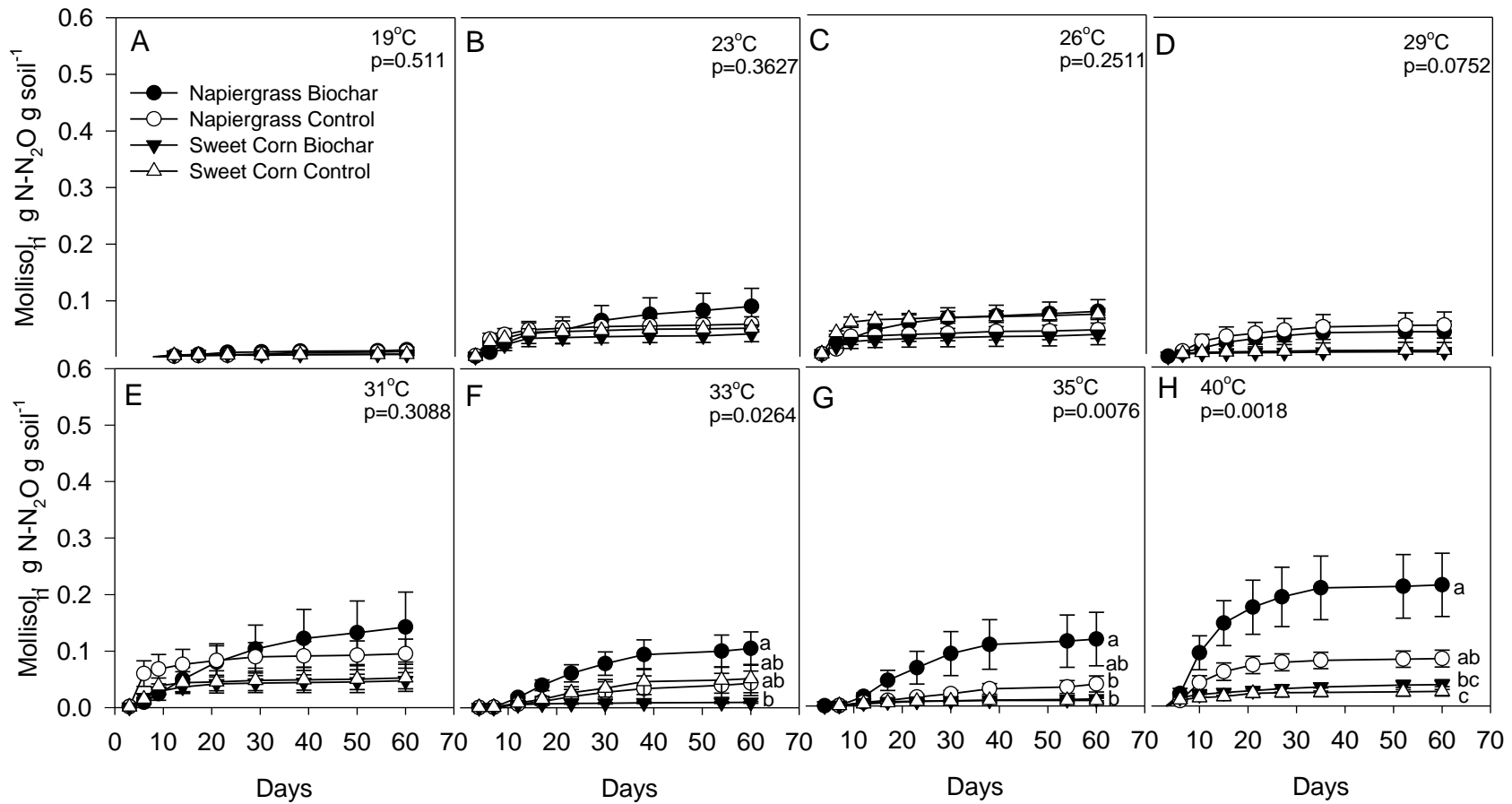
Soil respiration per g soil C at 26°C

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	162169474	162169474	13.2381
Error	30	367506513	12250217	Prob > F
C. Total	31	529675987		0.0010*

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	7486.5463	618.7239	12.10	<.0001*
Treatment[biochar]	-2251.177	618.7239	-3.64	0.0010*



Appendix Q: The cumulative emissions ($\mu\text{g n-N}_2\text{O g soil}^{-1}$) for the Oxisol soil for the treatments napiergrass biochar, napiergrass control, sweet corn biochar and sweet corn control at A) 19°C, B) 23°C, C) 26°C, D) 29°C, E) 31°C, F) 33°C, G) 35°C, and H) 40°C. Statistics are a two way ANOVA with an interaction between crop and biochar treatment. Statistics are for each individual graph and different lowercase letters denote significant differences at $\alpha=0.05$.



Appendix R: The cumulative emissions ($\mu\text{g n-N}_2\text{O g soil}^{-1}$) for the Mollisol soil for the treatments napiergrass biochar, napiergrass control, sweet corn biochar and sweet corn control at A) 19°C, B) 23°C, C) 26°C, D) 29°C, E) 31°C, F) 33°C, G) 35°C, and H) 40°C. Statistics are a two way ANOVA with an interaction between crop and biochar treatment. Statistics are for each individual graph and different lowercase letters denote significant differences at $\alpha=0.05$.

Appendix S: The cumulative emissions at day 60 of the incubation in $\mu\text{g N-N}_2\text{O kg soil}^{-1} \pm$ standard error for both the Oxisol soil and the Mollisol soil for napiergrass, sweet corn and bare plots with and without biochar in addition to the 1% biochar amended sand.

Soil	Crop	Treatment	19°C	23°C	26°C	29°C	31°C	33°C	35°C	40°C	
$\mu\text{g N-N}_2\text{O kg soil}^{-1} \pm$ standard error											
Oxisol	Napiergrass	Biochar	3.12±0.45	9.38±2.16	9.60±2.29	8.70±2.46	7.46±5.16	3.72±2.27	11.08±0.56	14.06±5.64	
		Control	4.87±2.78	5.46±1.04	8.94±1.88	10.71±9.16	5.56±0.85	14.36±6.09	6.67±2.91	11.06±3.05	
	Sweet Corn	Biochar	5.94±0.93	8.90±2.86	13.66±5.27	3.48±0.63	13.80±5.68	9.62±2.95	12.91±3.74	14.43±3.48	
		Control	11.67±1.86	32.77±13.40	35.78±11.80	9.22±1.89	25.39±9.83	17.67±6.40	30.17±7.15	19.25±5.09	
	Bare Soil	Biochar	1.56±0.69	8.14±4.11	22.57±15.08	1.55±0.29	11.06±1.54	3.30±0.93	3.37±0.96	6.00±0.73	
		Control	-0.70±0.27	0.75±0.35	4.25±1.00	-0.98±0.10	2.87±1.30	3.68±0.20	6.05±2.25	5.90±1.62	
	Baseline	N/A	2.92±0.87	7.23±0.79	2.69±3.12	0.36±0.17	6.53±1.74	4.63±1.43	5.86±1.32	1.14±1.94	
	Mollisol	Napiergrass	Biochar	13.49±4.25	89.16±32.01	75.39±20.80	43.54±18.93	142.59±61.84	104.81±29.55	118.35±47.33	179.18±46.49
			Control	11.34±7.56	57.87±12.82	42.67±26.15	55.46±22.99	95.42±25.71	43.23±21.67	38.92±14.15	70.82±12.38
		Sweet Corn	Biochar	5.70±1.65	40.73±13.85	34.57±7.18	8.08±0.92	47.55±14.36	9.48±1.69	12.42±3.43	32.46±7.16
Control			5.31±1.48	50.69±5.67	70.55±9.66	10.82±0.80	52.61±24.06	51.67±25.13	9.66±3.84	22.33±6.86	
Bare Soil		Biochar	2.70±1.00	61.22±35.60	81.12±44.73	14.34±9.69	52.23±23.71	23.36±10.36	38.44±13.32	16.06±1.29	
		Control	1.80±0.81	25.17±7.05	5.05±0.80	28.05±24.41	31.03±6.87	8.93±1.38	12.74±6.19	19.81±5.74	
Baseline		N/A	4.7±0.60	69.23±49.38	55.72±10.81	18.02±5.37	26.02±12.89	12.37±6.02	21.96±13.46	25.93±9.58	
Sand		N/A	Biochar	0.08±0.02	3.51±0.30	5.42±2.05	0.24±0.17	0.67±3.29	-0.09±0.62	1.53±2.37	2.49±0.21

Appendix T: The statistical output for soil N₂O flux per g soil at 26°C

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	12.581602	12.5816	20.8752
Error	30	18.081135	0.6027	Prob > F
C. Total	31	30.662738		<.0001*

Appendix U: The statistical output for individual soil respiration with temperature and the MANCOVA for comparing biochar and control treatments if both have significant relationships with temperature.

Oxisol napiergrass biochar

Linear Fit

respiration ug C-CO2 g soil = 36.169828 + 2.0759696*Temperature

Summary of Fit

RSquare	0.053058
RSquare Adj	0.021494
Root Mean Square Error	57.28619
Mean of Response	97.41093
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	5516.35	5516.35	1.6809
Error	30	98451.24	3281.71	Prob > F
C. Total	31	103967.59		0.2047

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	36.169828	48.3087	0.75	0.4599
Temperature	2.0759696	1.601198	1.30	0.2047

Oxisol napiergrass control

Linear Fit

respiration ug C-CO2 g soil = -9.413417 + 4.04705*Temperature

Summary of Fit

RSquare	0.198635
RSquare Adj	0.171923
Root Mean Square Error	53.09707
Mean of Response	109.9746
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	20964.63	20964.6	7.4361
Error	30	84578.95	2819.3	Prob > F
C. Total	31	105543.58		0.0106*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-9.413417	44.77606	-0.21	0.8349
Temperature	4.04705	1.484108	2.73	0.0106*

Oxisol sweet corn biochar

Linear Fit

respiration ug C-CO2 g soil = -82.50767 + 5.6368879*Temperature

Summary of Fit

RSquare	0.330547
RSquare Adj	0.307462
Root Mean Square Error	50.87092
Mean of Response	85.68979
Observations (or Sum Wgts)	31

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	37055.22	37055.2	14.3189
Error	29	75047.65	2587.9	Prob > F
C. Total	30	112102.87		0.0007*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-82.50767	45.37856	-1.82	0.0794
Temperature	5.6368879	1.48965	3.78	0.0007*

Oxisol sweet corn control

Linear Fit

respiration ug C-CO2 g soil = 2.232816 + 3.4844501*Temperature

Summary of Fit

RSquare	0.177079
RSquare Adj	0.149648
Root Mean Square Error	49.06526
Mean of Response	105.0241
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	15540.983	15541.0	6.4555
Error	30	72221.999	2407.4	Prob > F
C. Total	31	87762.981		0.0165*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	2.232816	41.37609	0.05	0.9573
Temperature	3.4844501	1.371416	2.54	0.0165*

MANCOVA for Oxisol sweet corn (biochar v control)

Summary of Fit

RSquare	0.278829
RSquare Adj	0.255184
Root Mean Square Error	49.61577
Mean of Response	94.72736
Observations (or Sum Wgts)	64

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	58058.89	29029.4	11.7923
Error	61	150165.21	2461.7	Prob > F
C. Total	63	208224.11		<.0001*

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	13	32824.19	2524.94	1.0329
Pure Error	48	117341.02	2444.60	Prob > F
Total Error	61	150165.21		0.4373
				Max RSq
				0.4365

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-37.29523	29.58558	-1.26	0.2123
Temperature	4.4753422	0.980618	4.56	<.0001*
Treatment[biochar]	-10.29673	6.201971	-1.66	0.1020

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Temperature	1	1	51273.441	20.8283	<.0001*
Treatment	1	1	6785.452	2.7564	0.1020

Mollisol napiergrass biochar

Linear Fit

respiration ug C-CO2 g soil = 2.232816 + 3.4844501*Temperature

Summary of Fit

RSquare	0.177079
RSquare Adj	0.149648
Root Mean Square Error	49.06526
Mean of Response	105.0241
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	15540.983	15541.0	6.4555
Error	30	72221.999	2407.4	Prob > F
C. Total	31	87762.981		0.0165*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	2.232816	41.37609	0.05	0.9573
Temperature	3.4844501	1.371416	2.54	0.0165*

Mollisol napiergrass control

Linear Fit

respiration ug C-CO2 g soil = -47.28145 + 5.7991691*Temperature

Summary of Fit

RSquare	0.260285
RSquare Adj	0.235628
Root Mean Square Error	63.8584
Mean of Response	123.794
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	43046.86	43046.9	10.5561
Error	30	122336.84	4077.9	Prob > F
C. Total	31	165383.70		0.0029*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-47.28145	53.85095	-0.88	0.3869
Temperature	5.7991691	1.784896	3.25	0.0029*

MANCOVA for Mollisol napiergrass (biochar v control)

Summary of Fit

RSquare	0.360495
RSquare Adj	0.339528
Root Mean Square Error	62.51615
Mean of Response	131.4363
Observations (or Sum Wgts)	64

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	134390.85	67195.4	17.1931
Error	61	238404.42	3908.3	Prob > F
C. Total	63	372795.28		<.0001*

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	13	46272.53	3559.43	0.8892
Pure Error	48	192131.90	4002.75	Prob > F
Total Error	61	238404.42		0.5694
				Max RSq
				0.4846

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-79.31088	37.278	-2.13	0.0374*
Treatment[biochar]	7.6422444	7.814519	0.98	0.3320
Temperature	7.1439715	1.235584	5.78	<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	3737.85	0.9564	0.3320
Temperature	1	1	130653.00	33.4299	<.0001*

Mollisol sweet corn biochar

Linear Fit

respiration ug C-CO2 g soil = -47.28145 + 5.7991691*Temperature

Summary of Fit

RSquare	0.260285
RSquare Adj	0.235628
Root Mean Square Error	63.8584
Mean of Response	123.794
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	43046.86	43046.9	10.5561
Error	30	122336.84	4077.9	Prob > F
C. Total	31	165383.70		0.0029*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-47.28145	53.85095	-0.88	0.3869
Temperature	5.7991691	1.784896	3.25	0.0029*

Mollisol sweet corn control

Linear Fit

respiration ug C-CO2 g soil = 54.801993 + 1.5371597*Temperature

Summary of Fit

RSquare	0.036578
RSquare Adj	0.004464
Root Mean Square Error	51.53035
Mean of Response	100.1482
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3024.461	3024.46	1.1390
Error	30	79661.312	2655.38	Prob > F
C. Total	31	82685.772		0.2944

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	54.801993	43.45487	1.26	0.2170
Temperature	1.5371597	1.440317	1.07	0.2944

The Oxisol napiergrass biochar with separate line fittings between 19-29 and 31-40°C

Lower temperature range

Linear Fit

respiration ug C-CO₂ g soil = -131.7016 + 9.9105126*Temperature

Summary of Fit

RSquare	0.365714
RSquare Adj	0.320408
Root Mean Square Error	51.6209
Mean of Response	108.6284
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	21509.799	21509.8	8.0721
Error	14	37306.045	2664.7	Prob > F
C. Total	15	58815.844		0.0131*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-131.7016	85.56804	-1.54	0.1461
Temperature	9.9105126	3.488217	2.84	0.0131*

Higher temperature range

Linear Fit

respiration ug C-CO₂ g soil = -204.4531 + 12.96345*Temperature

Summary of Fit

RSquare	0.766641
RSquare Adj	0.749972
Root Mean Square Error	28.28756
Mean of Response	109.9106
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	36803.178	36803.2	45.9933
Error	14	11202.602	800.2	Prob > F
C. Total	15	48005.780		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-204.4531	46.89013	-4.36	0.0007*
Temperature	12.96345	1.911496	6.78	<.0001*

The Mollisol sweet corn control with separate line fittings between 19-29 and 31-40C

Lower temperature range

Linear Fit

respiration ug C-CO₂ g soil = -204.4531 + 12.96345*Temperature

Summary of Fit

RSquare	0.766641
RSquare Adj	0.749972
Root Mean Square Error	28.28756
Mean of Response	109.9106
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	36803.178	36803.2	45.9933
Error	14	11202.602	800.2	Prob > F
C. Total	15	48005.780		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-204.4531	46.89013	-4.36	0.0007*
Temperature	12.96345	1.911496	6.78	<.0001*

Higher temperature range

Linear Fit

respiration ug C-CO₂ g soil = -374.1697 + 13.247863*Temperature

Summary of Fit

RSquare	0.763901
RSquare Adj	0.747037
Root Mean Square Error	26.33522
Mean of Response	86.1935
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	31415.549	31415.5	45.2971
Error	14	9709.615	693.5	Prob > F
C. Total	15	41125.164		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-374.1697	68.71757	-5.45	<.0001*
Temperature	13.247863	1.968387	6.73	<.0001*

Appendix V: The statistical output for individual soil N₂O flux with temperature and the MANCOVA for comparing biochar and control treatments if both have significant relationships with temperature.

Oxisol napiergrass biochar

Linear Fit

ox napier biochar = -0.000927 + 0.0003159*Temperature

Summary of Fit

RSquare	0.096052
RSquare Adj	0.065921
Root Mean Square Error	0.00633
Mean of Response	0.008392
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00012771	0.000128	3.1878
Error	30	0.00120188	0.000040	Prob > F
C. Total	31	0.00132959		0.0843

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-0.000927	0.005338	-0.17	0.8633
Temperature	0.0003159	0.000177	1.79	0.0843

Oxisol napiergrass control

Linear Fit

ox napier control = 0.000313 + 0.0002756*Temperature

Summary of Fit

RSquare	0.045251
RSquare Adj	0.013426
Root Mean Square Error	0.008269
Mean of Response	0.008443
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00009722	0.000097	1.4219
Error	30	0.00205118	0.000068	Prob > F
C. Total	31	0.00214840		0.2424

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.000313	0.006973	0.04	0.9645
Temperature	0.0002756	0.000231	1.19	0.2424

Oxisol sweet corn biochar

Linear Fit

ox corn biochar = 0.0004628 + 0.000335*Temperature

Summary of Fit

RSquare	0.083908
RSquare Adj	0.053371
Root Mean Square Error	0.00723
Mean of Response	0.010345
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00014363	0.000144	2.7478
Error	30	0.00156817	0.000052	Prob > F
C. Total	31	0.00171181		0.1078

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.0004628	0.006097	0.08	0.9400
Temperature	0.000335	0.000202	1.66	0.1078

Oxisol sweet corn control

Linear Fit

ox corn control = 0.022497 + 8.2989e-6*Temperature

Summary of Fit

RSquare	9.596e-6
RSquare Adj	-0.03332
Root Mean Square Error	0.017499
Mean of Response	0.022742
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	8.81548e-8	8.815e-8	0.0003
Error	30	0.00918672	0.000306	Prob > F
C. Total	31	0.00918680		0.9866

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.022497	0.014757	1.52	0.1379
Temperature	8.2989e-6	0.000489	0.02	0.9866

Mollisol napiergrass biochar

mol napier biochar 2 = -0.098357 + 0.006582*Temperature

Summary of Fit

RSquare	0.264089
RSquare Adj	0.239559
Root Mean Square Error	0.071769
Mean of Response	0.095812
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.05545298	0.055453	10.7658
Error	30	0.15452532	0.005151	Prob > F
C. Total	31	0.20997830		0.0026*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-0.098357	0.060522	-1.63	0.1146
Temperature	0.006582	0.002006	3.28	0.0026*

Mollisol napiergrass control

Linear Fit

mol napier control = -0.002579 + 0.0018447*Temperature

Summary of Fit

RSquare	0.08306
RSquare Adj	0.052495
Root Mean Square Error	0.040036
Mean of Response	0.05184
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00435579	0.004356	2.7175
Error	30	0.04808580	0.001603	Prob > F
C. Total	31	0.05244159		0.1097

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-0.002579	0.033762	-0.08	0.9396
Temperature	0.0018447	0.001119	1.65	0.1097

Mollisol sweet corn biochar

Linear Fit

mol corn biochar = 0.01797 + 0.0002001*Temperature

Summary of Fit

RSquare	0.003653
RSquare Adj	-0.02956
Root Mean Square Error	0.021588
Mean of Response	0.023873
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00005126	0.000051	0.1100
Error	30	0.01398099	0.000466	Prob > F
C. Total	31	0.01403225		0.7425

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.01797	0.018205	0.99	0.3315
Temperature	0.0002001	0.000603	0.33	0.7425

Mollisol sweet corn control

Linear Fit

mol corn control = 0.0425177 - 0.0002818*Temperature

Summary of Fit

RSquare	0.002968
RSquare Adj	-0.03027
Root Mean Square Error	0.033741
Mean of Response	0.034204
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00010165	0.000102	0.0893
Error	30	0.03415304	0.001138	Prob > F
C. Total	31	0.03425470		0.7671

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.0425177	0.028453	1.49	0.1455
Temperature	-0.000282	0.000943	-0.30	0.7671

Appendix W: The statistical output for temperature sensitivity of soil respiration with and without biochar.

Oneway Anova

Summary of Fit

Rsquare	0.233653
Adj Rsquare	0.19882
Root Mean Square Error	2.565002
Mean of Response	5.799579
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	44.13115	44.1312	6.7076	0.0167*
Error	22	144.74320	6.5792		
C. Total	23	188.87436			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
biochar	12	7.15560	0.74045	5.6200	8.6912
control	12	4.44356	0.74045	2.9080	5.9792

Std Error uses a pooled estimate of error variance

Appendix X: The statistical output for the day 60 16S for the Mollisol napiergrass at 23 and 31°C.

Oneway Anova

Summary of Fit

Rsquare	0.397594
Adj Rsquare	0.354565
Root Mean Square Error	62140.56
Mean of Response	412809.6
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Temperature	1	3.568e+10	3.568e+10	9.2401	0.0088*
Error	14	5.406e+10	3.8614e+9		
C. Total	15	8.9741e+10			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
23	8	460033	21970	412912	507154
31	8	365587	21970	318466	412707

Std Error uses a pooled estimate of error variance

Appendix Y: The statistical output for the ratio of *nosZ* community abundance to total community abundance for the day 60 Mollisol napiergrass at 23 and 31°C.

Oneway Anova

Summary of Fit

Rsquare	0.357738
Adj Rsquare	0.311862
Root Mean Square Error	0.001625
Mean of Response	0.00389
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Temperature	1	0.00002060	0.000021	7.7980	0.0144*
Error	14	0.00003698	2.642e-6		
C. Total	15	0.00005758			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
23	8	0.002755	0.00057	0.00152	0.00399
31	8	0.005024	0.00057	0.00379	0.00626

Std Error uses a pooled estimate of error variance

Appendix Z: The statistical output Figure 2.16.

2.16A

Oneway Anova

Summary of Fit

Rsquare	0.331306
Adj Rsquare	0.307424
Root Mean Square Error	551864.9
Mean of Response	763849.5
Observations (or Sum Wgts)	30

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
glucose	1	4.225e+12	4.225e+12	13.8727	0.0009*
Error	28	8.5275e+12	3.046e+11		
C. Total	29	1.2753e+13			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
60 day	16	412810	137966	130199	695420.6
Glucose	14	1165038	147492	862914	1467161.7

Std Error uses a pooled estimate of error variance

2.16 B and C

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	12459062	6229531	7.8551
Error	29	22998533	793053	Prob > F
C. Total	31	35457595		0.0019*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	2013.388	157.4259	12.79	<.0001*
Temperature[23]	-373.6304	157.4259	-2.37	0.0245*
glucose[60 day]	-499.746	157.4259	-3.17	0.0035*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Temperature	1	1	4467188.5	5.6329	0.0245*
glucose	1	1	7991873.9	10.0774	0.0035*

Level	Least Sq Mean	Std Error	Mean
23	1639.7576	222.63379	1639.76
31	2387.0183	222.63379	2387.02

Level	Least Sq Mean	Std Error	Mean
60 day	1513.6420	222.63379	1513.64
Glucose	2513.1339	222.63379	2513.13

2.16 D

Oneway Anova Summary of Fit

Rsquare	0.330045
Adj Rsquare	0.282191
Root Mean Square Error	0.001707
Mean of Response	0.003903
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
glucose	1	0.00002011	0.000020	6.8969	0.0199*
Error	14	0.00004081	2.915e-6		
C. Total	15	0.00006092			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
60 day	8	0.005024	0.00060	0.00373	0.00632
Glucose	8	0.002783	0.00060	0.00149	0.00408

Std Error uses a pooled estimate of error variance

REFERENCES

- Abdollahi, L., P. Schjønning, S. Elmholt, and L. J. Munkholm. 2014. "The Effects of Organic Matter Application and Intensive Tillage and Traffic on Soil Structure Formation and Stability." *Soil and Tillage Research* 136 (March): 28–37. doi:10.1016/j.still.2013.09.011.
- Ackerman, Frank, and Elizabeth Stanton. 2012. "Climate Risks and Carbon Prices: Revising the Social Cost of Carbon." SSRN Scholarly Paper ID 2056941. Rochester, NY: Social Science Research Network. <http://papers.ssrn.com/abstract=2056941>.
- Allison, Steven D., Matthew D. Wallenstein, and Mark A. Bradford. 2010. "Soil-Carbon Response to Warming Dependent on Microbial Physiology." *Nature Geoscience* 3 (5): 336–40. doi:10.1038/ngeo846.
- Anderson, Craig R., Leo M. Condron, Tim J. Clough, Mark Fiers, Alison Stewart, Robert A. Hill, and Robert R. Sherlock. 2011. "Biochar Induced Soil Microbial Community Change: Implications for Biogeochemical Cycling of Carbon, Nitrogen and Phosphorus." *Pedobiologia* 54 (5–6): 309–20. doi:10.1016/j.pedobi.2011.07.005.
- Anderson, Marti J. 2001. "A New Method for Non-Parametric Multivariate Analysis of Variance." *Austral Ecology* 26 (1): 32–46. doi:10.1111/j.1442-9993.2001.01070.pp.x.
- Anderson-Teixeira, Kristina J., Michael D. Masters, Christopher K. Black, Marcelo Zeri, Mir Zaman Hussain, Carl J. Bernacchi, and Evan H. DeLucia. 2013. "Altered Belowground Carbon Cycling Following Land-Use Change to Perennial Bioenergy Crops." *Ecosystems* 16 (3): 508–20. doi:10.1007/s10021-012-9628-x.
- Anderson, Tiffany R., Charles H. Fletcher, Matthew M. Barbee, L. Neil Frazer, and Bradley M. Romine. 2015. "Doubling of Coastal Erosion under Rising Sea Level by Mid-Century in Hawaii." *Natural Hazards* 78 (1): 75–103. doi:10.1007/s11069-015-1698-6.
- Atkinson, Christopher J., Jean D. Fitzgerald, and Neil A. Hipps. 2010. "Potential Mechanisms for Achieving Agricultural Benefits from Biochar Application to Temperate Soils: A Review." *Plant and Soil* 337 (1-2): 1–18. doi:10.1007/s11104-010-0464-5.
- Aulakh, M. S., J. W. Doran, and A. R. Mosier. 1992. "Soil Denitrification—Significance, Measurement, and Effects of Management." In *Advances in Soil Science*, edited by B. A. Stewart, 1–57. *Advances in Soil Science* 18. Springer New York. http://link.springer.com.eres.library.manoa.hawaii.edu/chapter/10.1007/978-1-4612-2844-8_1.
- Bamminger, Chris, Natalie Zaiser, Prisca Zinsser, Marc Lamers, Claudia Kammann, and Sven Marhan. 2014. "Effects of Biochar, Earthworms, and Litter Addition on Soil Microbial Activity and Abundance in a Temperate Agricultural Soil." *Biology and Fertility of Soils* 50 (8): 1189–1200. doi:10.1007/s00374-014-0968-x.
- Barrow, C.J. 2012. "Biochar: Potential for Countering Land Degradation and for Improving Agriculture." *Applied Geography* 34 (May): 21–28. doi:10.1016/j.apgeog.2011.09.008.
- Bayer, Cimelio, Juliana Gomes, Josileia Accordi Zanatta, Frederico Costa Beber Vieira, Marisa de Cassia Piccolo, Jeferson Dieckow, and Johan Six. 2015. "Soil Nitrous Oxide Emissions as Affected by Long-Term Tillage, Cropping Systems and Nitrogen Fertilization in Southern Brazil." *Soil & Tillage Research* 146 (March): 213–22. doi:10.1016/j.still.2014.10.011.
- Beddington, J, M Asaduzzaman, A Fernandez, M Clark, M Guillou, M Jahn, L Erda, et al. 2011. "Achieving Food Security in the Face of Climate Change: Summary for Policy Makers from the Commission on Sustainable Agriculture and Climate Change." CGIAR Research Program on Climate Change, Agriculture and Food Security (CAAFS). Copenhagen, Denmark.
- Blair, GJ, RDB Lefroy, and L Lisle. 1995. "Soil Carbon Fractions Based on Their Degree of Oxidation, and the Development of a Carbon Management Index for Agricultural Systems." *Australian Journal of Agricultural Research* 46 (7): 1459–66.

- Bommarco, Riccardo, David Kleijn, and Simon G. Potts. 2013. "Ecological Intensification: Harnessing Ecosystem Services for Food Security." *Trends in Ecology & Evolution* 28 (4): 230–38. doi:10.1016/j.tree.2012.10.012.
- Brady, Nyle C., and Ray R. Weil. 2008. *The Nature and Properties of Soils*. 14th ed. Upper Saddle River, New Jersey: Pearson Education, Inc.
- Braker, Gesche, Julia Schwarz, and Ralf Conrad. 2010. "Influence of Temperature on the Composition and Activity of Denitrifying Soil Communities." *FEMS Microbiology Ecology* 73 (1): 134–48. doi:10.1111/j.1574-6941.2010.00884.x.
- Bremner, John M. 1997. "Sources of Nitrous Oxide in Soils." *Nutrient Cycling in Agroecosystems* 49 (1-3): 7–16. doi:10.1023/A:1009798022569.
- Brewbaker, James L., and Chifume Nagai. 1992. "Breeding Tropical Supersweet Corn." *HortScience* 27 (6): 645–645.
- Brodowski, Sonja, Wulf Amelung, Ludwig Haumaier, Clarissa Abetz, and Wolfgang Zech. 2005. "Morphological and Chemical Properties of Black Carbon in Physical Soil Fractions as Revealed by Scanning Electron Microscopy and Energy-Dispersive X-Ray Spectroscopy." *Geoderma*, Mechanisms and regulation of organic matter stabilisation in soils, 128 (1–2): 116–29. doi:10.1016/j.geoderma.2004.12.019.
- Burney, Jennifer A., Steven J. Davis, and David B. Lobell. 2010. "Greenhouse Gas Mitigation by Agricultural Intensification." *Proceedings of the National Academy of Sciences* 107 (26): 12052–57. doi:10.1073/pnas.0914216107.
- Burzaco, Juan P., Doug R. Smith, and Tony J. Vyn. 2013. "Nitrous Oxide Emissions in Midwest US Maize Production Vary Widely with Band-Injected N Fertilizer Rates, Timing and Nitrapyrin Presence." *Environmental Research Letters* 8 (3): 035031. doi:10.1088/1748-9326/8/3/035031.
- Cardoso, Irene M., and Thomas W. Kuyper. 2006. "Mycorrhizas and Tropical Soil Fertility." *Agriculture, Ecosystems & Environment*, Nutrient Management in Tropical Agroecosystems, 116 (1–2): 72–84. doi:10.1016/j.agee.2006.03.011.
- Case, Sean D. C., Niall P. McNamara, David S. Reay, and Jeanette Whitaker. 2012. "The Effect of Biochar Addition on N₂O and CO₂ Emissions from a Sandy Loam Soil – The Role of Soil Aeration." *Soil Biology and Biochemistry* 51 (August): 125–34. doi:10.1016/j.soilbio.2012.03.017.
- . 2014. "Can Biochar Reduce Soil Greenhouse Gas Emissions from a Miscanthus Bioenergy Crop?" *GCB Bioenergy* 6 (1): 76–89. doi:10.1111/gcbb.12052.
- Cayuela, Maria Luz, Miguel Angel Sánchez-Monedero, Asunción Roig, Kelly Hanley, Akio Enders, and Johannes Lehmann. 2013. "Biochar and Denitrification in Soils: When, How Much and Why Does Biochar Reduce N₂O Emissions?" *Scientific Reports* 3 (April). doi:10.1038/srep01732.
- Cayuela, M. L., L. van Zwieten, B. P. Singh, S. Jeffery, A. Roig, and M. A. Sánchez-Monedero. 2014. "Biochar's Role in Mitigating Soil Nitrous Oxide Emissions: A Review and Meta-Analysis." *Agriculture, Ecosystems & Environment*, Environmental Benefits and Risks of Biochar Application to Soil, 191 (June): 5–16. doi:10.1016/j.agee.2013.10.009.
- Cheng, Chih-Hsin, and Johannes Lehmann. 2009. "Ageing of Black Carbon along a Temperature Gradient." *Chemosphere* 75 (8): 1021–27. doi:10.1016/j.chemosphere.2009.01.045.
- Cheng, Chih-Hsin, Johannes Lehmann, and Mark H. Engelhard. 2008. "Natural Oxidation of Black Carbon in Soils: Changes in Molecular Form and Surface Charge along a Climosequence." *Geochimica et Cosmochimica Acta* 72 (6): 1598–1610. doi:10.1016/j.gca.2008.01.010.
- Chen, Junhui, Xiaoyu Liu, Jinwei Zheng, Bin Zhang, Haifei Lu, Zhongzhi Chi, Genxing Pan, et al. 2013a. "Biochar Soil Amendment Increased Bacterial but Decreased Fungal Gene Abundance with Shifts in Community Structure in a Slightly Acid Rice Paddy from Southwest China." *Applied Soil Ecology* 71 (September): 33–44. doi:10.1016/j.apsoil.2013.05.003.
- . 2013b. "Biochar Soil Amendment Increased Bacterial but Decreased Fungal Gene Abundance with Shifts in Community Structure in a Slightly Acid Rice Paddy from Southwest China." *Applied Soil Ecology* 71 (September): 33–44. doi:10.1016/j.apsoil.2013.05.003.

- Clarke, K. R. 2006. "Non-Parametric Multivariate Analyses of Changes in Community Structure." <http://onlinelibrary.wiley.com/doi/10.1111/j.1442-9993.1993.tb00438.x/abstract>.
- Clarke, K. R., and R.N. Gorley. 1993. "PRIMER v6: User Manual/Tutorial." *Plymouth*.
- Clark, Jeffrey T. 1986. "Continuity and Change in Hawaiian Agriculture." *Agricultural History* 60 (3): 1–22.
- Clough, Tim J., Leo M. Condon, Claudia Kammann, and Christoph Müller. 2013. "A Review of Biochar and Soil Nitrogen Dynamics." *Agronomy* 3 (2): 275–93. doi:10.3390/agronomy3020275.
- Conant, Richard T., Michael G. Ryan, Göran I. Ågren, Hannah E. Birge, Eric A. Davidson, Peter E. Eliasson, Sarah E. Evans, et al. 2011. "Temperature and Soil Organic Matter Decomposition Rates – Synthesis of Current Knowledge and a Way Forward." *Global Change Biology* 17 (11): 3392–3404. doi:10.1111/j.1365-2486.2011.02496.x.
- Crombie, Kyle, Ondřej Mašek, Andrew Cross, and Saran Sohi. 2015. "Biochar – Synergies and Trade-Offs between Soil Enhancing Properties and C Sequestration Potential." *GCB Bioenergy* 7 (5): 1161–75. doi:10.1111/gcbb.12213.
- Davidson, Eric A., and Ivan A. Janssens. 2006. "Temperature Sensitivity of Soil Carbon Decomposition and Feedbacks to Climate Change." *Nature* 440 (7081): 165–73. doi:10.1038/nature04514.
- Deenik, Jonathan. 2006. "Nitrogen Mineralization Potential in Important Agricultural Soils of Hawai'i," July. <http://scholarspace.manoa.hawaii.edu/handle/10125/12455>.
- Dempewolf, Hannes, Ruth J. Eastwood, Luigi Guarino, Colin K. Khoury, Jonas V. Müller, and Jane Toll. 2014. "Adapting Agriculture to Climate Change: A Global Initiative to Collect, Conserve, and Use Crop Wild Relatives." *Agroecology and Sustainable Food Systems* 38 (4): 369–77. doi:10.1080/21683565.2013.870629.
- Deng, Lei, Guo-bin Liu, and Zhou-ping Shangguan. 2014. "Land-Use Conversion and Changing Soil Carbon Stocks in China's 'Grain-for-Green' Program: A Synthesis." *Global Change Biology* 20 (11): 3544–56. doi:10.1111/gcb.12508.
- Dicke, Christiane, Janet Andert, Christian Ammon, Jürgen Kern, Andreas Meyer-Aurich, and Martin Kaupenjohann. 2015. "Effects of Different Biochars and Digestate on N₂O Fluxes under Field Conditions." *Science of The Total Environment* 524–525 (August): 310–18. doi:10.1016/j.scitotenv.2015.04.005.
- Dick, Warren A., Basanthi Thavamani, Shannon Conley, Robert Blaisdell, and Aditi Sengupta. 2013. "Prediction of β -Glucosidase and β -Glucosaminidase Activities, Soil Organic C, and Amino Sugar N in a Diverse Population of Soils Using near Infrared Reflectance Spectroscopy." *Soil Biology and Biochemistry*, Special Issue: Interactions of Soil Minerals with Organic Components and Microorganisms VII and Enzymes in the Environment IV, 56 (January): 99–104. doi:10.1016/j.soilbio.2012.04.003.
- Don, Axel, Jens Schumacher, and Annette Freibauer. 2011. "Impact of Tropical Land-Use Change on Soil Organic Carbon Stocks - a Meta-Analysis." *Global Change Biology* 17 (4): 1658–70. doi:10.1111/j.1365-2486.2010.02336.x.
- Drijber, R. A., J. W. Doran, A. M. Parkhurst, and D. J. Lyon. 2000. "Changes in Soil Microbial Community Structure with Tillage under Long-Term Wheat-Fallow Management." *Soil Biology and Biochemistry* 32 (10): 1419–30. doi:10.1016/S0038-0717(00)00060-2.
- Elefsiniotis, P., and D. Li. 2006. "The Effect of Temperature and Carbon Source on Denitrification Using Volatile Fatty Acids." *Biochemical Engineering Journal* 28 (2): 148–55. doi:10.1016/j.bej.2005.10.004.
- Erisman, Jan Willem, James N. Galloway, Sybil Seitzinger, Albert Bleeker, Nancy B. Dise, A. M. Roxana Petrescu, Allison M. Leach, and Wim de Vries. 2013. "Consequences of Human Modification of the Global Nitrogen Cycle." *Phil. Trans. R. Soc. B* 368 (1621): 20130116. doi:10.1098/rstb.2013.0116.
- Fabrizzi, Karina P., Charles W. Rice, Telmo J. C. Amado, Jackson Fiorin, Pedro Barbagelata, and Ricardo Melchiori. 2008. "Protection of Soil Organic C and N in Temperate and Tropical Soils:

- Effect of Native and Agroecosystems.” *Biogeochemistry* 92 (1-2): 129–43. doi:10.1007/s10533-008-9261-0.
- Fang, Y., B. Singh, B. P. Singh, and E. Krull. 2014. “Biochar Carbon Stability in Four Contrasting Soils.” *European Journal of Soil Science* 65 (1): 60–71. doi:10.1111/ejss.12094.
- Fang, Yunying, Balwant Singh, and Bhupinder Pal Singh. 2015. “Effect of Temperature on Biochar Priming Effects and Its Stability in Soils.” *Soil Biology & Biochemistry* 80 (January): 136–45. doi:10.1016/j.soilbio.2014.10.006.
- Fang, Yunying, Bhupinder Pal Singh, and Balwant Singh. 2014. “Temperature Sensitivity of Biochar and Native Carbon Mineralisation in Biochar-Amended Soils.” *Agriculture, Ecosystems & Environment*, Environmental Benefits and Risks of Biochar Application to Soil, 191 (June): 158–67. doi:10.1016/j.agee.2014.02.018.
- Farrell, Mark, Thomas K. Kuhn, Lynne M. Macdonald, Todd M. Maddern, Daniel V. Murphy, Phillip A. Hall, Bhupinder Pal Singh, Karen Baumann, Evelyn S. Krull, and Jeff A. Baldock. 2013. “Microbial Utilisation of Biochar-Derived Carbon.” *Science of The Total Environment*, Soil as a Source & Sink for Greenhouse Gases, 465 (November): 288–97. doi:10.1016/j.scitotenv.2013.03.090.
- Fischer, Hubertus, J. Schmitt, S. Eggleston, R. Schneider, J. Elsig, F. Joos, M. Leuenberger, et al. 2015. “Ice Core-Based Isotopic Constraints on Past Carbon Cycle Changes.” *PAGES Magazine* 23 (1): 12–13.
- Foley, Michael, Scott Turn, Milton Staackmann, and Terry Surles. 2007. “A Scenario for Accelerated Use of Renewable Resources for Transportation Fuels in Hawaii.” Hawaii Natural Energy Institute. https://www.eere-pmc.energy.gov/states/Hawaii_Docs/Renewable_Fuel_Report.pdf.
- Food and Agriculture Organization. 2015. “Agricultural Land (% of Land Area) | Data | Graph.” <http://data.worldbank.org/indicator/AG.LND.AGRI.ZS/countries/1W-US?display=graph>.
- Fowler, David, Mhairi Coyle, Ute Skiba, Mark A. Sutton, J. Neil Cape, Stefan Reis, Lucy J. Sheppard, et al. 2013. “The Global Nitrogen Cycle in the Twenty-First Century.” *Phil. Trans. R. Soc. B* 368 (1621): 20130164. doi:10.1098/rstb.2013.0164.
- Frey, Serita D., Juhwan Lee, Jerry M. Melillo, and Johan Six. 2013. “The Temperature Response of Soil Microbial Efficiency and Its Feedback to Climate.” *Nature Climate Change* 3 (4): 395–98. doi:10.1038/nclimate1796.
- Galvez, A., T. Sinicco, M.L. Cayuela, M.D. Mingorance, F. Fornasier, and C. Mondini. 2012. “Short Term Effects of Bioenergy by-Products on Soil C and N Dynamics, Nutrient Availability and Biochemical Properties.” *Agriculture, Ecosystems & Environment* 160 (October): 3–14. doi:10.1016/j.agee.2011.06.015.
- Garnett, T, M.C. Appleby, A Balmford, I.J. Bateman, T.G. Benton, P. Bloomer, B. Burlingame, et al. 2013. “Sustainable Intensification in Agriculture: Premises and Policies” 341.
- Ghani, A, M Dexter, and K.W Perrott. 2003. “Hot-Water Extractable Carbon in Soils: A Sensitive Measurement for Determining Impacts of Fertilisation, Grazing and Cultivation.” *Soil Biology and Biochemistry* 35 (9): 1231–43. doi:10.1016/S0038-0717(03)00186-X.
- Glaser, Bruno, Ludwig Haumaier, Georg Guggenberger, and Wolfgang Zech. 2001. “The ‘Terra Preta’ Phenomenon: A Model for Sustainable Agriculture in the Humid Tropics.” *Naturwissenschaften* 88 (1): 37–41. doi:10.1007/s001140000193.
- Godfray, H. Charles J., and Tara Garnett. 2014. “Food Security and Sustainable Intensification.” *Phil. Trans. R. Soc. B* 369 (1639): 20120273. doi:10.1098/rstb.2012.0273.
- Goldberg, Carey. 1996. “As Sugar Fades, Hawaii Seeks a New Cash Crop.” *The New York Times*, August 9, sec. U.S. <http://www.nytimes.com/1996/08/09/us/as-sugar-fades-hawaii-seeks-a-new-cash-crop.html>.
- Gomiero, Tiziano, David Pimentel, and Maurizio G. Paoletti. 2011. “Environmental Impact of Different Agricultural Management Practices: Conventional vs. Organic Agriculture.” *Critical Reviews in Plant Sciences* 30 (1-2): 95–124. doi:10.1080/07352689.2011.554355.

- Greenstone, Michael, Elizabeth Kopits, and Ann Wolverton. 2013. "Developing a Social Cost of Carbon for US Regulatory Analysis: A Methodology and Interpretation." *Review of Environmental Economics and Policy* 7 (1): 23–46. doi:10.1093/reep/res015.
- Gruber, Nicolas, and James N. Galloway. 2008. "An Earth-System Perspective of the Global Nitrogen Cycle." *Nature* 451 (7176): 293–96. doi:10.1038/nature06592.
- Güereña, David, Johannes Lehmann, Kelly Hanley, Akio Enders, Charles Hyland, and Susan Riha. 2013. "Nitrogen Dynamics Following Field Application of Biochar in a Temperate North American Maize-Based Production System." *Plant and Soil* 365 (1-2): 239–54. doi:10.1007/s11104-012-1383-4.
- Güereña, David T., Johannes Lehmann, Janice E. Thies, Akio Enders, Nancy Karanja, and Henry Neufeldt. 2015. "Partitioning the Contributions of Biochar Properties to Enhanced Biological Nitrogen Fixation in Common Bean (*Phaseolus Vulgaris*)." *Biology and Fertility of Soils* 51 (4): 479–91. doi:10.1007/s00374-014-0990-z.
- Hagerty, Shannon B., Kees Jan van Groenigen, Steven D. Allison, Bruce A. Hungate, Egbert Schwartz, George W. Koch, Randall K. Kolka, and Paul Dijkstra. 2014. "Accelerated Microbial Turnover but Constant Growth Efficiency with Warming in Soil." *Nature Climate Change* 4 (10): 903–6. doi:10.1038/nclimate2361.
- Harter, Johannes, Hans-Martin Krause, Stefanie Schuettler, Reiner Ruser, Markus Fromme, Thomas Scholten, Andreas Kappler, and Sebastian Behrens. 2014. "Linking N₂O Emissions from Biochar-Amended Soil to the Structure and Function of the N-Cycling Microbial Community." *The ISME Journal* 8 (3): 660–74. doi:10.1038/ismej.2013.160.
- HÉNAULT, C., A. GROSSEL, B. MARY, M. ROUSSEL, and J. LÉONARD. 2012. "Nitrous Oxide Emission by Agricultural Soils: A Review of Spatial and Temporal Variability for Mitigation." *Pedosphere* 22 (4): 426–33. doi:10.1016/S1002-0160(12)60029-0.
- Higgins, Steven A., Allana Welsh, Luis H. Orellana, Konstantinos T. Konstantinidis, Joanne C. Chee-Sanford, Robert A. Sanford, Christopher W. Schadt, and Frank E. Löffler. 2016. "Detection and Diversity of Fungal Nitric Oxide Reductase Genes (p450nor) in Agricultural Soils." *Applied and Environmental Microbiology*, March, AEM.00243–16. doi:10.1128/AEM.00243-16.
- Hopkins, Francesca M., Timothy R. Filley, Gerd Gleixner, Markus Lange, Sara M. Top, and Susan E. Trumbore. 2014. "Increased Belowground Carbon Inputs and Warming Promote Loss of Soil Organic Carbon through Complementary Microbial Responses." *Soil Biology and Biochemistry* 76 (September): 57–69. doi:10.1016/j.soilbio.2014.04.028.
- IPCC. 2014. "Climate Change 2014: Impacts, Adaptations, and Vulnerability. Summary for Policymakers." http://www.ipcc.ch/pdf/assessment-report/ar5/wg2/ar5_wgII_spm_en.pdf.
- Ippolito, James A., David A. Laird, and Warren J. Busscher. 2012. "Environmental Benefits of Biochar." *Journal of Environment Quality* 41 (4): 967. doi:10.2134/jeq2012.0151.
- Jeffery, S., F.G.A. Verheijen, M. van der Velde, and A.C. Bastos. 2011. "A Quantitative Review of the Effects of Biochar Application to Soils on Crop Productivity Using Meta-Analysis." *Agriculture, Ecosystems & Environment* 144 (1): 175–87. doi:10.1016/j.agee.2011.08.015.
- Jin, Hongyan. 2010. "Characterization of Microbial Life Colonizing Biochar and Biochar-Amended Soils." Dissertation, Cornell University.
- Jones, Christopher M., Daniel R. H. Graf, David Bru, Laurent Philippot, and Sara Hallin. 2013. "The Unaccounted yet Abundant Nitrous Oxide-Reducing Microbial Community: A Potential Nitrous Oxide Sink." *The ISME Journal* 7 (2): 417–26. doi:10.1038/ismej.2012.125.
- Jones, D.L., J. Rousk, G. Edwards-Jones, T.H. DeLuca, and D.V. Murphy. 2012. "Biochar-Mediated Changes in Soil Quality and Plant Growth in a Three Year Field Trial." *Soil Biology and Biochemistry* 45 (February): 113–24. doi:10.1016/j.soilbio.2011.10.012.
- Kammann, Claudia, Stefan Ratering, Christian Eckhard, and Christoph Müller. 2012. "Biochar and Hydrochar Effects on Greenhouse Gas (Carbon Dioxide, Nitrous Oxide, and Methane) Fluxes from Soils." *Journal of Environment Quality* 41 (4): 1052. doi:10.2134/jeq2011.0132.

- Karhu, Kristiina, Marc D. Auffret, Jennifer A. J. Dungait, David W. Hopkins, James I. Prosser, Brajesh K. Singh, Jens-Arne Subke, et al. 2014a. "Temperature Sensitivity of Soil Respiration Rates Enhanced by Microbial Community Response." *Nature* 513 (7516): 81–84. doi:10.1038/nature13604.
- . 2014b. "Temperature Sensitivity of Soil Respiration Rates Enhanced by Microbial Community Response." *Nature* 513 (7516): 81–84. doi:10.1038/nature13604.
- Karhu, Kristiina, Tuomas Mattila, Irina Bergström, and Kristiina Regina. 2011. "Biochar Addition to Agricultural Soil Increased CH₄ Uptake and Water Holding Capacity – Results from a Short-Term Pilot Field Study." *Agriculture, Ecosystems & Environment* 140 (1–2): 309–13. doi:10.1016/j.agee.2010.12.005.
- Kell, Douglas B. 2012. "Large-Scale Sequestration of Atmospheric Carbon via Plant Roots in Natural and Agricultural Ecosystems: Why and How." *Phil. Trans. R. Soc. B* 367 (1595): 1589–97. doi:10.1098/rstb.2011.0244.
- Kerr, Rachel Bezner. 2012. "Lessons from the Old Green Revolution for the New: Social, Environmental and Nutritional Issues for Agricultural Change in Africa." *Progress in Development Studies* 12 (2/3): 213–29. doi:10.1177/146499341101200308.
- Khalil, K, B Mary, and P Renault. 2004. "Nitrous Oxide Production by Nitrification and Denitrification in Soil Aggregates as Affected by O₂ Concentration." *Soil Biology and Biochemistry* 36 (4): 687–99. doi:10.1016/j.soilbio.2004.01.004.
- Kloss, Stefanie, Franz Zehetner, Alex Dellantonio, Raad Hamid, Franz Ottner, Volker Liedtke, Manfred Schwanninger, Martin H. Gerzabek, and Gerhard Soja. 2012. "Characterization of Slow Pyrolysis Biochars: Effects of Feedstocks and Pyrolysis Temperature on Biochar Properties." *Journal of Environment Quality* 41 (4): 990. doi:10.2134/jeq2011.0070.
- Koranda, Marianne, Christina Kaiser, Lucia Fuchsluger, Barbara Kitzler, Angela Sessitsch, Sophie Zechmeister-Boltenstern, and Andreas Richter. 2014. "Fungal and Bacterial Utilization of Organic Substrates Depends on Substrate Complexity and N Availability." *FEMS Microbiology Ecology* 87 (1): 142–52. doi:10.1111/1574-6941.12214.
- Kozich, James J., Sarah L. Westcott, Nielson T. Baxter, Sarah K. Highlander, and Patrick D. Schloss. 2013. "Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform." *Applied and Environmental Microbiology* 79 (17): 5112–20. doi:10.1128/AEM.01043-13.
- Kuzyakov, Yakov, Irina Bogomolova, and Bruno Glaser. 2014. "Biochar Stability in Soil: Decomposition during Eight Years and Transformation as Assessed by Compound-Specific ¹⁴C Analysis." *Soil Biology and Biochemistry* 70 (March): 229–36. doi:10.1016/j.soilbio.2013.12.021.
- Kuzyakov, Yakov, Irina Subbotina, Haiqing Chen, Irina Bogomolova, and Xingliang Xu. 2009. "Black Carbon Decomposition and Incorporation into Soil Microbial Biomass Estimated by ¹⁴C Labeling." *Soil Biology and Biochemistry* 41 (2): 210–19. doi:10.1016/j.soilbio.2008.10.016.
- Laird, David A., Pierce Fleming, Dedrick D. Davis, Robert Horton, Baiqun Wang, and Douglas L. Karlen. 2010. "Impact of Biochar Amendments on the Quality of a Typical Midwestern Agricultural Soil." *Geoderma* 158 (3–4): 443–49. doi:10.1016/j.geoderma.2010.05.013.
- Lal, Rattan. 2008. "Carbon Sequestration." *Philosophical Transactions of the Royal Society B: Biological Sciences* 363 (1492): 815–30. doi:10.1098/rstb.2007.2185.
- Lehmann, Johannes. 2007a. "A Handful of Carbon." *Nature* 447 (7141): 143–44. doi:10.1038/447143a.
- . 2007b. "Bio-Energy in the Black." *Frontiers in Ecology and the Environment* 5 (7): 381–87.
- Lehmann, Johannes, John Gaunt, and Marco Rondon. 2006. "Bio-Char Sequestration in Terrestrial Ecosystems – A Review." *Mitigation and Adaptation Strategies for Global Change* 11 (2): 395–419. doi:10.1007/s11027-005-9006-5.
- Lehmann, Johannes, Matthias C. Rillig, Janice Thies, Caroline A. Masiello, William C. Hockaday, and David Crowley. 2011. "Biochar Effects on Soil Biota – A Review." *Soil Biology and Biochemistry* 43 (9): 1812–36. doi:10.1016/j.soilbio.2011.04.022.

- Lertrat, Kamol, and Taweesak Pulam. 2007. "Breeding for Increased Sweetness in Sweet Corn." *Internation Journal of Plant Breeding* 1 (1): 27–30.
- Liang, Biqing, Johannes Lehmann, Saran P. Sohi, Janice E. Thies, Brendan O'Neill, Lucerina Trujillo, John Gaunt, et al. 2010. "Black Carbon Affects the Cycling of Non-Black Carbon in Soil." *Organic Geochemistry*, 2008 Australian Organic Geochemistry Conference: A national conference held in association with the International Humic Substances Society and the Natural Organic Matter Interest Group, 41 (2): 206–13. doi:10.1016/j.orggeochem.2009.09.007.
- Liang, Biqing, Johannes Lehmann, Dawit Solomon, Saran Sohi, Janice E. Thies, Jan O. Skjemstad, Flavio J. Luizão, Mark H. Engelhard, Eduardo G. Neves, and Sue Wirick. 2008. "Stability of Biomass-Derived Black Carbon in Soils." *Geochimica et Cosmochimica Acta* 72 (24): 6069–78. doi:10.1016/j.gca.2008.09.028.
- Liang, B., J. Lehmann, D. Solomon, J. Kinyangi, J. Grossman, B. O'Neill, J. O. Skjemstad, et al. 2006. "Black Carbon Increases Cation Exchange Capacity in Soils." *Soil Science Society of America Journal* 70 (5): 1719. doi:10.2136/sssaj2005.0383.
- Liu, Xiao-yu, Jing-jing Qu, Lian-qing Li, A-feng Zhang, Zheng Jufeng, Jin-wei Zheng, and Gen-xing Pan. 2012. "Can Biochar Amendment Be an Ecological Engineering Technology to Depress N₂O Emission in Rice paddies?—A Cross Site Field Experiment from South China." *Ecological Engineering* 42 (May): 168–73. doi:10.1016/j.ecoleng.2012.01.016.
- Loope, Lloyd L., and Thomas W. Giambelluca. 1998. "Vulnerability of Island Tropical Montane Cloud Forests to Climate Change, with Special Reference to East Maui, Hawaii." *Climatic Change* 39 (2-3): 503–17. doi:10.1023/A:1005372118420.
- Luo, Chengwei, Luis M. Rodriguez-R, Eric R. Johnston, Liyou Wu, Lei Cheng, Kai Xue, Qichao Tu, et al. 2014. "Soil Microbial Community Responses to a Decade of Warming as Revealed by Comparative Metagenomics." *Applied and Environmental Microbiology* 80 (5): 1777–86. doi:10.1128/AEM.03712-13.
- Luo, Y., M. Durenkamp, M. De Nobili, Q. Lin, and P.C. Brookes. 2011. "Short Term Soil Priming Effects and the Mineralisation of Biochar Following Its Incorporation to Soils of Different pH." *Soil Biology and Biochemistry* 43 (11): 2304–14. doi:10.1016/j.soilbio.2011.07.020.
- Lupwayi, N. Z., W. A. Rice, and G. W. Clayton. 1998. "Soil Microbial Diversity and Community Structure under Wheat as Influenced by Tillage and Crop Rotation." *Soil Biology and Biochemistry* 30 (13): 1733–41. doi:10.1016/S0038-0717(98)00025-X.
- Major, Julie, Marco Rondon, Diego Molina, Susan J. Riha, and Johannes Lehmann. 2010. "Maize Yield and Nutrition during 4 Years after Biochar Application to a Colombian Savanna Oxisol." *Plant and Soil* 333 (1-2): 117–28. doi:10.1007/s11104-010-0327-0.
- Manyà, Joan J. 2012. "Pyrolysis for Biochar Purposes: A Review to Establish Current Knowledge Gaps and Research Needs." *Environmental Science & Technology* 46 (15): 7939–54. doi:10.1021/es301029g.
- Marquina, Sorena, Tibusay Pérez, Loreto Donoso, Adriana Giuliani, Rafael Rasse, and Francisco Herrera. 2014. "NO, N₂O and CO₂ Soil Emissions from Venezuelan Corn Fields under Tillage and No-Tillage Agriculture." *Nutrient Cycling in Agroecosystems* 101 (1): 123–37. doi:10.1007/s10705-014-9659-0.
- Mbuthia, Lilian Wanjiru, Veronica Acosta-Martínez, Jennifer DeBruyn, Sean Schaeffer, Donald Tyler, Evah Odoi, Molefi Mpheshea, Forbes Walker, and Neal Eash. 2015. "Long Term Tillage, Cover Crop, and Fertilization Effects on Microbial Community Structure, Activity: Implications for Soil Quality." *Soil Biology and Biochemistry* 89 (October): 24–34. doi:10.1016/j.soilbio.2015.06.016.
- McHenry, Mark P. 2011. "Soil Organic Carbon, Biochar, and Applicable Research Results for Increasing Farm Productivity under Australian Agricultural Conditions." *Communications in Soil Science and Plant Analysis* 42 (10): 1187–99. doi:10.1080/00103624.2011.566963.
- Melillo, J. M., P. A. Steudler, J. D. Aber, K. Newkirk, H. Lux, F. P. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau. 2002. "Soil Warming and Carbon-Cycle Feedbacks to the Climate System." *Science* 298 (5601): 2173–76. doi:10.1126/science.1074153.

- Mia, S., J. W. van Groenigen, T. F. J. van de Voorde, N. J. Oram, T. M. Bezemer, L. Mommer, and S. Jeffery. 2014. "Biochar Application Rate Affects Biological Nitrogen Fixation in Red Clover Conditional on Potassium Availability." *Agriculture, Ecosystems & Environment*, Environmental Benefits and Risks of Biochar Application to Soil, 191 (June): 83–91. doi:10.1016/j.agee.2014.03.011.
- Mitchell, David C., Michael J. Castellano, John E. Sawyer, and Jose Pantoja. 2013. "Cover Crop Effects on Nitrous Oxide Emissions: Role of Mineralizable Carbon." *Soil Science Society of America Journal* 77 (5): 1765. doi:10.2136/sssaj2013.02.0074.
- Morais, Rafael F. de, Diego M. Quesada, Veronica M. Reis, Segundo Urquiaga, Bruno J. R. Alves, and Robert M. Boddey. 2012. "Contribution of Biological Nitrogen Fixation to Elephant Grass (*Pennisetum Purpureum* Schum.)." *Plant and Soil* 356 (1-2): 23–34. doi:10.1007/s11104-011-0944-2.
- Mukherjee, A., R. Lal, and A. R. Zimmerman. 2014. "Effects of Biochar and Other Amendments on the Physical Properties and Greenhouse Gas Emissions of an Artificially Degraded Soil." *Science of The Total Environment* 487 (July): 26–36. doi:10.1016/j.scitotenv.2014.03.141.
- Mukherjee, Atanu, and Rattan Lal. 2013. "Biochar Impacts on Soil Physical Properties and Greenhouse Gas Emissions." *Agronomy* 3 (2): 313–39. doi:10.3390/agronomy3020313.
- Novak, Jeffrey M., Isabel Lima, Baoshan Xing, Julia W. Gaskin, Christoph Steiner, K. C. Das, Mohamed Ahmedna, Djaafar Rehrah, Donald W. Watts, and Warren J. Busscher. 2009. "Characterization of Designer Biochar Produced at Different Temperatures and Their Effects on a Loamy Sand." *Annals of Environmental Science* 3 (1): 2.
- O'Neill, B., J. Grossman, M. T. Tsai, J. E. Gomes, J. Lehmann, J. Peterson, E. Neves, and J. E. Thies. 2009. "Bacterial Community Composition in Brazilian Anthrosols and Adjacent Soils Characterized Using Culturing and Molecular Identification." *Microbial Ecology* 58 (1): 23–35. doi:10.1007/s00248-009-9515-y.
- Page, K. L., R. C. Dalal, M. J. Pringle, M. Bell, Y. P. Dang, B. Radford, and K. Bailey. 2013. "Organic Carbon Stocks in Cropping Soils of Queensland, Australia, as Affected by Tillage Management, Climate, and Soil Characteristics." *Soil Research* 51 (8): 596–607.
- Paudel, Shukra Raj, Ohkyung Choi, Samir Kumar Khanal, Kartik Chandran, Sungpyo Kim, and Jae Woo Lee. 2015. "Effects of Temperature on Nitrous Oxide (N₂O) Emission from Intensive Aquaculture System." *Science of The Total Environment* 518–519 (June): 16–23. doi:10.1016/j.scitotenv.2015.02.076.
- Paustian, K., J. Six, E. T. Elliott, and H. W. Hunt. 2000. "Management Options for Reducing CO₂ Emissions from Agricultural Soils." *Biogeochemistry* 48 (1): 147–63. doi:10.1023/A:1006271331703.
- Phillips, R. L., A. M. S. McMillan, T. Palmada, J. Dando, and D. Giltrap. 2015. "Temperature Effects on N₂O and N₂ Denitrification End-Products for a New Zealand Pasture Soil." *New Zealand Journal of Agricultural Research* 58 (1): 89–95. doi:10.1080/00288233.2014.969380.
- Pietikäinen, Janna, Oili Kiikkilä, and Hannu Fritze. 2000. "Charcoal as a Habitat for Microbes and Its Effect on the Microbial Community of the Underlying Humus." *Oikos* 89 (2): 231–42. doi:10.1034/j.1600-0706.2000.890203.x.
- Porter, J.R., L Xie, A.J Challinor, K Cochrane, S.M Howden, M.M Iqbal, D.B. Lobell, and M.I. Travesso. 2014. "Food Security and Food Production Systems. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change." Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., http://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-Chap7_FINAL.pdf.
- Powlson, David S., Clare M. Stirling, M. L. Jat, Bruno G. Gerard, Cheryl A. Palm, Pedro A. Sanchez, and Kenneth G. Cassman. 2014. "Limited Potential of No-till Agriculture for Climate Change Mitigation." *Nature Climate Change* 4 (8): 678–83. doi:10.1038/nclimate2292.

- Powlson, D. S., P. J. Gregory, W. R. Whalley, J. N. Quinton, D. W. Hopkins, A. P. Whitmore, P. R. Hirsch, and K. W. T. Goulding. 2011. "Soil Management in Relation to Sustainable Agriculture and Ecosystem Services." *Food Policy*, The challenge of global food sustainability, 36, Supplement 1 (January): S72–87. doi:10.1016/j.foodpol.2010.11.025.
- Qiao, Na, Douglas Schaefer, Evgenia Blagodatskaya, Xiaoming Zou, Xingliang Xu, and Yakov Kuzyakov. 2014. "Labile Carbon Retention Compensates for CO₂ Released by Priming in Forest Soils." *Global Change Biology* 20 (6): 1943–54. doi:10.1111/gcb.12458.
- Quilliam, Richard S., Helen C. Glanville, Stephen C. Wade, and Davey L. Jones. 2013. "Life in the 'charosphere' – Does Biochar in Agricultural Soil Provide a Significant Habitat for Microorganisms?" *Soil Biology and Biochemistry* 65 (October): 287–93. doi:10.1016/j.soilbio.2013.06.004.
- Quilliam, Richard S., Karina A. Marsden, Christoph Gertler, Johannes Rousk, Thomas H. DeLuca, and Davey L. Jones. 2012. "Nutrient Dynamics, Microbial Growth and Weed Emergence in Biochar Amended Soil Are Influenced by Time since Application and Reapplication Rate." *Agriculture, Ecosystems & Environment* 158 (September): 192–99. doi:10.1016/j.agee.2012.06.011.
- Ramnarine, R., R. P. Voroney, C. Wagner-Riddle, and K. E. Dunfield. 2015. "Conventional and No-Tillage Effects on the Distribution of Crop Residues and Light Fraction Organic Matter." *Soil Science Society of America Journal* 79 (1): 74. doi:10.2136/sssaj2014.05.0182.
- Revell, Kenneth T., Rory O. Maguire, and Foster A. Agblevor. 2012. "Field Trials With Poultry Litter Biochar and Its Effect on Forages, Green Peppers, and Soil Properties." *Soil Science* 177 (10): 573–79. doi:10.1097/SS.0b013e3182741050.
- Rondon, Marco A., Johannes Lehmann, Juan Ramírez, and Maria Hurtado. 2007. "Biological Nitrogen Fixation by Common Beans (*Phaseolus Vulgaris* L.) Increases with Bio-Char Additions." *Biology and Fertility of Soils* 43 (6): 699–708. doi:10.1007/s00374-006-0152-z.
- Roper, M. M., I. R. P. Fillery, R. Jongepier, P. Sanford, L. M. Macdonald, J. Sanderman, and J. A. Baldock. 2013. "Allocation into Soil Organic Matter Fractions of ¹⁴C Captured via Photosynthesis by Two Perennial Grass Pastures." *Soil Research* 51 (8): 748–59.
- Rotzoll, Kolja, and Charles H. Fletcher. 2013. "Assessment of Groundwater Inundation as a Consequence of Sea-Level Rise." *Nature Climate Change* 3 (5): 477–81. doi:10.1038/nclimate1725.
- Rousk, Johannes, Erland Bååth, Philip C. Brookes, Christian L. Lauber, Catherine Lozupone, J. Gregory Caporaso, Rob Knight, and Noah Fierer. 2010. "Soil Bacterial and Fungal Communities across a pH Gradient in an Arable Soil." *The ISME Journal* 4 (10): 1340–51. doi:10.1038/ismej.2010.58.
- Rousk, Johannes, Serita D. Frey, and Erland Bååth. 2012. "Temperature Adaptation of Bacterial Communities in Experimentally Warmed Forest Soils." *Global Change Biology* 18 (10): 3252–58. doi:10.1111/j.1365-2486.2012.02764.x.
- Rutigliano, F. A., M. Romano, R. Marzaioli, I. Baglivo, S. Baronti, F. Miglietta, and S. Castaldi. 2014. "Effect of Biochar Addition on Soil Microbial Community in a Wheat Crop." *European Journal of Soil Biology* 60 (January): 9–15. doi:10.1016/j.ejsobi.2013.10.007.
- Saleh-Lakha, Saleema, Michelle Miller, Rachel G. Campbell, Kim Schneider, Parastu Elahimanesh, Miranda M. Hart, and Jack T. Trevors. 2005. "Microbial Gene Expression in Soil: Methods, Applications and Challenges." *Journal of Microbiological Methods* 63 (1): 1–19. doi:10.1016/j.mimet.2005.03.007.
- Schlesinger, William H., and Jeffrey A. Andrews. 2000. "Soil Respiration and the Global Carbon Cycle." *Biogeochemistry* 48 (1): 7–20.
- Schnell, Ronnie W., Donald M. Vietor, Tony L. Provin, Clyde L. Munster, and Sergio Capareda. 2012. "Capacity of Biochar Application to Maintain Energy Crop Productivity: Soil Chemistry, Sorghum Growth, and Runoff Water Quality Effects." *Journal of Environment Quality* 41 (4): 1044. doi:10.2134/jeq2011.0077.
- Shakun, Jeremy D., Peter U. Clark, Feng He, Shaun A. Marcott, Alan C. Mix, Zhengyu Liu, Bette Otto-Bliesner, Andreas Schmittner, and Edouard Bard. 2012. "Global Warming Preceded by

- Increasing Carbon Dioxide Concentrations during the Last Deglaciation.” *Nature* 484 (7392): 49–54. doi:10.1038/nature10915.
- Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. “Stabilization Mechanisms of Soil Organic Matter: Implications for C-Saturation of Soils.” *Plant and Soil* 241 (2): 155–76. doi:10.1023/A:1016125726789.
- Six, J, E. T Elliott, and K Paustian. 2000. “Soil Macroaggregate Turnover and Microaggregate Formation: A Mechanism for C Sequestration under No-Tillage Agriculture.” *Soil Biology and Biochemistry* 32 (14): 2099–2103. doi:10.1016/S0038-0717(00)00179-6.
- Six, Johan, Stephen M. Ogle, F. Jay breidt, Rich T. Conant, Arvin R. Mosier, and Keith Paustian. 2004. “The Potential to Mitigate Global Warming with No-Tillage Management Is Only Realized When Practised in the Long Term.” *Global Change Biology* 10 (2): 155–60. doi:10.1111/j.1529-8817.2003.00730.x.
- Smil, Vaclav. 1999. “Detonator of the Population Explosion.” *Nature* 400 (6743): 415–415. doi:10.1038/22672.
- Smith, Jeffrey L., Harold P. Collins, and Vanessa L. Bailey. 2010. “The Effect of Young Biochar on Soil Respiration.” *Soil Biology and Biochemistry* 42 (12): 2345–47. doi:10.1016/j.soilbio.2010.09.013.
- Sohi, S.P., E. Krull, E. Lopez-Capel, and R. Bol. 2010. “Chapter 2 - A Review of Biochar and Its Use and Function in Soil.” In *Advances in Agronomy*, edited by Donald L. Sparks, Volume 105:47–82. Academic Press. <http://www.sciencedirect.com/science/article/pii/S0065211310050029>.
- Somerville, Chris, Heather Youngs, Caroline Taylor, Sarah C. Davis, and Stephen Long. 2010. “Feedstocks for Lignocellulosic Biofuels.” *Science* 329 (August): 790–92.
- Sparks, D.L., ed. 1996. *Methods of Soil Analysis Part 3-Chemical Methods*. SSSA Book Series 5. Madison, Wisconsin, USA: Soil Science Society of America, Inc.; American Society of Agronomy, Inc.
- Spokas, Keri B. Cantrell, Jeffrey M. Novak, David W. Archer, James A. Ippolito, Harold P. Collins, Akwasi A. Boateng, et al. 2012. “Biochar: A Synthesis of Its Agronomic Impact beyond Carbon Sequestration.” *Journal of Environment Quality* 41 (4): 973. doi:10.2134/jeq2011.0069.
- Spokas, K. A., J. M. Novak, C. A. Masiello, M. G. Johnson, E. C. Colosky, J. A. Ippolito, and C. Trigo. 2014. “Physical Disintegration of Biochar: An Overlooked Process.” *Environmental Science & Technology Letters* 1 (8): 326–32. doi:10.1021/ez500199t.
- Sradnick, André, Rajasekaran Murugan, Meike Oltmanns, Joachim Raupp, and Rainer Georg Joergensen. 2013. “Changes in Functional Diversity of the Soil Microbial Community in a Heterogeneous Sandy Soil after Long-Term Fertilization with Cattle Manure and Mineral Fertilizer.” *Applied Soil Ecology* 63 (January): 23–28. doi:10.1016/j.apsoil.2012.09.011.
- Stevenson, James R., Nelson Villoria, Derek Byerlee, Timothy Kelley, and Mywish Maredia. 2013. “Green Revolution Research Saved an Estimated 18 to 27 Million Hectares from Being Brought into Agricultural Production.” *Proceedings of the National Academy of Sciences* 110 (21): 8363–68. doi:10.1073/pnas.1208065110.
- Stewart, Catherine E., Ronald F. Follett, Elizabeth G. Pruessner, Gary E. Varvel, Kenneth P. Vogel, and Robert B. Mitchell. 2015. “Nitrogen and Harvest Effects on Soil Properties under Rainfed Switchgrass and No-till Corn over 9 Years: Implications for Soil Quality.” *GCB Bioenergy* 7 (2): 288–301. doi:10.1111/gcbb.12142.
- Strezov, Vladimir, Tim J. Evans, and Chris Hayman. 2008. “Thermal Conversion of Elephant Grass (*Pennisetum Purpureum* Schum) to Bio-Gas, Bio-Oil and Charcoal.” *Bioresource Technology* 99 (17): 8394–99. doi:10.1016/j.biortech.2008.02.039.
- Taghizadeh-Toosi, Arezoo, Tim J. Clough, Robert R. Sherlock, and Leo M. Condron. 2011. “Biochar Adsorbed Ammonia Is Bioavailable.” *Plant and Soil* 350 (1-2): 57–69. doi:10.1007/s11104-011-0870-3.

- Talbot, Jennifer M., and Kathleen K. Treseder. 2011. "Interactions among Lignin, Cellulose, and Nitrogen Drive Litter Chemistry–decay Relationships." *Ecology* 93 (2): 345–54. doi:10.1890/11-0843.1.
- Thiessen, Stefany, Gerd Gleixner, Thomas Wutzler, and Markus Reichstein. 2013. "Both Priming and Temperature Sensitivity of Soil Organic Matter Decomposition Depend on Microbial Biomass – An Incubation Study." *Soil Biology and Biochemistry* 57 (February): 739–48. doi:10.1016/j.soilbio.2012.10.029.
- Tscharntke, Teja, Yann Clough, Thomas C. Wanger, Louise Jackson, Iris Motzke, Ivette Perfecto, John Vandermeer, and Anthony Whitbread. 2012. "Global Food Security, Biodiversity Conservation and the Future of Agricultural Intensification." *Biological Conservation*, ADVANCING ENVIRONMENTAL CONSERVATION: ESSAYS IN HONOR OF NAVJOT SODHI, 151 (1): 53–59. doi:10.1016/j.biocon.2012.01.068.
- Uchida, R., and N. V. Hue. 2000. "Soil Acidity and Liming." *Plant Nutrient Management in Hawaiian Soils, Approaches for Tropical and Subtropical Agriculture*. Edited by JA Silva, and R. Uchida. University of Hawaii, Honolulu, 101–11.
- USDA. 2013a. "Official Series Description - WAHIAWA Series." Accessed July 26. https://soilseries.sc.egov.usda.gov/OSD_Docs/W/WAHIAWA.html.
- . 2013b. "Official Series Description - WAIALUA Series." Accessed July 26. https://soilseries.sc.egov.usda.gov/OSD_Docs/W/WAIALUA.html.
- US Department of Commerce, NOAA. 2016. "ESRL Global Monitoring Division - Global Greenhouse Gas Reference Network." Accessed March 6. <http://www.esrl.noaa.gov/gmd/ccgg/trends/weekly.html>.
- US EPA, Climate Change Division. 2016a. "Agriculture." Overviews & Factsheets,. Accessed February 2. <http://www3.epa.gov/climatechange/ghgemissions/sources/agriculture.html>.
- . 2016b. "Global Emissions." Overviews & Factsheets,. Accessed February 24. <http://www3.epa.gov/climatechange/ghgemissions/gwps.html>.
- . 2015c. "U.S. Tropical Islands Impacts & Adaptation." Overviews & Factsheets,. Accessed April 26. <http://www.epa.gov/climatechange/impacts-adaptation/islands.html>.
- Ussiri, David, and Rattan Lal. 2013. "Global Sources of Nitrous Oxide." In *Soil Emission of Nitrous Oxide and Its Mitigation*, 131–75. Springer Netherlands. http://link.springer.com.eres.library.manoa.hawaii.edu/chapter/10.1007/978-94-007-5364-8_5.
- Valentine, John, John Clifton-Brown, Astley Hastings, Paul Robson, Gordon Allison, and Pete Smith. 2012. "Food vs. Fuel: The Use of Land for Lignocellulosic 'next Generation' Energy Crops That Minimize Competition with Primary Food Production." *GCB Bioenergy* 4 (1): 1–19. doi:10.1111/j.1757-1707.2011.01111.x.
- Van Zwieten, L., B. P. Singh, S. W. L. Kimber, D. V. Murphy, L. M. Macdonald, J. Rust, and S. Morris. 2014. "An Incubation Study Investigating the Mechanisms That Impact N₂O Flux from Soil Following Biochar Application." *Agriculture, Ecosystems & Environment*, Environmental Benefits and Risks of Biochar Application to Soil, 191 (June): 53–62. doi:10.1016/j.agee.2014.02.030.
- Venterea, Rodney T., and Dennis E. Rolston. 2000. "Mechanisms and Kinetics of Nitric and Nitrous Oxide Production during Nitrification in Agricultural Soil." *Global Change Biology* 6 (3): 303–16. doi:10.1046/j.1365-2486.2000.00309.x.
- Vitousek, Peter M., John D. Aber, Robert W. Howarth, Gene E. Likens, Pamela A. Matson, David W. Schindler, William H. Schlesinger, and David G. Tilman. 1997. "Human Alteration of the Global Nitrogen Cycle: Sources and Consequences." *Ecological Applications* 7 (3): 737–50. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2.
- Vitousek, Peter M., Ken Cassman, Cory Cleveland, Tim Crews, Christopher B. Field, Nancy B. Grimm, Robert W. Howarth, et al. 2002. "Towards an Ecological Understanding of Biological Nitrogen Fixation." *Biogeochemistry* 57-58 (1): 1–45. doi:10.1023/A:1015798428743.

- Wang, Jinyang, Xiaojian Pan, Yinglie Liu, Xiaolin Zhang, and Zhengqin Xiong. 2012. "Effects of Biochar Amendment in Two Soils on Greenhouse Gas Emissions and Crop Production." *Plant and Soil* 360 (1-2): 287–98. doi:10.1007/s11104-012-1250-3.
- Wang, Xin, Lingli Liu, Shilong Piao, Ivan A. Janssens, Jianwu Tang, Weixing Liu, Yonggang Chi, Jing Wang, and Shan Xu. 2014. "Soil Respiration under Climate Warming: Differential Response of Heterotrophic and Autotrophic Respiration." *Global Change Biology* 20 (10): 3229–37. doi:10.1111/gcb.12620.
- Warnock, Daniel D., Johannes Lehmann, Thomas W. Kuyper, and Matthias C. Rillig. 2007. "Mycorrhizal Responses to Biochar in Soil – Concepts and Mechanisms." *Plant and Soil* 300 (1-2): 9–20. doi:10.1007/s11104-007-9391-5.
- Warren, R., J. VanDerWal, J. Price, J. A. Welbergen, I. Atkinson, J. Ramirez-Villegas, T. J. Osborn, et al. 2013. "Quantifying the Benefit of Early Climate Change Mitigation in Avoiding Biodiversity Loss." *Nature Climate Change* 3 (7): 678–82. doi:10.1038/nclimate1887.
- Warwick, R. M., K. R. Clarke, and Suharsono. 1990. "A Statistical Analysis of Coral Community Responses to the 1982–83 El Niño in the Thousand Islands, Indonesia." *Coral Reefs* 8 (4): 171–79. doi:10.1007/BF00265008.
- "Web Soil Survey." 2016. Accessed January 20. <http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx>.
- West, Paul C., Holly K. Gibbs, Chad Monfreda, John Wagner, Carol C. Barford, Stephen R. Carpenter, and Jonathan A. Foley. 2010. "Trading Carbon for Food: Global Comparison of Carbon Stocks vs. Crop Yields on Agricultural Land." *Proceedings of the National Academy of Sciences* 107 (46): 19645–48. doi:10.1073/pnas.1011078107.
- West, Tristram O., and Gregg Marland. 2002. "A Synthesis of Carbon Sequestration, Carbon Emissions, and Net Carbon Flux in Agriculture: Comparing Tillage Practices in the United States." *Agriculture, Ecosystems & Environment* 91 (1–3): 217–32. doi:10.1016/S0167-8809(01)00233-X.
- WHO. 2014. "Food Security." *Trade, Foreign Policy, Diplomacy and Health*. <http://www.who.int/trade/glossary/story028/en/>.
- Woolf, Dominic, James E. Amonette, F. Alayne Street-Perrott, Johannes Lehmann, and Stephen Joseph. 2010. "Sustainable Biochar to Mitigate Global Climate Change." *Nature Communications* 1 (August): 56. doi:10.1038/ncomms1053.
- Xiong, Zheng-Qin, Guang-Xi Xing, and Zhao-Liang Zhu. 2007. "Nitrous Oxide and Methane Emissions as Affected by Water, Soil and Nitrogen." *Pedosphere* 17 (2): 146–55. doi:10.1016/S1002-0160(07)60020-4.
- Xu, Gang, Yingchun Lv, Junna Sun, Hongbo Shao, and Linlin Wei. 2012. "Recent Advances in Biochar Applications in Agricultural Soils: Benefits and Environmental Implications." *CLEAN – Soil, Air, Water* 40 (10): 1093–98. doi:10.1002/clen.201100738.
- Yanai, Yosuke, Koki Toyota, and Masanori Okazaki. 2007. "Effects of Charcoal Addition on N₂O Emissions from Soil Resulting from Rewetting Air-Dried Soil in Short-Term Laboratory Experiments." *Soil Science and Plant Nutrition* 53 (2): 181–88. doi:10.1111/j.1747-0765.2007.00123.x.
- Yao, H., Z. He, M. J. Wilson, and C. D. Campbell. 2000. "Microbial Biomass and Community Structure in a Sequence of Soils with Increasing Fertility and Changing Land Use." *Microbial Ecology* 40 (3): 223–37. doi:10.1007/s002480000053.
- Yao, Ying, Bin Gao, June Fang, Ming Zhang, Hao Chen, Yanmei Zhou, Anne Elise Creamer, Yining Sun, and Liuyan Yang. 2014. "Characterization and Environmental Applications of Clay–biochar Composites." *Chemical Engineering Journal* 242 (April): 136–43. doi:10.1016/j.cej.2013.12.062.
- Yonemura, Seiichiro, Isamu Nouchi, Seiichi Nishimura, Gen Sakurai, Kazuki Togami, and Kazuyuki Yagi. 2013. "Soil Respiration, N₂O, and CH₄ Emissions from an Andisol under Conventional-Tillage and No-Tillage Cultivation for 4 Years." *Biology and Fertility of Soils* 50 (1): 63–74. doi:10.1007/s00374-013-0831-5.

- Zhang, Afeng, Liqiang Cui, Gengxing Pan, Lianqing Li, Qaiser Hussain, Xuhui Zhang, Jinwei Zheng, and David Crowley. 2010. "Effect of Biochar Amendment on Yield and Methane and Nitrous Oxide Emissions from a Rice Paddy from Tai Lake Plain, China." *Agriculture, Ecosystems & Environment* 139 (4): 469–75. doi:10.1016/j.agee.2010.09.003.
- Zhang, Shuangfu, Yayi Wang, Weitao He, Min Wu, Meiyang Xing, Jian Yang, Naiyun Gao, and Mianli Pan. 2014. "Impacts of Temperature and Nitrifying Community on Nitrification Kinetics in a Moving-Bed Biofilm Reactor Treating Polluted Raw Water." *Chemical Engineering Journal* 236 (January): 242–50. doi:10.1016/j.cej.2013.09.086.
- Zhao, Rudong, Neil Coles, and Jiaping Wu. 2015. "Carbon Mineralization Following Additions of Fresh and Aged Biochar to an Infertile Soil." *CATENA* 125 (February): 183–89. doi:10.1016/j.catena.2014.10.026.
- Zimmerman, Andrew R., Bin Gao, and Mi-Youn Ahn. 2011. "Positive and Negative Carbon Mineralization Priming Effects among a Variety of Biochar-Amended Soils." *Soil Biology and Biochemistry* 43 (6): 1169–79. doi:10.1016/j.soilbio.2011.02.005.