Assessment of Pyrogenic Carbonaceous Soil Amendments on Greenhouse Gas Emissions in relation to Crop Productivity

（作物生産に関与する温室効果ガス排出における

土壌改良材としての熱分解炭素質の評価）

BASALIRWA DANIEL

2020

Assessment of Pyrogenic Carbonaceous Soil Amendments on Greenhouse Gas Emissions in relation to Crop Productivity

（作物生産に関与する温室効果ガス排出における

土壌改良材としての熱分解炭素質の評価）

BASALIRWA DANIEL

2020

The United Graduate School of Agricultural Sciences, Tottori University

Assessment of Pyrogenic Carbonaceous Soil Amendments on Greenhouse Gas Emissions in relation to Crop Productivity

A Doctoral thesis

By

BASALIRWA DANIEL

D17A4110Y

Global Arid Land Science

Examination committee

Major supervisor:	Prof. Eiji NISHIHARA (Ph.D.)
Co-supervisor:	Prof. Sadahiro YAMAMOTO (Ph.D.)
Co-supervisor:	Prof. Tsugiyuki MASUNAGA (Ph.D.)
Co-examiner:	Prof. Satoshi YAMADA (Ph.D.)
Co-examiner:	Prof. Yasuomi IBARAKI (Ph.D.)

Submitted to the United Graduate School of Agricultural Sciences, Tottori University in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Agriculture

2020

DEDICATION

I dedicate this thesis to my loving family.

God bless you all!!

ACKNOWLEDGEMENTS

Special thanks to the Almighty God for the good health, wisdom and knowledge throughout this research.

I am very grateful to the Government of Japan, through the Ministry of Education, Culture, Sports, Science and Technology (MEXT) for the financial support during my Masters and Doctoral degree studies through the *Monbukagakusho* scholarship. I also thank the administration of Tottori University, Japan for all the support and assistance given to me during my stay in Japan.

My sincere appreciation to my major supervisor Prof. Eiji Nishihara, and co-supervisors Prof. Sadahiro Yamamoto and Prof. Tsugiyuki Masunaga for the guidance, support and all the other forms of advice given to me during my studies. Special thanks to Prof. Satoshi Yamada and Prof. Yasuomi Ibaraki for their valuable comments and suggestions during my doctoral defense.

I also thank Dr. Frank Kalemelawa and all members of the Arid land crop production laboratory from 2015–2020 especially Ms. Caroline Namirembe, Dr. Cosmas Wacal, Mr. Daisuke Sasagawa, Ms. Fuyumi Akae and Mr. Sho Koyama for the support, advice and words of encouragement during this study.

Sincere thanks to Dr. Shigeto Sudo and all the laboratory members at the Institute for Agro-Environmental Sciences (NIAES), NARO, Tsukuba, Japan, and also to Dr. Aung Zaw Oo at the Japan International Research Center for Agricultural Science (JIRCAS) for the technical assistance and support during my stay at NIAES in Tsukuba.

Lastly, I thank the administration of Makerere University, Kampala, Uganda for encouraging me to study at Tottori University, Japan.

God bless you all!!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

General introduction

1.1. Overview of greenhouse gas emissions

Achieving zero hunger (goal 2) and climate action (goal 13) are among the 17 Sustainable Development Goals (SDGs) stipulated by the United Nations (UN) in its 2030 agenda. The current global population of about 7.8 billion people is expected to increase to 8.5, 9.7, and 10.9 billion in 2030, 2050, and 2100 respectively with more than half of the population growth up to 2050 concentrated in nine countries; India, Nigeria, Pakistan, DRC, Ethiopia, Tanzania, Indonesia, Egypt and USA (UN DESA, 2019). The increase in the world's population implies that the arable land per capita is becoming lower. With the increasing demands for food and animal feed aimed at tackling goal 2 (zero hunger) of the SDGs, the society will be pressed to increase agricultural production either by increasing yields on already cultivated lands or by cultivating currently natural areas, and also by changing current crop consumption patterns (Licker et al., 2010). However, the need to conserve our natural ecosystems, and the rising amounts of greenhouse gas (GHG) emissions which have made climate change to occur at rates higher than we expected imply that the only feasible option would be increasing agricultural production through increasing yields on already cultivated lands (Tilman et al., 2011). This will be through increasing farm inputs such as pesticides, water and fertilizers (organic and inorganic), among others but one of the challenges mankind is now facing is the quest for methods of sustainable food production which could increase food production with minimum effects on the environment through reducing GHG emissions in the atmosphere.

Greenhouse gases are linked with the phenomenon of the greenhouse effect because they absorb the emitted infrared radiation thereby trapping the radiation from escaping out of the earth's atmosphere and reradiating some back to the earth's surface hence causing an increase in the temperature of the earth's surface; without the greenhouse effect, the average temperature of our planet would be less than −17°C (Tuckett, 2016). This could make it difficult for our planet to support biological processes of plants and animals, including human beings. However, anthropogenic activities in agriculture such as cattle farming (enteric fermentation), rice cultivation, use of synthetic fertilizers, manures, incorporation of crop residues in soil, increased biological nitrogen fixation (BNF) through cultivation of leguminous crops partly contribute to the

general increase in GHG emissions especially carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) emissions (Mosier et al., 1998; IPCC, 2013; FAO, 2016b). The share of agricultural emissions by source and at global level are shown in **Figure 1.** With an increase in these activities inorder to match the global food demand, there could be further increase in the earth's surface temperatures due to the greenhouse effect which has resulted in global warming. By 2018, the global annual surface mean abundances of CO_2, CH_4 and N_2O gases had reached 407.8 ppm, 1869 ppb and 331.1 ppb compared to those of the pre-industrial (before 1750) levels of 278 ppm, 722 ppb, and 270 ppb respectively which are closely linked with anthropogenic activities (WMO, 2019). Global Warming Potential (GWP) is a default metric for comparing the emissions of different gases (based on the radiative forcing) on a common scale called CO_2-equivalent emissions and is usually intergrated up to a chozen time horizon which can be 20, 100 or 500 years (IPCC, 2013). This report further showed that in a 100 year time horizon, N_2O has a GWP 298 times that of CO_2 with a life time of 121 years while CH_4 has a GWP of 34 times that of CO_2 with a life time of 12.4 years. This means that a single molecule of N_2O can cause 298 times as much damage as one molecule of CO_2 while that of CH_4 can cause a damage equivalent to 34 times that of CO_2.

1.1.1. CO_2 emissions

CO_2 is a major anthropogenic GHG accounting for 76% of the total anthropogenic GHG emissions **(Figure 2)** (IPCC, 2014). Fossil fuels and industrial processes are the primary sources of CO_2 emissions accounting for 65% while forestry and other land uses such as deforestration and agriculture account for 11% of the total GHG emissions. In agriculture, the CO_2 is absorbed from the atmosphere by plants through photosynthesis where by sunlight energy is trapped in the C bonds of organic molecules (carbohydrates, proteins, e.t.c.) which are used as a source of energy (via respiration) by plants (especially the plant roots), microorganisms and animals with the C being returned to the amosphere as CO_2 (Weil and Brady, 2016). The authors further explained that organic matter can accumulate in soil when the partially decomposed plant tissues and microbial cell debris are adsorbed on soil colloids or occluded inside soil aggregates where it is protected from further microbial metabolism for decades before the C in them is returned to the atmosphere as CO_2.

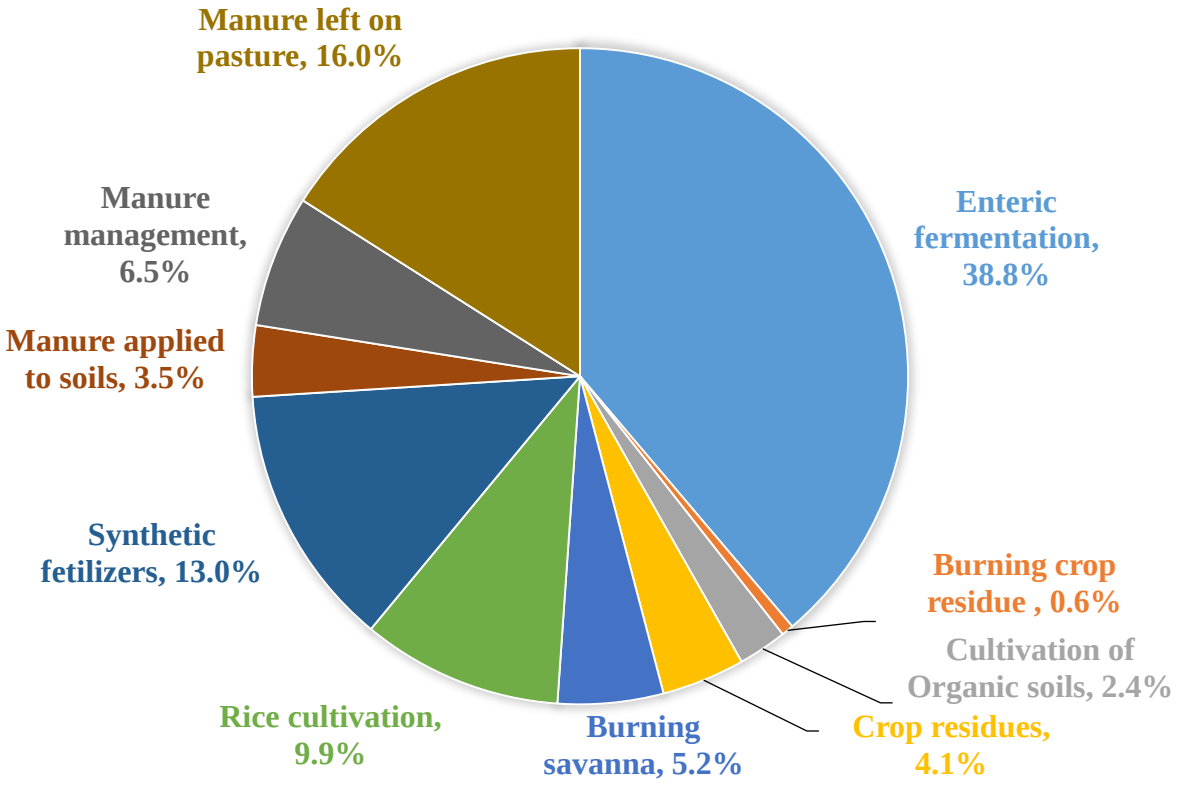

Figure 1. Global agricultural emissions (CO_2 equivalent) by source in 2017 **(**FAO, 2019).

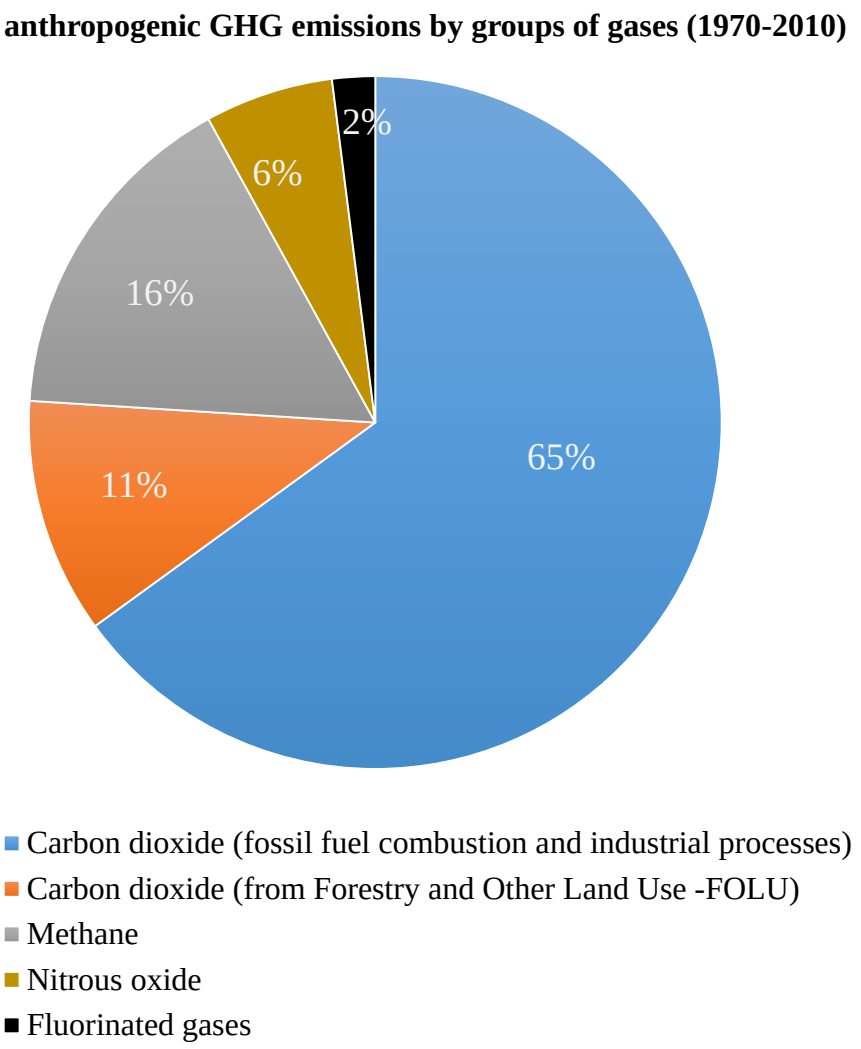

Figure 2. Global anthropogenic GHG emissions by groups of gases (1970–2010) (IPCC, 2014).

Oertel et al. (2016) separated CO_2 fluxes into three types: (i) Soil respiration which includes root, anaerobic and aerobic microbial respiration; (ii) Ecosystem respiration which includes aboveground plant respiration; (iii) Net ecosystem exchange (NEE) which is the difference between photosynthesis and ecosystem respiration where by a positive NEE indicates a CO_2 source while a negative NEE reveals a CO_2 sink. Soil respiration is considered the second largest terrestrial C flux and is crucial in regulating atmospheric CO_2 concentration and climate dynamics in the earth system, and also associated with nutrient processes such as decomposition and mineralization (Luo and Zhou, 2006; Bond-Lamberty and Thomson, 2010).

Regarding soil respiration, there are five major sources of CO_2 efflux from soil; (i) microbial decomposition of soil organic matter (SOM) in root free soil without undecomposed plant remains (basal respiration); (ii) microbial decomposition of SOM in root affected or plant residue affected soil (priming effect); (iii) microbial decomposition of dead plant remains; (iv) microbial decomposition of rhizodeposits (exudates, secretions and sloughed-off root cells) from living roots (rhizomicrobial respiration); (v) Root respiration (Kuzyakov et al., 2006). The first four sources are termed as microbial respiration or respiration by heterotrophs since they are produced by soil microorganisms (bacteria, fungi, and actinomycetes) while the last source is termed as respiration by autotrophs since it is produced from actual root respiration (determined by root biomass growth). In a study by Shi et al. (2020), root respiration was the main source of soil respiration in a cropland under soybean, which contributed 70% of CO_2 emissions from all land-use types. Unlike plant-derived CO_2 sources (iii, iv, v) which have a high turnover rate and low residence time in soil, SOM derived CO_2 sources (i and ii) are important in contributing to changes in atmospheric CO_2 concentration because of a long residence time in soil and lower turnover rate and are considered long-term sinks for C in soil. C sequestration is one of the relatively effective ways in which CO_2 can be removed from the atmosphere thus mitigating climate change. Mitigation of atmospheric CO_2 emissions by increased C sequestration in soil is more beneficial given other global challenges that include reducing land degradation, soil quality and productivity enhancement as well as the preservation of biodiversity (Batjes, 1999). Soil management practices such as increasing soil organic carbon content, reduced tillage, manuring, residue incorporation and mulching can play an important role in sequestering C in soil there by reducing CO_2 emission (Rastogi et al., 2002). However, unlike CH_4 and N_2O emissions, the many sources of atmospheric CO_2 emissions from soil makes it difficult to determine whether soil is a

net sink or source of atmospheric CO_2 (Kuzyakov et al., 2006). For instance, a given soil management practice such as residue incorporation could either be source or sink of CO_2 emissions depending turnover rate and residence time in soil. This is because decomposition depends on the C/N ratios of the material where by those with a low C/N ratio (N- rich legumes and vegetables residues) will decompose faster and release more CO_2 than those with a higher C/N ratio (straw of cereal crops) which decompose at a much slower rate. Since CO_2 emissions from soil to the atmosphere are a product of soil respiration from the decomposition of SOM by soil microbes and respiration of plant roots and soil animals (Fang et al., 1998), the factors affecting soil respiration will influence CO_2 efflux from the soil. Soil temperature and moisture are important for CO_2 efflux from soil (Longdoz et al., 2000). Soil respiration rates are positively correlated to ambient temperature which increases at higher temperatures by accelerating decomposition rates with optimum temperatures at 35–40°C (Weil and Brady, 2016). After temperature and light availability, soil moisture is a main driver of net primary productivity and thus strongly affects the accumulation and cycling of soil carbon (Moyano et al., 2013). Moinet et al. (2016) found a positive correlation between soil moisture content and soil respiration. Increase in soil moisture content (up to 60% water-filled pore space-WFPS) enhances soil respiration but further increase in soil moisture content (mostly >80% WFPS) results in reduction of soil respiration due to the reduction of soil aeration (Linn and Doran, 1984; Joo et al., 2012). Therefore, medium textured soils (loam soils) favor soil respiration because they are well drained and aerated. However, the low SOM content and poor water holding capacity of sandy soils limits soil respiration while in clay soils, most of the SOM is protected from decomposition hence low rates of soil respiration. Available C (labile and non-labile forms of SOM) in soil provide decomposition substrates for microorganisms and directly affect soil respiration (Shi et al., 2020). Since high N additions in soil can suppress soil respiration by reducing microbial activity and biomass (Wu et al., 2020), moderate N addition is necessary to balance the C/N ratio to avoid competition among soil microbes for available soil N when the C/N ratio of organic materials is high. Although biochemical metabolisms in soil result mainly in CO_2 production, there exists other soil processes that could either consume or produce CO_2 such as methanogenesis, phototrophs, or carbonic reactions (Luo and Zhou, 2006). Under strongly anaerobic conditions, such as wetlands and rice paddies, bacteria produce CH_4 rather than CO_2 as they decompose organic matter (Weil and Brady, 2016).

1.1.2. CH_4 emissions

Atmospheric CH_4 is the second most important GHG after CO_2, and it accounts for 16% of the total global anthropogenic emissions **(Figure 2)** (IPCC, 2014; WMO, 2019). The major anthropogenic sources of CH_4 include enteric fertmentation and manure, rice cultivation, biomass burning, among others (Saunois et al., 2019). In enteric fermentation, the microbes in the rumen of ruminant animals such as cattle, goats, and sheep decompose and ferment food thereby producing CH_4 as a by-product. Soils can act as sources and sinks of CH_4 depending on the net balance between methanogenesis and methanotrophy processess. Methanogenesis refers to microbial production of CH_4 especially in anaerobic conditions in wetland soils and rice paddies, but can also occur in upland soils inside soil aggregates where anaerobic microsites occur; methanotrophy refers to microbial consumption of CH_4 especially in upland soils by organisms having methane monooxygenase (MMO) enzyme that uses O_2 and CH_4 for their metabolism under aerobic conditions (Dutaur and Verchot, 2007; Weil and Brady, 2016). Soil pH, redox potential (less than −0.2 V), higher temperature (0–35°C; optimum at 25°C) and soil management practices such as landfilling of organic matter or plant residues can result in significant CH_4 emissions (Topp and Pattey, 1997). Cultural practices like inorganic N fertilization of crop lands inhibit CH_4 oxidation by agricultural soils because the availability of NH_4^+ from the fertilizer stimulates the ammonium-oxidizing bacteria (AOB) at the expense of methane-oxidizing bacteria (MOB) (Topp and Pattey, 1997; Seghers et al., 2005; Weil and Brady, 2016). However, soils treated with organic fertilizers such as compost have higher CH_4 oxidation rates compared to soils receiving mineral fertilizer, attributed to the enhanced abundance of methanotrophs in the organic fertilized soil (Seghers et al., 2005). In rice paddies, disturbance of anaerobic conditions by puddling, transplanting, fertilizer application and weeding releases soil-entrapped CH_4 to the atmosphere (Setyanto et al., 2002). Since upland soils are major sinks for CH_4 gas, mitigation measures of CH_4 from the agricultural sector should focus more on the rice paddies and other waterlogged ecosystems since they are the major sources of atmospheric CH_4.

1.1.3. N_2O emissions

N_2O is the most significant ozone-depleting substance and third most important GHG released in the atmosphere and it accounts for 6% of the total anthropogenic GHG emissions **(Figure 2)** (IPCC, 2014), with two-thirds of the total gross anthropogenic N_2O emissions originating from agriculture mainly as a consequence of the lack of sychronization between crop N demand and soil

N supply (UNEP, 2013). N_2O emissions in agriculture arises mainly from synthetic N fertilizer, manure application and management, crop residue incorporation in soil, and legume N fixation (Mosier et al., 1998). Several processes are responsible for N_2O emission from agricultural soils and they include nitrification, denitrification, nitrifier denitrification, Dissimilatory Nitrate Reduction to Ammonia (DNRA) and Codenitrification (Signor and Cerri, 2013; Cayuela et al., 2013; Weil and Brady, 2016). However, the relative contribution of each process to total N_2O emissions depends not only on the soil characteristics like texture, available carbon, pH, aerobicity and microbial activity but also the prevailing environmental conditions such as temperature and rainfall (Cayuela et al., 2014). The detailed mechanisms of how N_2O emissions occur from these biological processes are described below;

1.1.3.1. Nitrification

Nitrification is a microbially mediated process where ammonium ions (NH_4^+) are oxidized to nitrites (NO_2^-) and then to nitrates (NO_3^-). This process occurs in presence of NH_4^+ (from crop residues and N fertilizers), oxygen, neutral pH (6.5–8.8), low moisture content (<60% WFPS), temperatures (20–30°C) and carbon sources (bicarbonates and CO_2). Nitrification occurs in two steps; (i) ammonia oxidation to hydroxylamine (NH_2OH) (Eq. 1) and then to NO_2^- (Eq. 2) aided by ammonia oxidizing bacteria (AOB) such as *Nitrosomonas*. Ammonia monoxgenase (AMO) mediates the first step while hydroxylamine oxido-reductase (HAO) mediates the second step; (ii) NO_2^- oxidation to NO_3^- by autotrophic nitrite oxidizing bacteria in genera *Nitrobacter* mediated by nitrite oxidoreductase (Eq. 3) (Thangarajan et al., 2013).

$$NH_3 + O_2 + 2H^+ + 2e^- \longrightarrow NH_2OH + H_2O \quad (1)$$

$$NH_2OH + H_2O \longrightarrow NO_2^- + 5H^+ + 4e^- \quad (2)$$

$$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^- \quad (3)$$

During nitrification, N_2O is released through two pathways; nitrifier nitrification and nitrifier denitrification. In the ***nitrifier-nitrification*** pathway, N_2O is released directly from the oxidation of NH_2OH (Hooper and Terry, 1979; Sanchez-Garcia et al., 2014). During hydroxylamine oxidation, the hydroxylamine produced from ammonia oxidation is subsequently oxidized first to NO by HAO and then reduced to N_2O which is catalyzed by nitric oxide reductase (Thangarajan et al., 2013).

When conditions are favourable, the second step of nitrification (Eq. 3) is thought to follow the first closely to avoid accumulation of toxic NO_2^-. However, when oxygen supplies are marginal, the nitrifying bacteria may produce some NO and N_2O (Weil and Brady, 2016). Under anaerobic conditions, the concentration of NO_2^- increases in the soil (Khalil et al., 2004). The NO_2^- is then alternatively used by the nitrifying bacteria as a final electron acceptor resulting into N_2O and NO during nitrification (Snyder et al., 2009). This is termed as ***nitrifier-denitrification*** and in this nitrification pathway, ammonia is oxidized to nitrite (NO_2^-) followed by the reduction of nitrite (NO_2^-) to nitric oxide (NO), nitrous oxide (N_2O) and molecular nitrogen (N_2) (Wrage et al., 2001). Furthermore, in addition to anaerobic conditions, the authors added that this pathway is also favoured by the low soil organic carbon contents and low soil pH. Therefore, during oxidation of high NH_4^+ concentrations (>80 mg N kg^{-1}), the soil matrix will actively consume oxygen and accumulate high concentrations of NO_2^-, leading to suboxic conditions hence inducing nitrifier-denitrification (Huang et al., 2014).

1.1.3.2. Denitrification

Denitrification refers to the reduction process of nitrate ions (NO_3^-) to dinitrogen gas (N_2) mediated by facultative anaerobic bacteria of the genera *Pseudomonas, Bacillus, Micrococcus,* and *Achromobacter* (Weil and Brady, 2016). The reduction of N_2O to N_2 is the last step in the denitrification process of the geo-biological nitrogen cycle (Paraskevopoulos et al., 2006). When denitrification is complete, it yields N_2 which is stable in the atmosphere but partial denitrification results in a variable fraction of N_2 which is emitted as N_2O gas. N_2O release is favoured over N_2 if the soil is acidic (pH<5.0), relatively low C supply, soil isn't overly wet (some O_2 is present) and when the concentration of NO_2^- and NO_3^- are high (Weil and Brady, 2016).

All the reactions involved in denitrification are catalysed by metalloenzymes (Fujita et al., 2007). These enzymes include nitrate reductase (NO_3^- to NO_2^-), nitrite reductase (NO_2^- to NO), nitric oxide reductase (NO to N_2O), and nitrous oxide reductase (N_2O to N_2), and are usually induced under increasingly high anaerobic conditions. This enables the organisms to sustain respiratory metabolism during oxygen limitation, with NO_x as terminal electron acceptors (Bakken et al., 2012), using organic C as the electron donor (Morley and Baggs, 2010). The NO is produced as an intermediate during the denitrification process but due to its high cyto-toxicity, it is rapidly decomposed to N_2O by the nitric oxide reductase enzyme immediately after its production by nitrite reductase (Shiro, 2012). Therefore, respiratory nitric oxide reductase found in denitrifying

bacteria and in some ammonia oxidizing organisms is the major contributor of biological production of N_2O (Spiro, 2012). Since N_2O is non-toxic and microrganisms can tolerate relatively high (millimolar) concentrations and the fact that the reduction potential of the N_2O/N_2 couple is high (+1.35V at pH 7), some bacteria can exploit this property by using N_2O as the terminal electron acceptor in energy conserving respiratory metabolism; the nitrous oxide reductase enzyme found in denitrifying bacteria uses N_2O as a substrate (Spiro, 2012) hence producing N_2 gas. Therefore, changes in the soil physicochemical properties that affect the activity of N_2O reductase enzyme will influence the final gaseous product released into the atmosphere.

1.1.3.3. Dissimilatory Nitrate Reduction to Ammonia (DNRA) or nitrate ammonification

DNRA is an anaerobic bacterial process that reduces NO_3^- to NO_2^- and then to NH_4^+ (Weil and Brady, 2016). N_2O is argued to be produced at the nitrite reduction stage during nitrate ammonification (Schmidt et al., 2011). A soil investigation study of denitrification and DNRA showed that DNRA was a faster process than denitrification and that it accounted for 14.9% of the total reduction of ^{15}N-labeled nitrate added to soil from Griffith (NSW Australia) under anaerobic incubation without any exogenous C source addition, but only 5% for the soil from Yangzhou, China (Yin et al., 2002). The authors concluded that the available C supply had far stronger influence on DNRA compared to the redox potential. Schmidt et al. (2011) demonstrated strong relationships between potential DNRA and soil NO_3^- and NO_2^- concentrations, sand content, pH and bulk density. However, the possible role of DNRA as an N_2O source in soil is only recently being realized and still frequently ignored in process studies and models (Baggs, 2011).

1.1.3.4. Codenitrification

In codenitrification, one N atom from NO or N_2O in denitrification combines with one atom from another source (co-substrate like amino acids) forming a hybrid product and N_2O is formed if the formal oxidation state of the nucleophilic N is −1 (e.g. hydroxylamine-NH_2OH) (Spott et al., 2011). This process is carried out by bacteria such as *Streptomyces spp* and fungi like *Fusarium oxysporum* and has been measured in various aerobic soils including agroecosystems and grasslands (Weil and Brady, 2016). Utilizing the co-substrate within denitrification implies that it is possible to produce two molecule of N_2O for every two molecules of nitrate reduced as opposed to one molecule produced during the conventional denitrification pathway (Baggs, 2011). Codenitrification also acts as an N immobilizing process due to bonding of inorganic N (e.g from

NO_3^- or NO_2^-) onto organic compounds due to N- or even C- nitrosation reactions (Spott et al., 2011).

1.2. Main sources of N_2O emissions

1.2.1. Fertilizers and manures

The decline in soil fertility has made the use of fertilizers inevitable as a neccessity to feed the world's increasing population. Fertilizers refer to any organic or inorganic material of natural or synthetic origin (except liming materials) that is added to the soil to supply one or more plant nutrients essential for plant growth (Sabry, 2015). In comparison with livestock manures which are either left on pasture or applied to soil, there has been a gradual increase in the use of synthetic fertilizers in agriculture; in 2017, synthetic fertilizers (mineral and chemical fertilizers) consumption reached 191 million tonnes of nutrients as input to agricultural soil with 57% from nutrient nitrogen (**Figure 3**). Nitrogen is an essential plant nutrient required by the plant and depending on the plant species, it can be absorbed from soil as either NH_4^+ or NO_3^-. However, if the fertilizers are applied in excess of plant requirement, they result in environmental pollution especially water pollution, soil acidification and N_2O emissions to the atmosphere. Moreover, synthetic fertilizers account for 13.0% of the agricultural GHG emisions **(Figure 1)**. Asia, Americas, Europe, Africa and Oceania utilizes 60.1%, 21.3%, 13.9%, 3.3% and 1.4% of the total N input from synthetic fertilizers with each continent contributing to 58.3%, 21.2%, 16%, 3.2% and 1.3% respectively of the GHG emissions (CO_2-equivalents) from the use of synthetic fertilizer (FAO, 2019). These statistics show a positive correlation between N fertilizer use and GHG emissions which provide evidence that synthetic fertilizers are significant sources of N_2O emissions. Cereals and vegetables are among the crops which consume large amounts of fertilizers. In 2014/2015, vegetable production accounted for 7.4% which ranked fourth after wheat, maize and rice with 18.2%, 17.8% and 15.2% of the total N inputs respectively (Heffer et al., 2017).

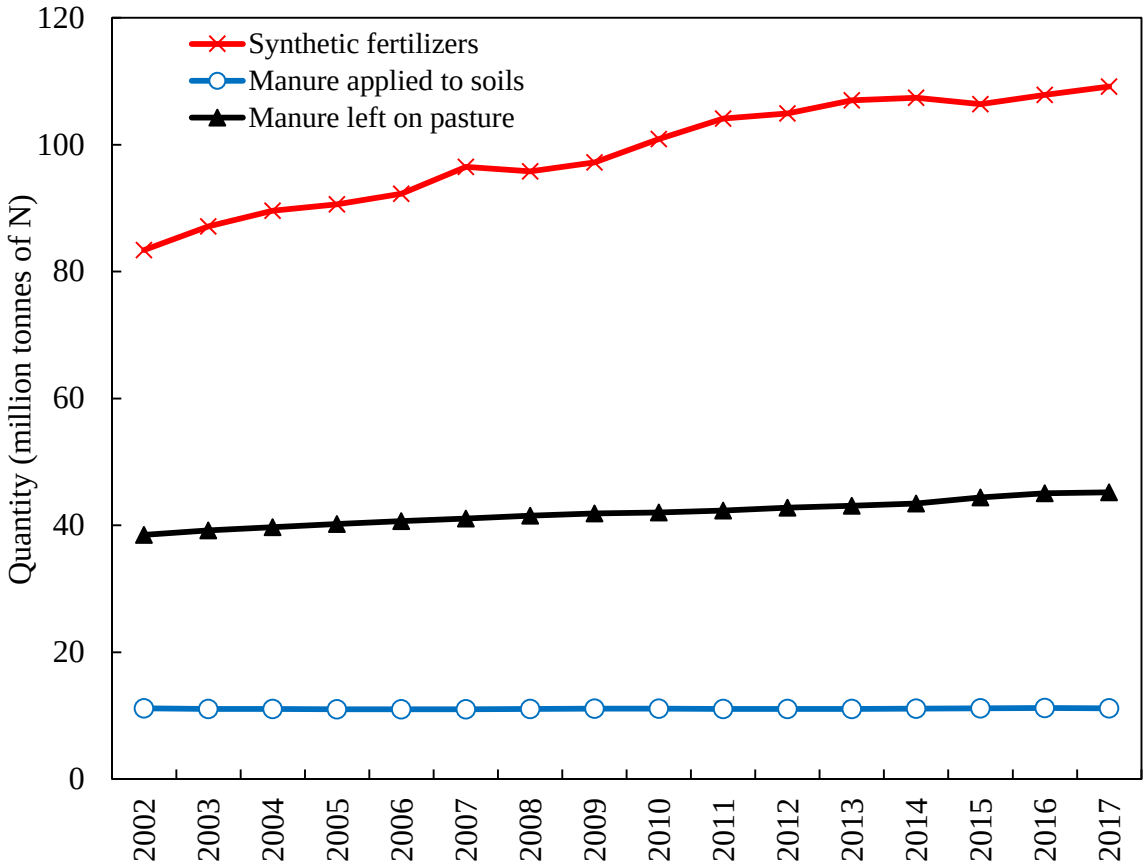

Figure 3. Global N inputs from synthetic fertilizer and livestock manure from 2002 and 2017 (FAO, 2019).

However, in terms of application rates, vegetables were reported to be among the crops with high fertilizer application of 240 kg ha^{-1} which is higher than for cereals at 102 kg ha^{-1} (FAO, 2006). In addition, the short life cycles of vegetable crops which favour multiple croppings per year results in larger annual N inputs as compared to that in other crops. Therefore, with the current increase in intensive vegetable production, it is necessary to minimize the likely effects of excessive N fertilizer use especially soil acidification and N_2O emissions.

1.2.2. Crop residue mineralization

Crop residues are plant materials that are left on cultivated land after crop harvest and their addition to soil is one of the strategies aimed at increasing SOM which subsequently increases soil fertility. They are important sources of CO_2 and N_2O emissions and they account for 4.1% of the total emissions from agriculture (**Figure 1**). Crop residues increase N_2O emissions from soil by; (i) supplying easily mineralizable N which provides additional substrate for nitrification and denitrification, (ii) increasing mineralizable C which stimulates denitrification of soil mineral N and crop residue N, (iii) increased oxygen consumption which creates anaerobic conditions thus stimulating N_2O emission from denitrification (Velthof et al., 2002). GHG emissions from crop residues vary with environment factors, type of crop, soil properties and residue management factors (Novoa and Tejeda, 2006). The factors affecting soil respiration also affect the rate of crop crop decomposition. In terms of management, crop residues may be removed from the fields, left on soil surface as mulch or incorporated in soil but the incorporation of crop residues in soil appears to result in the highest N_2O emissions (Nett et al., 2016; Li et al., 2016; Scheer et al., 2014). Nett et al. (2015) found that surface-application of cauliflower residues may produce as high or even higher N_2O emissions than incorporation by homogenous mixing in top soil due to soil moisture conservation and creation of anaerobic conditions as well as increase in C and N in upper soil layers which favours denitrification. Microbial immobilization of soil and fertilizer N results during the decomposition of poor quality residues having low N content, high C/N ratios, high lignin and polyphenol contents (Singh et al., 2005). Contrary, high quality plant residues (high N content, low C/N ratios, low lignin, cellulose and polyphenol contents) have higher decomposition and N mineralization rates (Kamkar et al., 2014) which results in higher CO_2 and N_2O emissions. Crop residues can be grouped into three categories based on their N_2O emission factors i.e. crop residues with low emission (<0.5%) such as straw of cereals, those with high emission (> 1.5%) such as N-rich residues of vegetables and leguminosae, and crop residues with

moderate emissions (0.5–1.5%) which includes most of the other crops (Velthof et al., 2002). Since vegetable production is characterised by excessive application of N fertilizers, they can leave large amounts of post-harvest crop residues (Nett et al., 2015). Therefore, more focus is needed to reduce post-harvest GHG emissions from crop residues especially those with high emission factors including vegetables such as broccoli.

1.2.3. Biological Nitrogen fixation

Biological Nitrogen fixation (BNF) is a biological process which involves the conversion of inert atmospheric dinitrogen gas (N_2) to reactive N forms such as ammonia that becomes available to all forms of life through the nitrogen cycle (Weil and Brady, 2016). This process is important for global agricultural productivity and is considered one of the most important biological processes on the planet (FAO, 2016a) because it provides the earth's ecosystems with about 200 million tonnes of N per year (Rascio and Rocca, 2008). BNF is carried out mostly by leguminous plants such as soybean, common beans, clover, alfalfa which form symbiotic relatonships with rhizobial bacteria such as *Rhizobia* and *Bradyrhizobia*. Under low N conditions, plant roots secrete flavonoid molecules in soil which attract compatible rhizobia and stimulates them to synthesize a highly specific signal molecule called the Nod factor which are perceived by the plant to allow symbiotic infection of the root hence forming root nodules which serve as sites of BNF (Liu and Murray, 2016; Ferguson, 2013). BNF not only increases crop yield through providing plants with N, but it also replenishes the N reserves in soil after the decomposition of the plant residues following crop harvest. Legume-Rhizobium symbiosis is also important for environmental reasons because the fixed atmospheric N can replace synthetic N fertilizers that are used in large quantities (Sugiyama et al., 2007) thus reducing environmental pollution including N_2O emissions. Legume crops do not significantly influence N_2O emissions because the emissions during their growth are considered negligible (Rochette and Janzen, 2005) and N_2O can be produced during later stages of crop growth through the decomposition of the N-rich plant residues such as root nodules (Yang and Cai, 2006; Shah, 2014). Although BNF has a smaller contribution to N_2O emissions, different genotypes having varying nodulation capacities could have varying effects on GHG emissions. For instance, an upland field experiment involving three soybean genotypes of varying nodulation i.e. normal nodulating, super-nodulating and non-nodulating genotypes revealed that the genotype with high nodulating abilities resulted in the highest N_2O emissions especially during the full bloom (R2) and full pod stages (R4) but insignificant during the seed filling stage (Kim et al.,

2005). Although super-nodulation is an important trait in terms of the abundant supply of fixed atmospheric N, they have been regarded as inferior in growth and seed yield due to their high energy requirements which requires a high consumption of carbohydrates to form root nodules (Takahashi et al., 2003; Ferguson, 2013). However, there could be a potential for improved super-nodulating soybean genotypes to perform better in soils with low fertility that might need abundunt inputs especially synthetic N fertilizers to improve their productivity in the absence of BNF. To date, little work has been done to investigate GHG emissions from legume fields. Therefore, GHG mitigation strategies in legume production systems should mainly focus on the sustainability of improved super-nodulating soybeans in low nutrient soils through increasing their productivity with minimal impacts on the environment.

1.3. N_2O mitigation strategies

The three broad strategies of reducing N_2O from agriculture include; (i) changing diet and reducing food loss/wastes; (ii) Increasing nitrogen use efficiency (NUE) in crop and animal production, including manure NUE; (iii) Adopting technologies and management practices such as using enhanced efficiency fertilizers and nitrification inhibitors in crop production to decrease the fraction of input N that is released as N_2O (emission factors) (UNEP, 2013). However, adoption of these fertilizers and nitrification inhibitors remains a challenge especially to developing nations where farmer adoption will most likely be limited by the high cost of these technologies; therefore, cheaper and available technologies need to be used to solve this problem. One of the relatively cheaper technologies to achieve GHG mitigation is the incorporation of pyrogenic carbonaceous materials (such as biochar) in soil because they can be available on farms after crop harvest, sequester carbon in soil and improve crop productivity.

1.3.1. Use of pyrogenic carbonaceous materials in soil

Pyrogenic Carbonaceous Material (PCM) is an umbrella term for all materials such as biochar, charcoal, activated carbon, char, black carbon and soot that are produced by thermochemical conversion and contains some organic C (Lehmann and Joseph, 2015). The authors define biochar as a solid material obtained from thermochemical conversion of biomass (such as wood, manure or leaves) in an oxygen-limited environment to above 250°C, a process called pyrolysis (also used for making charcoal). Biochar is designed for use in soil application to address soil issues and ***environmental management*** while charcoal is a product obtained from thermochemical

conversion of biomass (mainly but not exclusively wood) for ***energy generation***. On the other hand, they defined activated carbon (AC) as a PCM that has undergone *activation* by steam or addition of chemicals and it is mainly used for filtration, restoration or for specialized experiments in soil such as inoculation. Some of the promising biochar applications include char gasification and combustion for energy production, soil remediation, C sequestration, catalysis, and development of AC and specialty materials with biomedical and industrial uses (Lehmann and Joseph, 2015; Nanda et al., 2016).

The inspiration of using biochar as a supplement in soil stems from the observations made in the ancient agricultural management practices that created "*terra preta"* deep black soils (Sohi et al., 2009; Lone et al., 2015). The high fertility associated with these anthropogenic soils, the "*terra preta*," in the Amazon is in relation to the high concentration of nutrients such as N, P, K, Ca and high content of organic C in the form of char; and the practice of 'slash and char' by the pre-Columbian indigenous people of the Amazon (Glaser et al., 2001). From their investigations, they showed that the "*terra preta*" soils contained up to 70 times more black carbon than the surrounding soils **(Figure 4)** and that due to its polycyclic aromatic structure, black carbon is chemically and microbially stable and persists in the environment over centuries; with its oxidation producing carboxylic groups on the edges of the aromatic backbone, which increases its nutrient-holding capacity. The history and evident value of the "*terra preta*" has led to the suggestion that investment in biochar and its application to agricultural soil may be economically viable and beneficial (Sohi et al., 2009). Recently, biochar is mainly used in agronomy with an aim of enhancing soil fertility, mitigation of GHG emission and land reclamation.

Sohi et al. (2009) reported that biochar is produced mainly from the following processes; (i) fast pyrolysis (anhydrous) which involve short duration pyrolysis at higher temperatures; (ii) slow pyrolysis (low temperature 450–550°C, oxygen free, sometimes steam); (iii) slow pyrolysis (high temperature 600–900°C, oxygen free); (iv) gasification (high temperature >800°C, fast heating rate, with presence of oxygen). The suitable process to adopt depends on the feed stock; with process (i & ii) being suitable for biomass energy crops (cereals, wood pellets, palm oil), processes (i, ii & iv) for agricultural waste (wheat straw, hazelnut and peanut shells, waste wood, etc.), process (iii) for compost (green waste) while manure/animal wastes, kitchen wastes and sewage sludge being suitable for gasification.

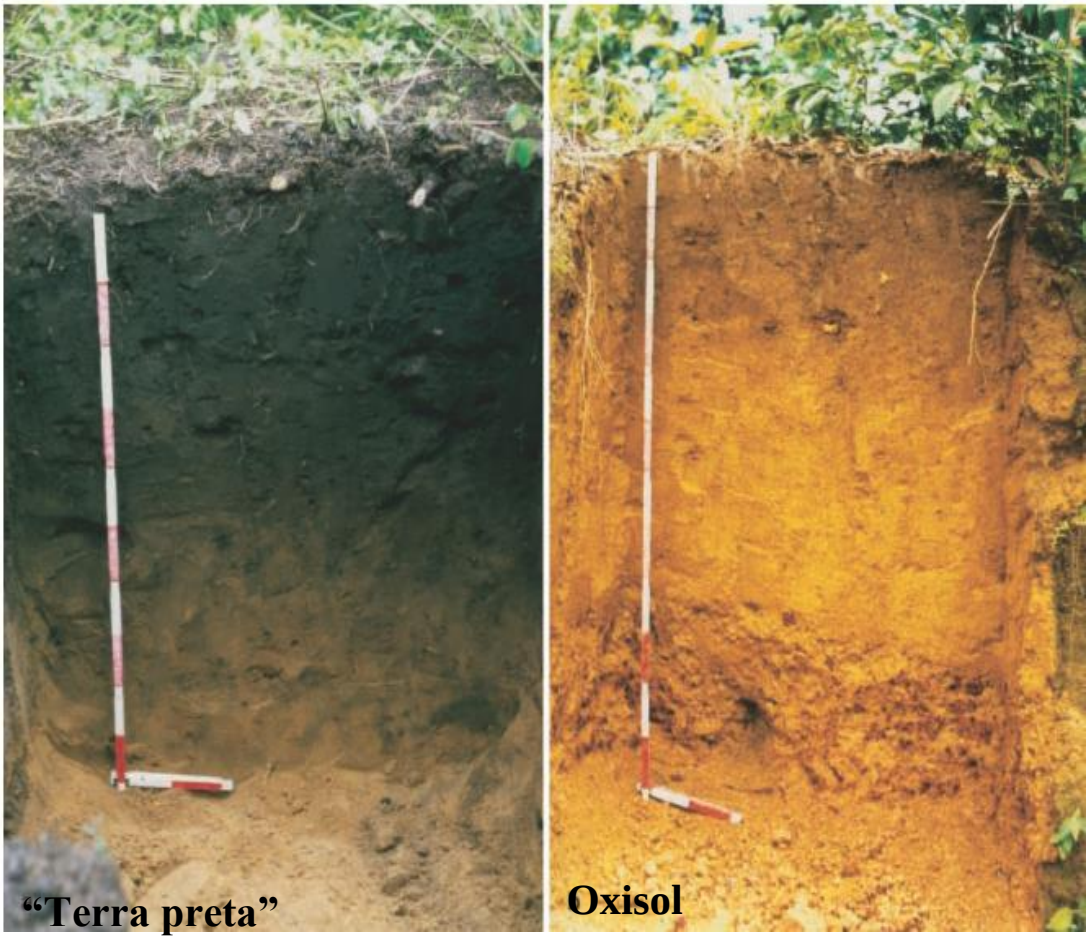

Figure 4. Typical profiles of the “terra preta” and oxisol (From Glaser et al., 2001).

The conversion of biomass C to biochar C sequesters 50% of the initial C as compared to low amounts that are retained after burning (3%) and biological decomposition (<10–20% after 5–10 years) (Lehmann et al., 2006). Pyrolysis methods have a significant impact on biochar properties such as biochar yield, pH, particle size, and surface area (Hussain et al., 2017). As pyrolysis temperatures increase from 300°C to 700°C, the aromatic C, ash, pH, surface area and pore volume of biochar increases while the yield, volatiles, electrical conductivity, cation exchange capacity decrease (Nanda et al., 2016).

It is necessary to consider biochar's C sequestration potential after applying it in soil through investigating CO_2 emissions. A meta-analysis by Song et al. (2016) revealed that biochar application stimulates CO_2 emissions from upland fields and laboratory incubations but reduced CO_2 emissions from paddy fields. The increased CO_2 emissions with rate and time of biochar application was explained as follows; (i) biochar increases biomass and activities of microbes thus enhancing decomposition of native SOM; (ii) The portion of labile organic C pool of amended biochar may be consumed by the microorganisms thereby increasing CO_2 emissions; (iii) Biochar increases plant growth and root biomass which may promote root respiration and provide additional organic matter for decomposition. On the other hand, the authors attributed the reduction in CO_2 emissions following biochar application in paddy field to; (i) Stimulation of CH_4 production and consequently reducing CO_2 emissions; (ii) Biochar improves soil pH and consequently increases the solubility of CO_2 and formation of bicarbonate acid leading to reduction in CO_2 emissions. Furthermore, other authors (Lin et al., 2015; Zhang et al., 2016) have shown non-significant effects of biochar and fertilizer application on CO_2 emissions.

Biochar application has also been reported to increase, decrease or have no effect on CH_4 emissions. Reduction in CH_4 emissions by biochar is due to the increasing soil aeration and soil pH which inhibits methanogenic activity and promotes methanotrophy, and also limiting N availability to microbes through adsorption of NH_4^+ hence reducing competitive inhibition and ultimately supporting methanotrophy; and lastly, by adsorption of CH_4 onto biochar surfaces (Pal, 2016). Findings from Jeffery et al. (2016) revealed that when biochar is applied with <120 t ha^{-1} N fertilizer, it can reduce CH_4 fluxes while no effect occurred when applied with >120 t ha^{-1} N fertilizer but this is still highly controversial. Lin et al. (2015) observed no significant effects of biochar on CH_4 emissions and attributed it to the failure of increased moisture content following biochar addition; in their study, biochar amendment could increase anaerobic conditions without

having an impact on soil pH which would favor CH_4 oxidation. Soil pH is one of the main determinant underlying biochar's effect on soil CH_4 fluxes (Jeffery et al., 2016). Wang et al. (2015b) reported that CH_4 emissions were negatively correlated with soil pH and they suggested that methanogens may have been better adapted to the acidic paddy soil. It seems that biochar application has varying responses under different soil types. For example, a meta-analysis by He et al. (2017) revealed that biochar application decreased CH_4 fluxes in coarse soils while it increased CH_4 fluxes in fine soils due to increased soil aeration (in coarse soils) which makes soil more favorable for aerobic methanotrophs and increases CH_4 oxidation. The mechanisms of biochar application on CH_4 production in soils still remain unclear.

The role of biochar to reduce N_2O emissions has been attributed to the increased soil aeration, increased soil pH and also due to the reduced N pools in the soil by N immobilization and adsorption (Clough et al., 2013; Li et al., 2015b). Biochar functions as an "*electron shuttle*" that facilitates the transfer of electrons to soil denitrifying microorganisms and this, in addition to the alkalinity of the biochars (liming ability in soil), increases abundance of *nosZ* (nitrous oxide reductase) genes in microbial communities that promotes the reduction of N_2O to N_2 (Cayuela et al., 2013, 2014; Harter et al., 2014). Increase in N_2O emissions following biochar application has been attributed to the release of biochar embodied-N or priming effects on SOM following biochar addition; increased soil water content which improves conditions for denitrification and at times, the provision of inorganic-N and/or C substrate for microbes (Clough et al., 2013; Petter et al., 2016). In addition, biochar induced plant growth promotes plant competition effect for N on N_2O fluxes with lower N_2O emissions in the presence of plants due to the plant N uptake competing with microbes for N (Saarnio et al., 2013).

The increase in crop yield following biochar application is due to the improvement in soil physico-chemical properties (Castellini et al., 2015), increased microbial population (Jones et al., 2012) and the reduced N nutrient leaching (Kanthle et al., 2016; Xu et al., 2016) while the negative effects on crop productivity can be attributed mainly to nutrient imbalances, N limitation (Borchard et al., 2014) and phytotoxicity (Rogovska et al., 2012; Marchand et al., 2016).

To date, the effects of biochar on GHG emissions and crop productivity are still contradictory because the variation in the functioning of biochar as a soil amendment depends on biochar feedstock type, biochar application rates, soil properties, and climatic conditions (Hussain et al., 2017). The roles of biochar on C sequestration, GHG mitigation, water retention, sorption

of chemicals are largely dependent on surface properties of biochar such as the specific surface area, porosity and morphology (size, shape and structure which differs as a function of pyrolysis temperature and feedstock) (Mukome and Parikh, 2016). For instance, the scanning electron microscopy (SEM) images of rice husk biochar (**Figure 5a**) and palm shell biochar (**Figure 5b**) show variation in morphology, textures and pores of the two biochars which could result in differences in their functioning.

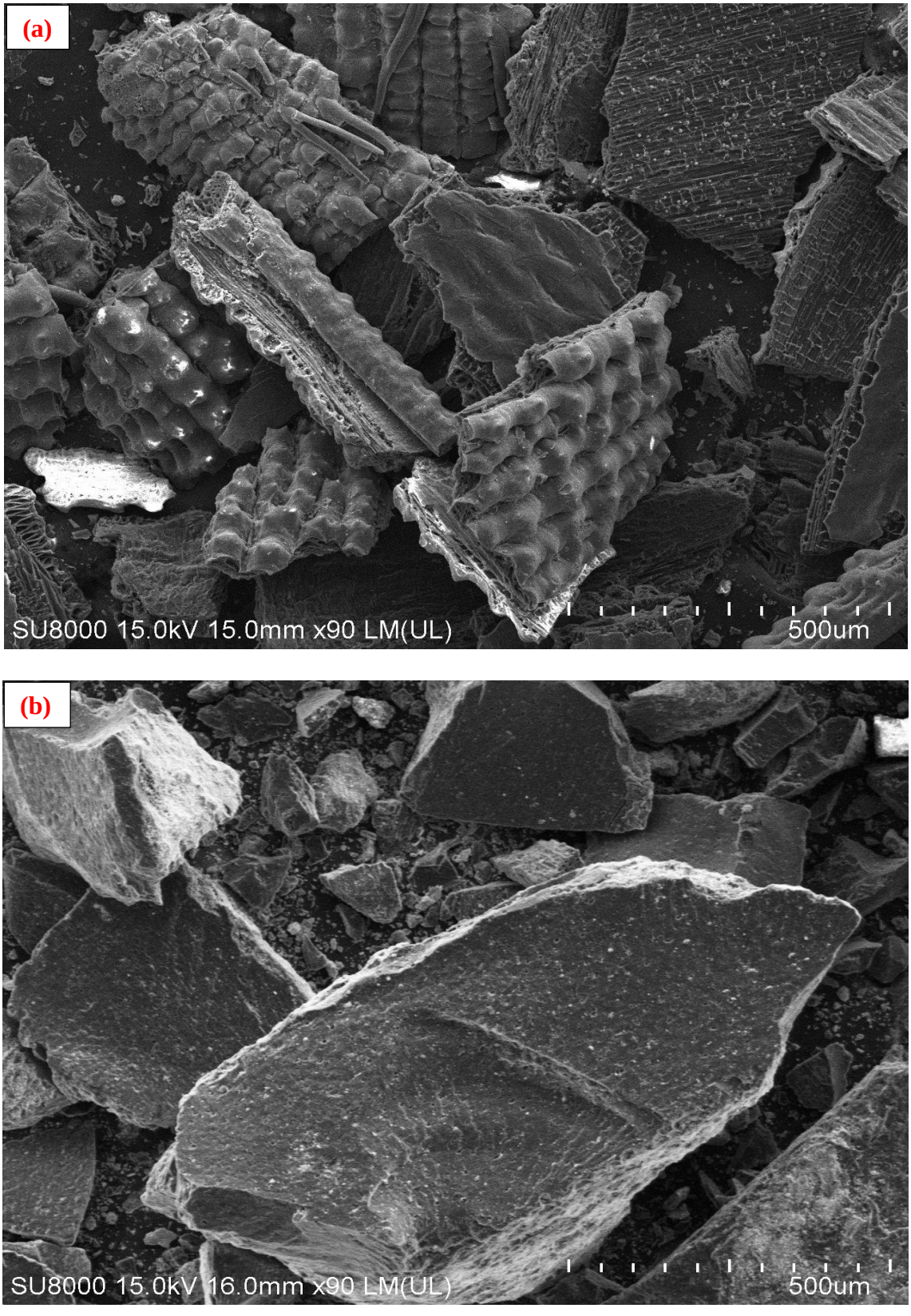

Figure 5. SEM images of rice husk biochar **(a)** and palm shell biochar **(b)**.

Filiberto and Gaunt (2013) reported that the recommended application rates of biochar as a soil amendment are quite variable given the insufficient field data available to make general recommendations on biochar application rates according to soil types and crops; with biochar feedstock materials influencing the application rates. The impact of biochar in soil depends on soil texture, with coarser textured loamy soils requiring more biochar and time to produce any significant increase in aggregate stability (Obia et al., 2016). For the feedstock, plant derived materials have been considered most important feedstocks in mitigating N_2O emissions (Cayuela et al., 2014). However, biochar from plant residues like hazelnut shell, pine and oak have shown little effects on soil improvement and crop yield while nutrient rich biochars especially those from animal derived manures have improved crop growth (Rajkovich et al., 2012). Therefore, unless derived from manure or blended with nutrient rich materials, biochars do not substitute for conventional fertilizer, and hence, addition of biochar without necessary amounts of N, P and K should not be expected to provide improvements to crop yield (Filiberto and Gaunt, 2013). Plant derived materials seem to be most promising feedstock in mitigating N_2O emissions but more studies are still needed for those other important groups of feedstock for which scarce or no information is currently available (Cayuela et al., 2014). Moreover, these should focus on the biochar application rates in the soil, biochar residual effects and biochar aging or weathering in the soils, pyrolysis temperatures among others.

To date, the use of PCMs as soil amendments still remains doubtful since there are many feedstock, produced under different pyrolysis conditions and also the different soil types in which biochar is applied. This has resulted in to many knowledge gaps on how we can sustainably harness the benefits of this valuable resource which would result into increased volumes of food required to feed the increasing population with less environmental effects. Moreover, more information is still needed to further advance knowledge on mitigation of GHG emissions from fertilizers, crop residues and BNF as major sources of GHG emissions with emphasis of Brassica vegetables and soybean cropping systems by using PCM as soil amendments.

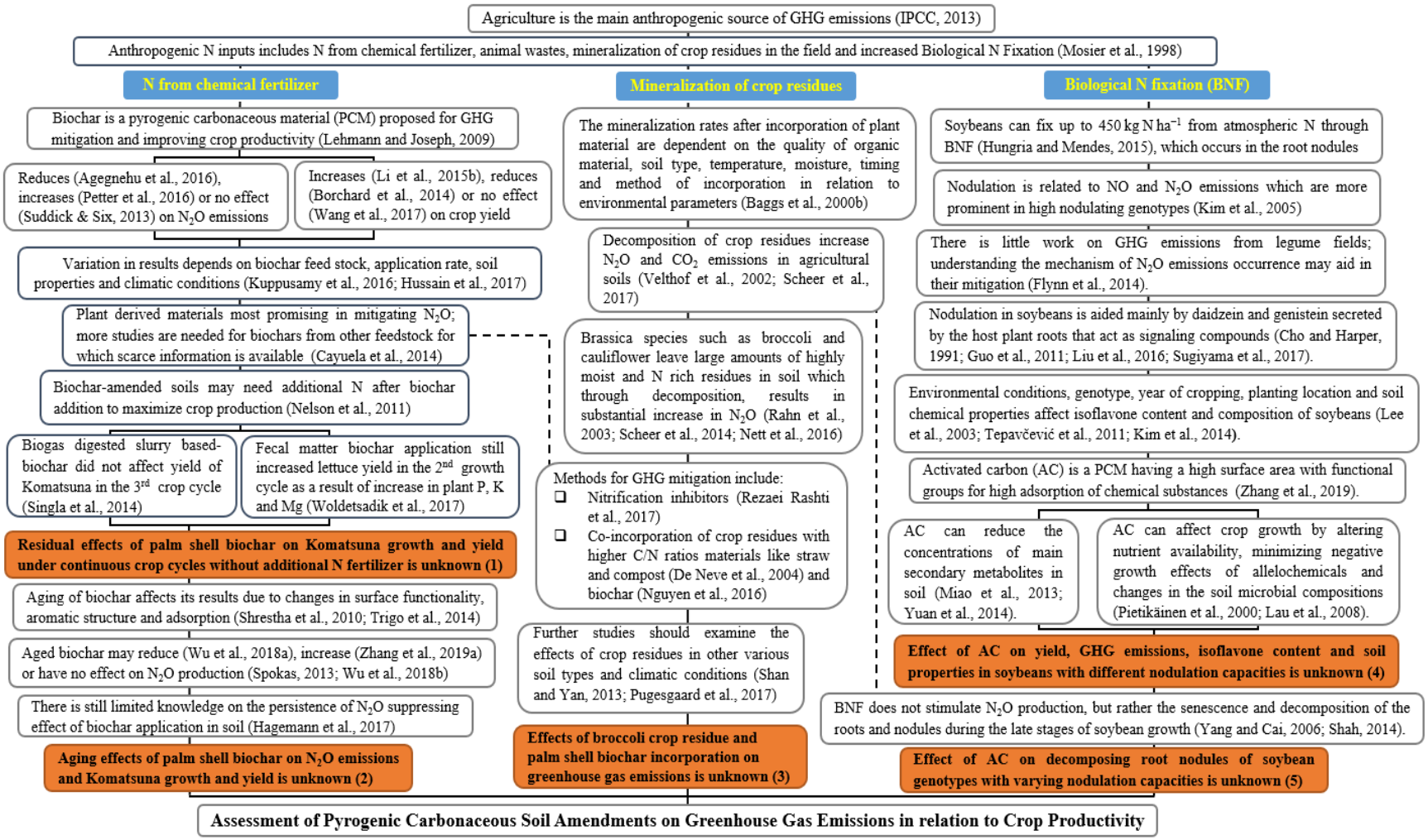

Figure 6. Literature review flow chart.

1.4. Major objective

To assess the effect of pyrogenic carbonaceous soil amendments on greenhouse gas emissions in relation to crop productivity.

1.4.1. Specific objectives

1. To assess the residual effects of palm shell biochar (PSB) on growth and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under three continuous crop cycles without additional N fertilizer application after the first crop cycle.
2. To assess the impact of fresh and aged PSB on N_2O emissions, soil properties, nutrient content and yield of Komatsuna under sandy soil conditions.
3. To assess the effect of crop residue and PSB incorporation on GHG emissions during the fallow and crop growing seasons of broccoli (*Brassica oleracea* var. *italica*).
4. To assess the effect of activated carbon (AC) on GHG emissions, seed yield, soil chemical properties and isoflavone content of soybean genotypes with varying nodulation capacities under sandy soil conditions.
5. To assess the effect of AC on N_2O and CO_2 emissions from decomposing root nodules of soybean genotypes with varying nodulation capacities under sandy soil conditions.

1.5. Hypotheses

1. Biochar application in presence of fertilizer would maintain higher crop yield during the second and third crop cycles due to the increased mineralization of biochar nutrients.
2. Biochar can suppress N_2O emissions, improve soil properties and plant nutrient content without having negative effects on crop yield even after one year of application in soil.
3. Biochar can reduce GHG emissions from broccoli residues; the fallow season would have higher GHG emissions than the crop growing season.
4. AC reduces GHG emissions, reduces nodulation, increases seed yield, soil chemical properties and seed protein content, and also reduce isoflavone content in the seeds, roots and soil. The effect of AC on the studied variables can vary depending on the genotypes.
5. N_2O and CO_2 emissions from nodules of the high nodulating genotype are higher than those of the normal nodulation genotype; AC reduces N_2O but not CO_2 emissions.

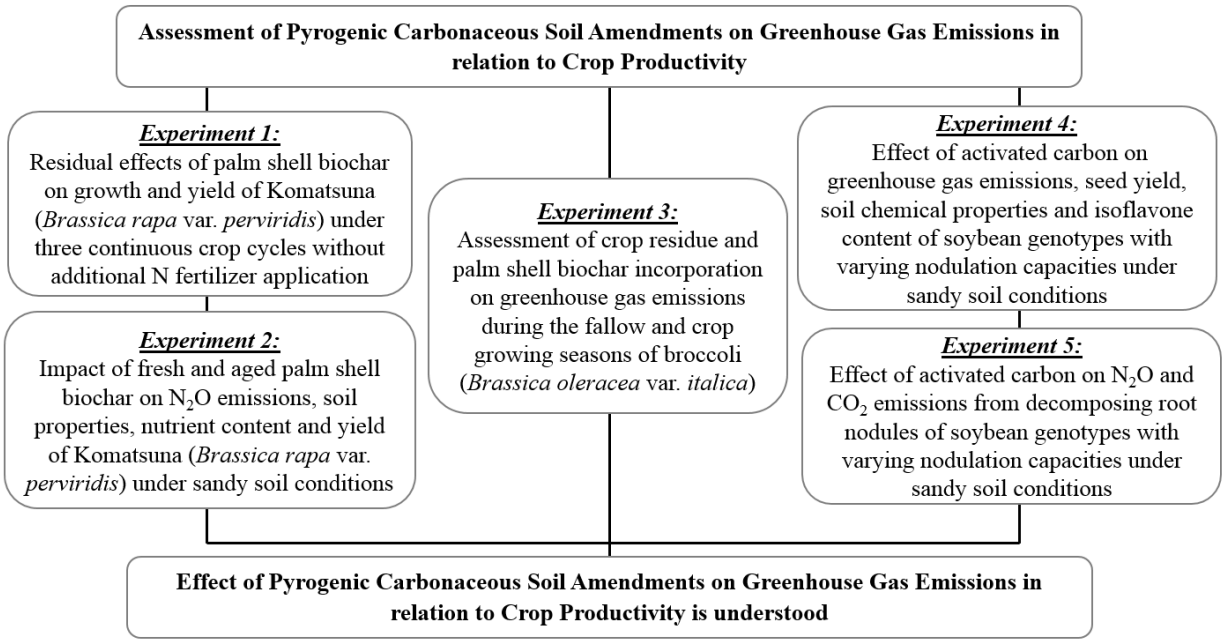

Figure 7. Experiment flow chart.

CHAPTER TWO

Residual effects of palm shell biochar on growth and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under three continuous crop cycles without additional N fertilizer application

2.1. Introduction

Biochar plays a major role in sustainable soil management by improving crop productivity and reducing environmental impacts on soil and water resources (Lehmann and Joseph, 2009). However, reports on biochar application in soils are still contradictory with some reporting increase (Mete et al., 2015; Li et al., 2015b; Agegnehu et al., 2016), reduction (Borchard et al., 2014; Marchand et al., 2016) or no significant effect on crop yield (Suddick and Six, 2013; Wang et al., 2017).

Increase in crop yield following biochar application has been attributed to the increased nitrogen use efficiency (NUE) as a result of applying biochar in combination with N fertilizers (Li et al., 2015b) which results in a steady supply of plant available N, improvement in soil physical properties like water retention (Castellini et al., 2015), increased microbial population (Jones et al., 2012) and reduced N leaching (Kanthle et al., 2016; Xu et al., 2016). Addition of biochar to soil also improves soil organic carbon, soil pH, cation exchange capacity, porosity, water holding capacity, nutrient retention, soil aggregation and lowers soil bulk density and tensile strength thereby facilitating plant growth as a result of improved root growth and higher nutrient uptake (Abrishamkesh et al., 2015; Hussain et al., 2017). Biochar mainly contains high concentration of N, P, K and Ca which may provide soil with nutrients directly or used as nutrients for microorganisms (Cha et al., 2016). Nigussie et al. (2012) reported that application of maize stalk biochar up to 10 t ha^{-1} increased N, P and K uptake of lettuce due to the presence of plant nutrients and ash in the biochar, high surface area and porous nature of the biochar, and the capacity of biochar to act as a medium for microorganisms.

The data in this chapter is published as Supplementary data to the article:

Basalirwa, D., Sudo, S., Wacal, C., Oo A.Z., Sasagawa, D., Yamamoto, S., Masunaga, T., Nishihara, E., 2020. Impact of fresh and aged palm shell biochar on N_2O emissions, soil properties, nutrient content and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under sandy soil conditions. Soil Science and Plant Nutrition 66 (2), 328–343. https://doi.org/10.1080/00380768.2019.1705737.

The negative effects of biochar on crop productivity can be attributed to nutrient imbalances and N limitation as a result of higher biochar application rates (Borchard et al., 2014), due to nutrient oversupply (Kuppusamy et al., 2016) and phytotoxicity (Rogovska et al., 2012; Marchand et al., 2016) whereby biochar chemicals released especially after the first few weeks might alter microbial processes (Cayuela et al., 2013) which indirectly affects crop growth.

The contradictions in the use of biochar are as a result of the broad range of feedstock, produced under different pyrolysis conditions and also the different soil types in which biochar is applied (Kuppusamy et al., 2016; Hussain et al., 2017). The effects of biochar also depends on application rates (Singla et al., 2014; Hagner et al., 2016) but these application rates also vary with the initial soil properties, biochar characteristics and feedstock (Filiberto and Gaunt, 2013). Biochar-amended soils may need additional N after biochar addition to maximize crop production (Nelson et al., 2011). The investigation of the effects of biochar in a long run is necessary by studying its residual effects on nutrient availability and crop growth in soils like sandy soils that have very poor nutrient retention. It is necessary to consider those farmers who would not be able to supply additional fertilizer following biochar application in the next crop growing season. This problem is most likely to affect farmers who lack enough funds to purchase fertilizers every season especially those small scale farmers in Sub Saharan Africa and South East Asia. Therefore, before advising them to apply biochar in their fields, a thorough study of the residual effects of biochar in soils is mandatory.

To date, very few studies have focused on the residual effects of biochar especially on crop yield; the residual effects at different biochar application rates need to be assessed. More research is also required on how biochar properties change overtime in soil (Ameloot et al., 2013; Cayuela et al., 2014; Zhang et al., 2016) to affect its functioning as a soil amendment. Woldetsadik et al. (2017) reported that fecal matter biochar application up to 30 t ha^{-1} still increased lettuce yield in the second growth cycle as a result of increase in plant P, K, Mg and to a lesser extent N mineralization in soils. In another study using Komatsuna, a common leafy vegetable grown in Japan, Singla et al. (2014) showed that application of biogas digested slurry based-biochar in a sand dune Regosol did not affect the yield of Komatsuna in the third crop cycle conducted after fertilizer application in the second crop cycle when compared to the treatment with only chemical fertilizer. On the basis of their research, the present study focused on further exploring their findings using palm shell biochar, one of the plant derived biochars that currently have limited or

scarce information regarding their effects in sandy soils. Moreover, increasing plant N uptake through biochar addition could improve N fertilizer use efficiency in poor sandy soils where N loss is a major environmental and agronomic problem (Uzoma et al., 2011).

This study aimed at examining the residual effects of palm shell biochar on the growth and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under three continuous crop cycles without additional N fertilizer application after the first crop cycle. It was hypothesized that biochar application in presence of fertilizer would maintain higher crop yield during the second and third crop cycles due to the increased mineralization of biochar nutrients.

2.2. Materials and methods

2.2.1. Establishment of pot experiment

The pot experiment was conducted in a greenhouse (vinyl house) at Tottori University, Tottori, Japan (35°30' 55"N 134°10'12"E) from 27th November 2015 to 10th June 2016 with three crop cycles. The first crop cycle was conducted from 27th November 2015 to 18th January 2016, the second cycle from 1st April 2016 to 26th April 2016 and third crop cycle from 16th May 2016 to 10th June 2016. At the start of the first crop cycle, Komatsuna (*Brassica rapa* var. *perviridis*) seedlings were transplanted into 1/2000a Wagner pots (29.3 cm height, 25.6 cm outer diameter and 24.0 cm inner diameter) filled with 18 kg of sandy soil. Prior to potting the soil, it was air dried and passed through a 2 mm sieve to remove weed residues and other impurities. The sandy soil was collected from Tottori sand dunes, Tottori, Japan while palm shell biochar, pyrolyzed at 400–550°C was purchased from a commercial company (King Coal Co. Ltd, Tokyo, Japan). **Table 1** shows the physico-chemical properties of the sandy soil and biochar used. Five treatments in triplicate included: (1) only 6% biochar (B); (2) only fertilizer (F); (3) fertilizer + 6% biochar (FB); (4) fertilizer + 12% biochar (F2B) and (5) fertilizer + 18% biochar (F3B). The biochar and inorganic fertilizer NPK (15:15:15) at 225 kg N ha^{-1}, 225 kg P_2O_5 ha^{-1} and 225 kg K_2O ha^{-1}, with dolomite at 1000 kg ha^{-1} were incorporated into the top 10 cm of soil (7.2 kg air dry basis). For each pot, biochar and soil were mixed manually and added into polythene bags, then gently shaken to obtain a uniform soil-biochar mixture. Fertilizer was then added to the soil-biochar mixture and was further shaken to ensure complete homogeneity. There was no fertilizer and biochar addition during the second and third crop cycle; fertilizer was applied once at the start of the first crop cycle. Transplanting was done the same day when fertilizer and biochar were mixed in the soil. One Komatsuna seedling at the 3–4 leaf stage was transplanted in each pot. All pots received equal

amounts of water and this was based on daily temperatures and vegetable growth throughout the crop growing periods. After each harvest, the soils in pots were occasionally irrigated with equal amounts of water to provide suitable conditions for soil chemical reactions. During the crop growing period, the air temperatures inside the greenhouse ranged between 0.5 to 35.9°C with an average of 9.3°C for the first crop cycle, 2.4 to 52.1°C with an average of 18.9°C for the second crop cycle and 9.4 to 47.7°C with an average of 23.6°C for the third crop cycle.

Table 1. Initial nutrient composition of soil and biochar used in this experiment.

Properties	**Units**	**Soil**	**Biochar**
pH (H_2O)		7.45	7.99
EC	dS m^{-1}	0.01	0.39
Total C	g kg^{-1}	0.05	350
Total N	g kg^{-1}	0.03	5.20
C/N		1.67	67.4
Available P	mg kg^{-1}	2.6	135
Exchangeable K	mg kg^{-1}	81.4	1540
Exchangeable Ca	mg kg^{-1}	63.6	3103
Exchangeable Mg	mg kg^{-1}	64.7	185
Sand	%	97.2	-
Silt	%	2.8	-
Clay	%	<0.0	-
Texture		Sand	-
Bulk density	g cm^{-3}	1.4	-

2.2.2. Crop growth and yield

At the end of each cycle, crop growth was determined by measuring plant height and leaf area. Plant height was measured from the soil surface to the tip of the longest leaf. The leaf chlorophyll content, expressed in SPAD values was measured by the chlorophyll meter (SPAD-502, Minolta Co. Ltd, Osaka, Japan) prior to removal of plants from the soil. Each plant was then divided into leaf blades, leaf stalks, roots and their fresh weights measured. Leaf blades were taken for measurement of leaf area using the leaf area meter (LI-3100, LI-COR, Lincoln, NE USA). Before measurement, the roots were carefully removed from soil, washed with tap water, rinsed with RO (Reverse Osmosis) water and then blotted dry between absorbing papers. The plant parts were oven dried at 72°C until they attained constant dry weight and later used for measurement of plant nutrient concentration. The yield was measured by adding dry weights of all the above ground parts.

2.2.3. Soil and biochar analysis

At the end of the three crop cycles, soil samples were taken from 10 cm depth, mixed homogeneously, air-dried, sieved (<2mm), packed and stored for analysis. Soil and biochar pH and EC were measured at a 1:5 (w/v) soil to water ratio using pH and EC electrodes (F-74 pH/ION/COND meter, Horiba Ltd, Kyoto, Japan). Soil texture was determined using the pipette method. Total C, total N were determined by dry combustion using the C/N corder (JM1000CN, J-SCIENCE LAB, Kyoto, Japan) with an auto sampler (JMA1000, J-SCIENCE LAB, Kyoto, Japan) and the results used for calculating the C/N ratio. Available P was determined by using 0.002N H_2SO_4 buffered with $(NH_4)_2SO_4$ (Truog, 1930). Briefly, 0.5 g of air dried soil or biochar was extracted with 100 ml of extraction solution for 30 min on a shaker and then P in the soil filtrate determined by phosphomolybdate blue method (Murphy and Riley, 1962) using a spectrophotometer at 710 nm (U-5100, Hitachi, Tokyo, Japan). Exchangeable K, Ca and Mg were determined by extraction of 1.0 g of soil with 15 ml of 1N ammonium acetate (NH_4OAC) buffered at pH 7.1 for 15 min on a shaker. After which, samples were centrifuged at 3,000 rpm for 3 min, supernatant filtered into 50 ml flasks and again 15 ml NH_4OAC added and centrifugation repeated twice and collected into flasks and final solution topped up with NH_4OAC to the 50 ml mark. The soil extracts were then measured by the atomic absorption spectrophotometer (Z-2300, Hitachi, Tokyo, Japan). Biochar chemical analysis was done following similar methods used for soil analysis.

2.2.4. Plant nutrient uptake

Plant nutrient uptake was determined at the end of each crop cycle. The dry samples were finely ground into powder by use of a stainless steel wonder blender and later analyzed for N, P, K, Ca and Mg concentration. The total N was determined by the dry combustion method using the C/N corder described above. The other elements were determined by the H_2SO_4-H_2O_2 digestion method (Thomas et al., 1967). Plant P was determined by colorimetry (vanadate-molybdate yellow method) using a spectrophotometer at 420 nm (U-5100, Hitachi, Tokyo, Japan) while K, Ca and Mg were determined using the atomic absorption spectrophotometer (Z-2300; Hitachi, Tokyo, Japan). Nutrient uptake for the different plant parts was determined by multiplying their dry weights with the corresponding nutrient contents and then adding up all the nutrient uptakes for all the plant parts.

2.2.5. Statistical analysis

All data were analyzed statistically using IBM SPSS statistics (Version 20.0). Tukey's honestly significant (HSD) test was used to determine whether there were significant differences between the treatments and crop cycles at 0.05 probability level unless otherwise specified.

2.3. Results

2.3.1. Crop growth and yield

Figure 8a shows the variation in plant height between treatments across the crop cycles. The lowest crop growth and yield in all crop cycles was observed in the B treatment. Plant height did not significantly vary among FB, F2B and F3B treatments within and cross the crop cycles. In addition, no significant differences were observed between the F treatment and FB, F2B, F3B treatments during the first and second crop cycles. However, in the third crop cycle, plant height in the F treatment was significantly higher ($P < 0.01$) than in the FB, F2B and F3B treatments by 27.8%, 31.9% and 38.5% respectively. Moreover, plant height for the F treatment significantly increased with crop cycle. In the third crop cycle, the positive residual effects from the inorganic fertilizer resulted in an increase in plant height as compared to biochar amended soils where plant height did not vary significantly.

In all treatments, the leaf chlorophyll content significantly ($P < 0.001$) reduced with increase in cropping (**Figure 8b**). No significant differences in leaf chlorophyll content were observed between F, FB, F2B and F3B treatments during the first and second crop cycles.

However, in the third crop cycle, the F treatment had significantly (P<0.001) higher leaf chlorophyll content than in treatments with biochar. Therefore, in the third crop cycle, biochar application in soils with fertilizer resulted in negative residual effects on the leaf chlorophyll content with a reduction of 13.2% in the FB, 20.8% in the F2B and by 20% in the F3B treatments as compared to non-biochar amended soil (F treatment).

In the first crop cycle, there were no significant differences in leaf area and yield among the F, FB, F2B and F3B treatments (**Figure 8c and 8d**). However, in the third crop cycle, the highest leaf area and yield were observed in the F treatment. In the first and second crop cycles, there was no significant variation in crop yield for the F treatment but significantly reduced by 38.3% in the third crop cycle. In the FB, F2B and F3B treatments, leaf area and yield significantly reduced with increased cropping. In the second crop cycle, the residual effects of biochar resulted into a significant yield decline by 38.5% in F3B when compared to the F treatment. The yield of F did not significantly differ from that of the FB and F2B treatments in the second crop cycle. Biochar addition at 6% (FB treatment) did not have any significant residual effects on yield during the second cycle. However, in the third crop cycle, the residual effects of biochar resulted into yield decline by 21.5% in FB (P > 0.05), 48.7% in F2B (P=0.001) and by 53% in F3B treatment (P=0.001) when compared to the F treatment.

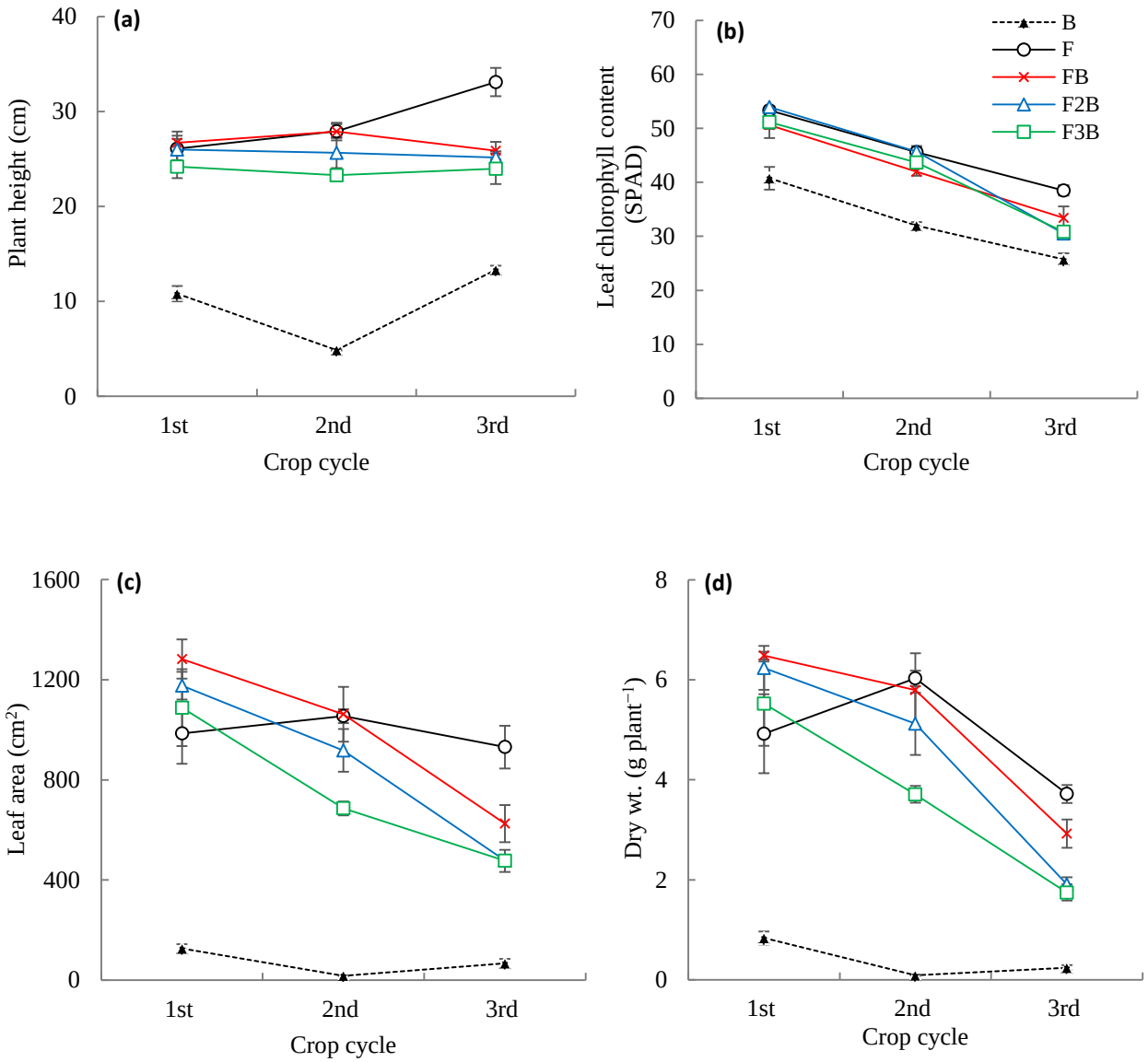

Figure 8. Variations in plant height **(a)**, Leaf chlorophyll content **(b)**, leaf area **(c)**, and shoot dry weight **(d)** across the three crop cycles. F represents fertilizer; B, 2B and 3B represent biochar application at 6, 12 and 18% (w/w) respectively. Data points represent mean ± standard error (n=3).

2.3.2. Plant nutrient uptake

The lowest plant uptake for all the nutrients was observed in the B treatment. For all the treatments with biochar, plant uptake for all nutrients was generally highest in the first crop cycle and lowest in the third crop cycle (**Table 2**). However, in the absence of biochar, N uptake was highest in the first crop cycle and lowest in the third crop cycle while P, K, Ca and Mg were highest during the second crop cycle. In the first crop cycle, biochar application in the presence of fertilizer did not have any significant effects on plant N and Mg uptake. However, in the second and third crop cycles, biochar application significantly reduced plant N and Mg uptake but this was more evident during the third crop cycle. Biochar did not generally affect plant P uptake in the FB, F2B and F3B treatments as compared to the F treatment. Biochar application significantly increased plant K uptake only in the first crop cycle. Plant Ca uptake was significantly increased by biochar in the FB treatment during the first crop cycle but in the second and third crop cycles, biochar significantly reduced Ca uptake especially in the F3B treatment.

2.3.3. Soil chemical properties

Biochar significantly increased soil pH, EC, total N, NO_3^--N, available P and exchangeable K, Ca and Mg content (**Table 3**). However, biochar significantly reduced exchangeable NH_4^+-N content. Except for exchangeable NH_4^+-N content, the B treatment had significantly higher soil pH, EC, exchangeable K and Ca than the F treatment.

Table 2. Plant nutrient uptake among treatments at the end of each crop cultivation cycle.

Nutrient	Treatment	1st crop cycle	2nd crop cycle	3rd crop cycle
			mg plant^{-1}	
N	B	20.6 ± 4.2b	-	-
	F	310.8 ± 49.7a	239.7 ± 18.5a	144.3 ± 28.8a
	FB	397.3 ± 3.7a	207.2 ± 27.2a	62.5 ± 9.3b
	F2B	390.3 ± 19.9a	163.9 ± 21.1ab	45.6 ± 3.1b
	F3B	344.4 ± 43.2a	112.9 ± 9.6b	44.2 ± 5.3b
P	B	4.3 ± 0.8b	-	-
	F	35.4 ± 5.4a	53.2 ± 2.1a	33.7 ± 1.2ab
	FB	44.1 ± 0.9a	55.3 ± 6.8a	35.2 ± 3.0a
	F2B	42.2 ± 2.9a	50.1 ± 6.2a	27.9 ± 2.4ab
	F3B	37.8 ± 4.3a	36.0 ± 1.0a	24.7 ± 1.8b
K	B	32.2 ± 4.8c	-	-
	F	263.4 ± 29.5b	320.6 ± 3.4a	194.5 ± 22.4a
	FB	433.9 ± 13.4a	365.0 ± 44.2a	163.1 ± 19.8a
	F2B	448.9 ± 24.5a	322.7 ± 52.6a	125.4 ± 10.9a
	F3B	403.9 ± 54.0ab	232.5 ± 12.8a	126.9 ± 17.8a
Ca	B	14.6 ± 2.1c	-	-
	F	86.7 ± 13.4b	120.4 ± 3.4a	80.9 ± 7.2a
	FB	155.6 ± 3.1a	128.6 ± 16.1a	60.8 ± 8.2ab
	F2B	119.4 ± 2.9ab	99.4 ± 9.9ab	41.3 ± 2.6b
	F3B	89.9 ± 22.5b	71.8 ± 2.5b	38.6 ± 3.1b
Mg	B	4.2 ± 0.7b	-	-
	F	34.1 ± 4.2a	47.8 ± 2.4a	36.1 ± 4.4a
	FB	39.2 ± 2.3a	37.4 ± 5.2ab	17.0 ± 1.6b
	F2B	30.7 ± 1.6a	33.2 ± 4.3ab	12.5 ± 0.9b
	F3B	25.5 ± 4.2a	22.2 ± 1.2b	11.0 ± 0.7b

For each plant nutrient, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. Mean ± standard error (n=3). Dash (-): No samples analyzed due to inadequate sample size. F represents fertilizer; B, 2B and 3B represent biochar application at 6, 12 and 18% (w/w) respectively.

Table 3. Soil chemical properties at the end of the three crop cycles.

	Soil pH (H_2O)	Soil EC (dS m^{-1})	Total N (g kg^{-1})	NH_4^+-N	NO_3^--N	P	K	Ca	Mg
				mg kg^{-1}					
B	8.4 ± 0.1a	0.05 ± 0.00b	0.49 ± 0.02ab	2.44 ± 0.05b	2.16 ± 0.78b	15.3 ± 0.8c	137.2 ± 2.9c	414.1 ± 14.7b	56.7 ± 0.9c
F	7.2 ± 0.0c	0.02 ± 0.00c	0.32 ± 0.00b	3.63 ± 0.22a	1.49 ± 0.17b	3.5 ± 0.3c	25.4 ± 7.9d	147.9 ± 6.0c	62.3 ± 1.7bc
FB	8.0 ± 0.2b	0.05 ± 0.00b	0.56 ± 0.06ab	2.23 ± 0.12b	2.99 ± 0.61b	37.2 ± 2.4b	108.0 ± 4.2cd	481.1 ± 42.2b	63.8 ± 3.6bc
F2B	8.4 ± 0.0a	0.08 ± 0.00a	0.73 ± 0.05ab	2.28 ± 0.11b	7.12 ± 0.98a	70.6 ± 1.2a	234.3 ± 4.6b	912.4 ± 57.4a	83.0 ± 3.8ab
F3B	8.5 ± 0.0a	0.09 ± 0.01a	1.05 ± 0.30a	1.85 ± 0.09b	7.68 ± 1.27a	78.2 ± 8.0a	335.4 ± 38.9a	1032.7 ± 98.4a	98.9 ± 10.1a

Different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. Mean ± standard error (n=3). F represents fertilizer; B, 2B and 3B represent biochar application at 6, 12 and 18% (w/w) respectively.

2.4. Discussion

The general reduction in crop growth and yield with increase in crop cycles was a result of nutrient depletion by the preceding crops since there was no biochar and fertilizer application during the second and third crop cycles. However, the lower crop growth and yield observed in the biochar treatments during the second and third crop cycles showed that biochar had negative residual effects on crop growth and yield and this was more significant in the third crop cycle. With the exception of K which can be taken up by the plants as long as it is available (luxury consumption), the soil nutrients (from biochar) did not have a significant impact on crop growth. Hence the yield of Komatsuna did not depend on the nutrients from palm shell biochar even in the third crop cycle, as there could have been an increase in crop growth in the B treatment. Plant growth depended on the nutrient supply from chemical fertilizer. Moreover, Dharmakeerthi et al. (2012) concluded that the supply of N and Mg as chemical fertilizers with biochar is necessary to promote better plant growth. The positive correlation between crop yield, leaf chlorophyll content and leaf area with plant tissue N concentration showed that the negative residual effects of biochar on crop yield were mainly due to changes in plant N uptake. Therefore, the negative effects of higher biochar application rates on crop yield in the second and third crop cycles can be attributed to N limitation (Borchard et al., 2014) due to immobilization of the available N due to higher C/N ratios or as a result of reduction in N pools by biochar through adsorption of NH_4^+-N (Clough et al., 2013) as observed in **Table 3**. The higher crop yield in the non-biochar amended soils (F treatment) during the third crop cycle could partly be attributed to the increased N mineralization which made N available to plants in soils without biochar. This also explains the higher plant height which was observed in the F treatment during the third crop cycle (**Figure 8a**). The positive correlation between N uptake and leaf chlorophyll content also shows that with increase in crop cycles, N became the limiting factor hence resulting in lower chlorophyll content in the plants grown in biochar amended soils. In sites with low native N supply, biochar reduced leaf chlorophyll contents suggesting that biochar may reduce grain yield in N-deficient soils if additional N is not applied (Asai et al., 2009; Nelson et al., 2011). In addition, findings from Akhtar et al. (2014) showed that biochar application significantly decreased leaf N content and chlorophyll content index (CCI) of tomato.

In all the three crop cycles, biochar application alone without fertilizer had the lowest leaf chlorophyll content, crop growth and yield indicating that the mineral N in biochar was not

available for plant uptake and can be partly attributed to the nutrient content of the original feedstock and the pre-existing soil nutrient status (Woldetsadik et al., 2017). The effect of biochar application on crop yield depends on the initial soil properties. In this study, the sandy soil had a very low concentration of nutrients and could not support plant growth despite having higher amounts of nutrients in biochar alone (B) as compared to fertilizer alone (F) treatments leading to significantly lower crop yields compared to biochar treatments with fertilizer. This was expected because sandy soil with a low nutrient status was used in this study meaning that the major source of nutrients for crop growth ought to have been from external sources such as biochar or fertilizer. Rajkovich et al. (2012) showed that plant residue biochars such as hazelnut shell, pine and oak showed little effects on plant growth. Palm shell biochar is also a plant residue derived biochar and therefore, it showed little effects on crop yield not only in the first crop cycle, but even for the residual effects in the second and third crop cycles. In contrast, Woldetsadik et al. (2017) showed that in soils with initial higher nutrient amounts, there were no significant differences in lettuce yield between soils amended with biochar alone and those with biochar amended with fertilizer. This is because the authors used biochar from fecal matter which is proposed to supply nutrients directly for crop growth. Animal manure based biochars may directly supply more nutrients to the plant (Rajkovich et al., 2012), hence higher crop yields. However, the results from the present study imply that the nutrients in palm shell biochar are not available to plants and that biochar benefits on crop yield are realized when it is applied with fertilizer. Chan et al. (2007) evaluated the effect of green waste biochar on radish growth and reported that in the absence of N fertilizer, biochar application in the soil even at its highest rate (100 t ha^{-1}) did not increase radish yield. Therefore, benefits from plant derived biochars seem to be significant only when applied with inorganic fertilizers; biochar ammendment has a synergistic effect with fertilizers in improving crop yields (Hussain et al., 2017) because most of the nutrients contained in biochar are not available to plants (Filiberto and Gaunt, 2013) as observed in the present study.

The consistent positive effects of biochar application on plant K concentration in the second and third crop cycles shows that biochar acted as a source of K even after the first crop cycle. However, Butnan et al. (2015) explained that the high K content in biochar leads to *luxury consumption* that adversely impacts on Ca and Mg nutrition in maize. This could explain the reduced Ca and Mg uptake following biochar application in the present study. Similarly, Pavlíková et al. (2017) reported that biochar reduced Ca, Mg and Na content of spinach plants but increased

K content while an inconsistent effect was observed for P content. The results also revealed that biochar significantly increases soil chemical properties and this was consistent with the findings from other studies (Chan et al., 2007; Chintala et al., 2013; Yue et al., 2017) which reported increase in soil chemical properties with increase in biochar application rates. The increase in soil nutrient concentrations was due to the higher inherent nutrient composition of biochar used in this study **(Table 1)** which were expected to increase with increasing biochar amounts in the soil. Biochar contains high concentrations of N, P, K and Ca which may provide nutrients directly in soil or may be a source of nutrients for microorganisms (Akhtar et al., 2014; Cha et al., 2016; Buss et al., 2016). The presence of higher nutrient amounts in the B treatment than in the F treatment indicates that biochar has a potential to supply vast amounts of nutrients to the soil as compared to fertilizer application. However, these nutrients were not available to the plants since the results of crop growth and yield showed that the B treatment had significantly lower yield as compared to the F treatment. Komatsuna belongs to the brassica family which prefers NO_3^--N to NH_4^+-N (Ikeda and Osawa, 1981; Xu et al., 2017). Hence, the significant increase in soil NO_3^--N content in the biochar treatments with fertilizer as compared to the non-biochar amended soil can be related to the low N uptake (**Table 2**) in plants grown in biochar-amended soils. However, it could also be that biochar facilitated nitrification but the NO_3^--N obtained from this process was not taken up by the plants at higher biochar rates.

2.5. Conclusion

In this study, biochar application in soils with fertilizer did not significantly influence crop yield and N uptake during the first crop cycle. However, with increased cultivation in these soils, biochar hindered N availability to the plants resulting in negative residual effects on crop growth, yield and leaf chlorophyll content during the second and third crop cycles. Biochar application improved soil chemical properties. The negative residual effects of biochar necessitate the need for more seasonal N fertilizer application in sandy soils where it has been previously used as a soil amendment. Moreover, while considering the need for seasonal N fertilizer application in biochar amended soils, it is necessary to assess the role of biochar in mitigating the negative effects especially N_2O emissions and soil acidification that might result from fertilizer application.

CHAPTER THREE

Impact of fresh and aged palm shell biochar on N_2O emissions, soil properties, nutrient content and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under sandy soil conditions

3.1. Introduction

Agriculture is the main anthropogenic source of nitrous oxide (N_2O) (IPCC, 2013) mainly as a result of biological transformation of nitrogen (N) contained in fertilizers (Konsolakis, 2015). In intensive vegetable production, N_2O emissions are mainly associated with inorganic N fertilizer and manure which are often applied in excess and not fully absorbed by plants (Jia et al., 2012b). Komatsuna (*Brassica rapa* var. *perviridis*) is one of the common leafy vegetables grown in Japan, utilizing about 120–150 kg N ha^{-1} per cultivation cycle which usually lasts 1 month (Amkha et al., 2009; Amkha and Inubushi, 2009; Singla et al., 2013, 2014). Since Komatsuna can be grown for seven times in a single year, the fertilizer N inputs to soil are high and can significantly decrease soil quality. For instance, vegetable fields intensively managed for more than 10 years with large inputs of N (>1,000 kg N ha^{-1} yr^{-1}) have an average soil pH of 5 (Jia et al., 2012b; Li et al., 2015a, 2015b; Wang et al., 2015a) which may be lower than that in other agricultural production systems, resulting in higher N_2O emitted from the soils.

The use of biochar as a soil amendment may reduce N_2O emissions and improve crop productivity (Lehmann and Joseph, 2009; Hussain et al., 2017). Although the mechanisms are not well understood, the application of N fertilizers in combination with biochar can improve the temporal synchrony between crop-N demand and soil-N availability, thereby enhancing N use efficiency for crop growth and reducing environmental impacts (Cayuela et al., 2014). Biochar can be applied once in the soil but N fertilizers are often applied every crop season. However, the effects of biochar on N_2O emissions are contradictory since it can reduce (Nguyen et al., 2014; Agegnehu et al., 2016; Zhang et al., 2016), increase (Verhoeven and Six, 2014; Li et al., 2015a; Petter et al., 2016) or have no effect (Suddick and Six, 2013; Liu et al., 2015; Niu et al., 2018) on N_2O emissions.

The same has been reported for the effects of biochar on crop yield, with increase (Li et al., 2015b), reduction (Borchard et al., 2014) and no effects (Suddick and Six, 2013; Wang et al., 2017; Niu et al., 2018). The role of biochar to reduce N_2O emissions has been attributed to the increased soil aeration, increased soil pH, reduced N pools in soil (Clough et al., 2013; Li et al.,

2015b) and also through acting as an *electron shuttle* by facilitating electron transfer to denitrifying microorganisms in soil thereby promoting a reduction of N_2O to N_2 (Cayuela et al., 2013). Water-filled pore space (WFPS), which is a measure of the water-air contents in soil, regulates soil aeration and the oxygen availability for microorganisms; the increased oxygen availability at low WFPS (less than 80% WFPS) inhibits activity of denitrifiers, with nitrifiers being mainly responsible for N_2O emissions (Cayuela et al., 2014). Increase in crop yield following biochar application and N fertilizers has been attributed to the increased nitrogen use efficiency (NUE) (Uzoma et al., 2011; Li et al., 2015b) and the reduced N leaching (Kanthle et al., 2016) resulting in a steady supply of plant available N. Furthermore, the increase in crop yield is due to increased plant nutrient uptake resulting from the presence of inherent nutrients, ash, high surface area and porous nature of biochar and its capacity to act as a medium for microorganisms (Nigussie et al., 2012). On the contrary, the reduction in crop yield following biochar application is reported in silty soils due to the nutrient imbalances and N immobilization resulting especially at high biochar application rates of approximately 300 t ha^{-1} (Borchard et al., 2014). Generally, the addition of biochar to soil also improves soil properties thereby facilitating plant growth, as a result of improved root growth and higher nutrient uptake (Abrishamkesh et al., 2015; Hussain et al., 2017), and reduces bulk density due to biochar-induced soil aggregation which may aid root growth and if more water is available, it can increase crop growth and yield (Obia et al., 2016). However, the impacts of biochar on plant productivity in soils vary depending on the soil characteristics, plant species, environmental conditions, biochar properties (Saarnio et al., 2013) and application rates (Singla et al., 2014; Hagner et al., 2016). Generally, biochar application can increase soil pH, organic C, exchangeable cations and available P contents and reduce tensile strength, especially at higher rates of more than 50 t ha^{-1} (Chan et al., 2007).

The effects of biochar in soil depend on its aging/weathering (Lehmann and Joseph, 2009; Trigo et al., 2014), which affects the aromatic structure, surface functionality and adsorption of minerals, and organic compounds in biochar that are responsible for its resistance to losses via degradation, leaching, chemical oxidation and recalcitrance in soil (Shrestha et al., 2010). Compared to fresh biochar, aged biochar has a better oxygen-containing functionality, patchy mineral coatings and presence of more microorganisms that can increase degradation to water-soluble molecules resembling oxidized polycyclic aromatic hydrocarbons (Hockaday et al., 2007). Biochar aging in soil enhances NH_4^+-N in soil due to the higher acidic functional groups, increases

microbial and enzymatic activities, and facilitates higher rates of N mineralization due to the increased N demand for the microorganisms (Yadav et al., 2019). The analysis of variation among biochar aging, soil amendment, and the potential for C sequestration is recommended to further elucidate the role of biochar in soil processes (Zhao et al., 2015). Hagemann et al. (2017) reported that N_2O emissions were still effectively reduced by powdered beech wood biochar in the third year after application in the field while Spokas (2013) showed that wood and macadamia nut shell biochars weathered for 3 years under field conditions did not reduce N_2O production which was reduced in fresh biochars in laboratory incubations. One of the challenges hindering the large application of biochar for climate change mitigation is the limited knowledge on the persistence of N_2O suppressing effect following biochar addition (Hagemann et al., 2017) which may vary with the biochar feedstock.

Palm shells are among the agricultural wastes obtained from the palm oil industry and they have a highly complex pore structure and fiber matrix which could be a good feedstock for production of pyrogenic carbonaceous materials such as activated carbon and biochar (Arami-Niya et al., 2010; Abdullah and Sulaiman, 2013; Martinsen et al., 2015). However, the agronomic and environmental effects of palm shell biochar are poorly known especially in sandy soils. Since aging affects biochar functioning, it is still unclear as to whether the large application of palm shell biochar could still mitigate N_2O emissions and maintain soil properties even when basal N fertilizers are applied 1 year after the initial biochar application in the soil. Therefore, the aim of this study was to assess the impact of fresh and aged palm shell biochar on N_2O emissions, soil properties, nutrient content and yield of Komatsuna under sandy soil conditions. It was hypothesized that even after 1 year of application in soil, biochar could still suppress N_2O emissions, improve soil properties and plant nutrient content without having negative effects on crop yield.

3.2. Materials and methods

3.2.1. Establishment of pot experiment

This experiment was conducted in a greenhouse (vinyl house) at Tottori University, Tottori, Japan (35°30' 55"N 134°10'12"E) from November 2016 to March 2017. Komatsuna (*Brassica rapa* var. *perviridis*) was grown for two crop cycles; the first crop cycle lasted for 50 days while the second crop cycle lasted for 44 days. At the start of each crop cycle, one seedling with 3–4 leaves was transplanted into each Wagner pot (1/2000a–29.3 cm height, 25.6 cm outer diameter and 24.0 cm

inner diameter). After harvesting plants and the subsequent removal of root residues at the end of the first crop cycle, new seedlings were immediately transplanted into the same pots on the same day. On 28th November 2016, fertilizers were added to soils in the F (only fertilizer), FB (fertilizer + 6% biochar), F2B (fertilizer + 12% biochar) and F3B (fertilizer + 18% biochar) pots which had been used in the previous experiment in 2015 (Chapter two) while the B (only 6% biochar) pots in that study did not receive any fertilizer. The percentages (6%, 12% and 18%) were calculated on a weight by weight (w/w) biochar on an air dry soil weight basis corresponding to approximately 85, 170 and 250 t ha^{-1} biochar, respectively. Similar to the previous experiment, NPK was added at 225 kg N ha^{-1}, 225 kg P_2O_5 ha^{-1}, and 225 kg K_2O ha^{-1}, with dolomite at 1000 kg ha^{-1} incorporated into the top 10 cm of soil in the F, FB, F2B and F3B pots. No biochar was added into these pots because it had been applied in November 2015 and Komatsuna had been continuously grown in the soils for three crop cycles without applying basal fertilizer and biochar at the start of the second and third crop cycles. The soil chemical properties at the end of the third crop cycle were also measured (**Table 3**). Therefore, in the current study, the soils in these pots will be referred to as sandy soils containing 1-year aged palm shell biochar (PSB_{aged}).

In addition, five new treatments involving fresh/new palm shell biochar were added in sieved sandy soil in Wagner pots of similar size as described above. Fertilizer and biochar were mixed at 10 cm soil depth (7.2 kg air dry basis) at similar rates as described above. The fertilizer types used in these pots were also similar to that described above. The soils in these pots are referred to as sandy soils mixed with fresh palm shell biochar (PSB_{fresh}). The sandy soil was collected from Tottori sand dunes, Tottori, Japan and its properties are shown in **Table 1**. Palm shell biochar (pyrolyzed at 400–550°C) was purchased from a commercial company (King Coal Co. Ltd, Tokyo, Japan), with a particle size of <3 mm and had the following properties: pH (H_2O) 7.99; electrical conductivity (EC) 0.39 dS m^{-1}; total C 350.2 g kg^{-1}; total N 5.2 g kg^{-1}; C/N ratio 67.4; H/C ratio 0.04; available P 135.2 mg kg^{-1}; exchangeable K 1540.2 mg kg^{-1}; exchangeable Ca 3103.8 mg kg^{-1}; exchangeable Mg 185.2 mg kg^{-1} and CEC 12.8 cmol (+) kg^{-1}. Therefore, this research includes treatments with fresh and aged palm shell biochar at 0%, 6%, 12%, and 18% (w/w) replicated three times. Prior to transplanting, 1500 ml of water were added to each pot. After transplanting, irrigation was done with 200–250 ml of water which were added to the pots using a watering can once every 2–3 days and this was based on the crop growth and daily temperatures. Thermo recorders (TR-71wf, T & D Corporation, Nagano, Japan) were inserted at 5 cm soil depth

to monitor the mean daily soil temperature while the air temperature inside the greenhouse was monitored by a wireless thermo recorder (RTR-500B1, T & D Corporation, Nagano, Japan) throughout the crop growing period.

3.2.2. Gas sampling and analysis

The manual closed chamber (**Figure 9**) was used to collect air samples from each replicate, across the two crop cycles after 3, 8, 19, 32, 49, 58, 79 and 93 days between 10:00 am and 1:00 pm. The frustum shaped sampling chambers had an outer radius (R) of 13.5 cm, inner radius (r) of 11.9 cm and height of 26 cm. Each chamber was equipped with three ports; one for sampling, the other for measuring air temperature inside the chamber and the other fitted with an air buffer bag (1-L Tedlar® bag) to compensate for the pressure differences. The headspace concentrations of gases were measured after 0, 20, and 40 min of closing the pots by sampling 35 ml of gas using a 60 ml plastic syringe. 5 ml of gas were used to flush the syringe needle and 30 ml were immediately injected into 15 ml pre-evacuated vials (Nichiden-Rika Glass Co. Ltd, Kobe, Japan) fitted with butyl rubber stoppers and then stored in the laboratory until analysis. The air temperature inside the chamber was simultaneously measured by a digital thermo recorder (TR-71U*i*, T & D Corporation, Nagano, Japan). To measure the N_2O concentration, 1 ml of the gas was manually injected into the gas chromatograph (GC-14A; Shimadzu Corporation, Kyoto, Japan) equipped with an electron capture detector (ECD). All samples were analyzed in a laboratory at the Institute for Agro-Environmental Sciences NARO, Tsukuba, Japan. The gas fluxes were calculated by considering the linear increase of N_2O (mg N_2O-N m^{-2} h^{-1}) concentrations in the chamber headspace over the 40 min using the following equation (Minamikawa et al., 2015).

$$N_2O\ Flux = \rho \text{ X } \frac{dC}{dt} \text{ X } \frac{V}{A} \text{ X } \frac{273}{(273+T)} \text{ X } \frac{28}{44}$$

where ρ is gas density for N_2O (1.96 kg m^{-3}) dC/dt is the rate of the change in the gas concentration over time (ppm h^{-1}); V is the volume occupied by the headspace (m^3); A is the chamber area (m^2) and T is the mean air temperature inside the chamber (°C).

The total seasonal fluxes were calculated using the following equation according to Ding et al. (2015):

Cumulative N_2O emissions =

$$\sum_{i=1}^{n} (F_i + F_{i+1})/2 \text{ X } (t_{i+1} - t_i) \text{ X } 24$$

where F is the N_2O flux (μg N_2O-N m^{-2} h^{-1}), i is the ith measurement, $(t_{i+1} - t_i)$ is the number of days between the two measurements while n is the total number of measurements.

On each gas sampling day, soil moisture at 12 cm depth was measured using time-domain reflectometer (TDR) probes and then expressed as water-filled pore space (WFPS) by the equation;

$$\text{WFPS (\%)} = \frac{Volumetric\ water\ content\ (\%)}{Total\ soil\ porosity\ (\%)} \text{ X } 100$$

Where total soil porosity = $1 - (\frac{soil\ bulk\ density}{2.65})$ with 2.65 being the assumed soil particle density.

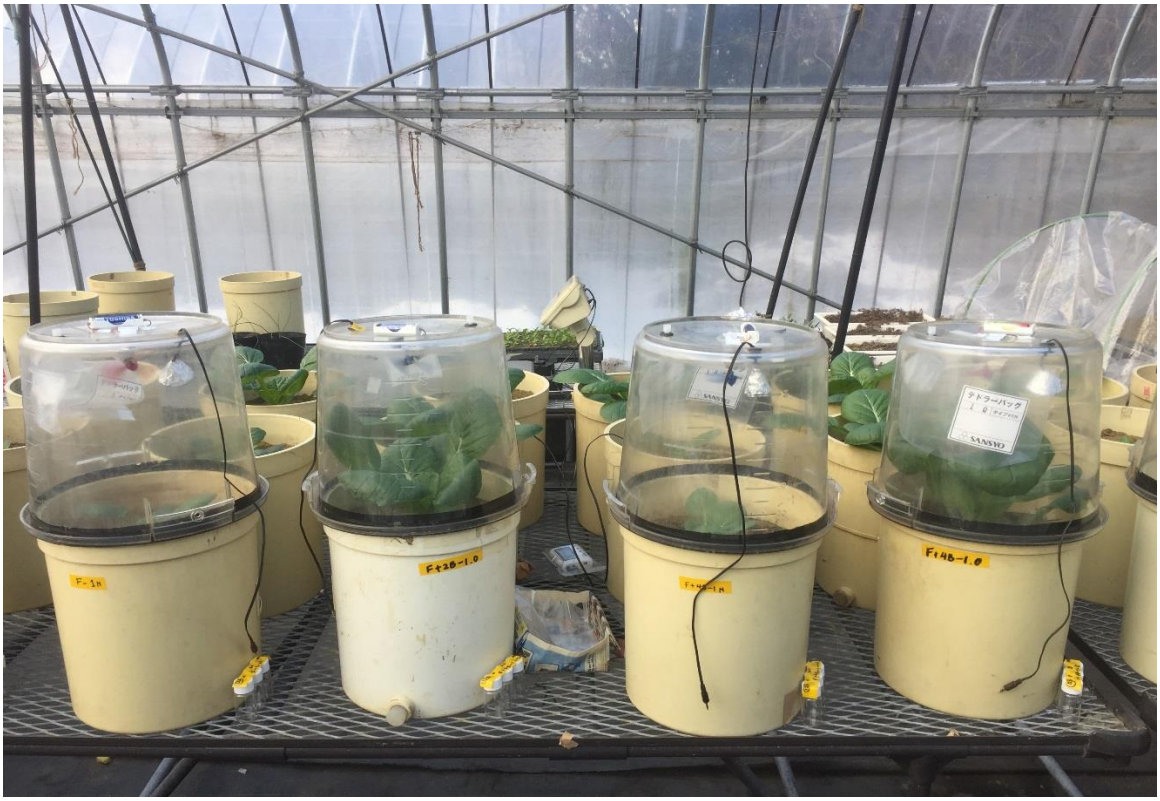

Figure 9. Chambers loaded on Wagner pots during gas sampling.

3.2.3. Crop growth and yield

At the end of each crop growth cycle, crop growth was determined by measuring plant height and leaf area. The leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA) was used for leaf area measurement. Leaf chlorophyll content was measured on standing plants and expressed as SPAD values using the chlorophyll meter (SPAD-502, Minolta Co. Ltd, Osaka, Japan). The plant parts were oven-dried at 72°C until they attained constant dry weight and later used for measurement of plant nutrient concentration. Yield was determined by adding up the dry weight of above-ground parts/plant shoot (leaf blades and leaf stalks) for each plant.

3.2.4. Soil and biochar analysis

To determine the inorganic N (exchangeable NH_4^+-N and NO_3^--N) content of soil in the different treatments on each gas sampling day, soil samples were collected at 0–10 cm depth from each Wagner pot at three different positions by 5 ml Eppendorf tips which had been cut at the tip end. The soil samples were thoroughly mixed and 20 g were stored in an ice cooler box and later stored at −80°C until analysis. To determine exchangeable NH_4^+-N, 5 g dry weight equivalent of wet soil were weighed in 100 ml bottles and extracted with 50 ml 10% potassium chloride (KCl) solution by shaking the soil slurry for 1 h using a mechanical shaker; then, samples were filtered and stored at 4°C before analysis which was done within 5 days. NO_3^--N was determined by weighing 1 g dry weight equivalent of wet soil in 250 ml bottles and extracted with 100 ml of 0.01% $AlCl_3 \cdot 6H_2O$ solution by shaking for 30 min in a mechanical shaker and the filtered samples were analyzed on the same day. Exchangeable NH_4^+-N was determined by the Indophenol blue method (Smith and Cresser, 2004) at 693 nm while NO_3^--N was determined by the ultraviolet absorption method (Yamaki, 2003) at 210 nm using a spectrophotometer (U-5100, Hitachi, Tokyo, Japan).

At the end of the two crop cycles, soil sampling and analysis of pH, EC, total N, C/N ratio, available P, exchangeable K, Ca and Mg were also done following similar procedures described in section 2.2.3. Before analysis, soil samples at 0–10 cm depth were taken, mixed homogeneously, air-dried and sieved (< 2 mm). Soil texture was determined using the pipette method. The bulk density of soil before its addition to the pots was determined using 100 cm^3 soil cores after oven drying at 105°C for 24 h. Biochar chemical analysis was done following similar procedures used in soil analysis. The H/C ratio of the biochar was obtained after C and H analysis using an elemental analyzer (vario EL cube, CHNS Elemental Analyzer, Elementar, Germany); aged biochar was picked from the sandy soil before H and C analysis. After analysis, there was no

change in the H/C ratio of fresh to aged biochar in all treatments. Scanning electron microscopy (SEM) images of fresh and aged PSB showed that the surface of fresh PSB was generally rough with some small particles adhering to it and most of the pores blocked while 1-year aged PSB had smoother surfaces with many unblocked pores and new small pores formed (**Figure 10**).

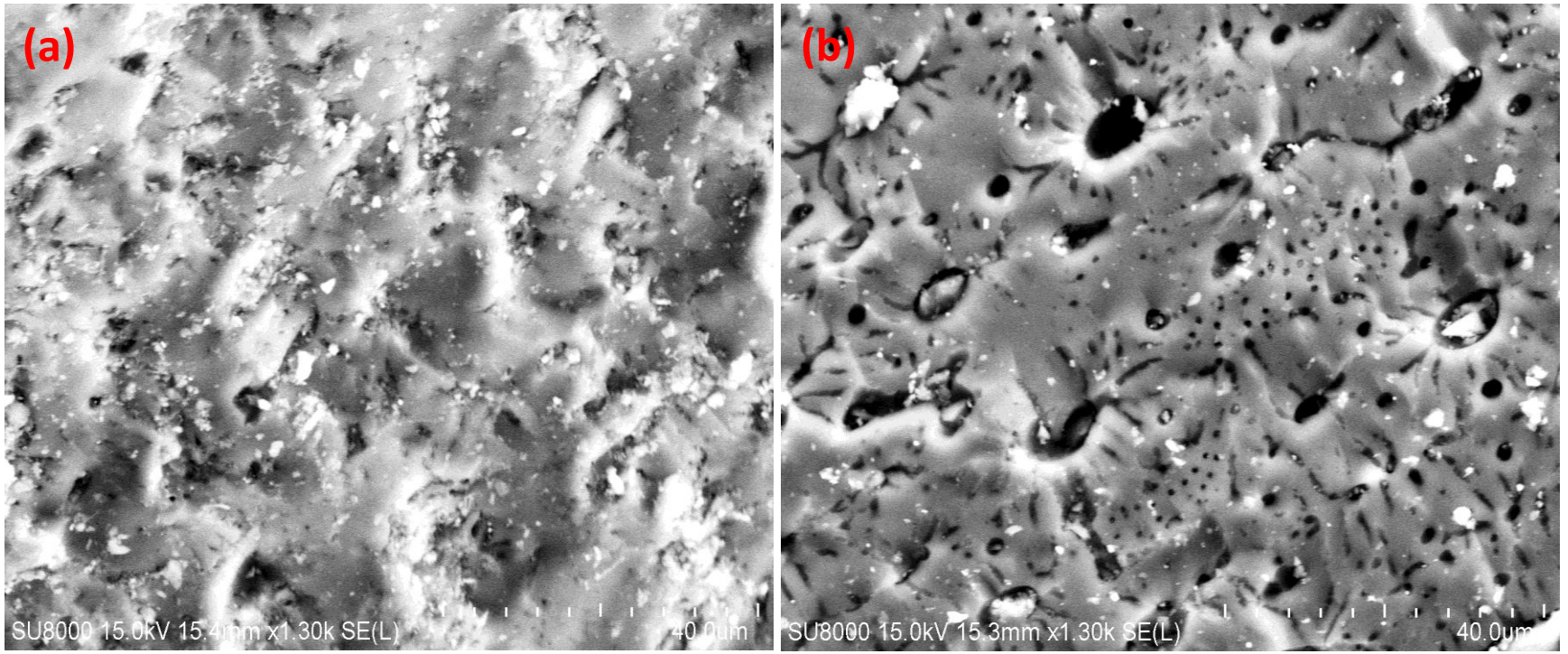

Figure 10. Representative scanning electron microscopy images of fresh (**a**) and 1-year aged (**b**) palm shell biochar.

3.2.5. Plant analysis

At the end of each crop cycle, the entire shoot (leaf blades and leaf stalks) were cut, oven dried at 72°C for 7 days, mixed and ground into fine powder using a stainless steel wonder blender and later analyzed for N, P, K, Ca and Mg concentration following similar procedures described in section 2.2.4.

3.2.6. Statistical analyses

All data were analyzed statistically using IBM SPSS statistics (Version 20.0). Analysis of variance (ANOVA) determined the effects of biochar on crop growth and yield, N_2O emissions, soil chemical properties and plant nutrient concentration followed by Tukey's HSD test at $P < 0.05$ unless otherwise specified. Correlation analysis using the Pearson's correlation coefficients was used to calculate the linear correlations between N_2O fluxes and soil temperature, WFPS, exchangeable NH_4^+-N and NO_3^--N.

3.3. Results

3.3.1. Air temperature, soil temperature and soil moisture

The mean daily air temperatures inside the greenhouse ranged from 2.4°C to 17.1°C and 2.8°C to 16.7°C for the first and second crop cycles, respectively (**Figure 11a**). The mean daily air temperatures were generally high in December but then continuously declined in January and thereafter gradually increased in February and March. The mean daily soil temperatures for both PSB_{fresh} and PSB_{aged} soils followed a similar trend, ranging from 7.3°C to 15.3°C during the two crop cycles (**Figure 11b and 11c**). There were no significant differences in soil temperature between the biochar types and treatments. Generally, the mean daily soil temperature for all the treatments in both PSB_{fresh} and PSB_{aged} soils on 2nd December was 14.9°C but then it continuously declined to 7.5°C on 17th January and thereafter gradually increased to 12.3°C on 2nd March. The variation in soil temperatures for all the treatments followed a similar pattern to that of the air temperature. The moisture levels were below 30% WFPS and ranged from 12.2% to 27.1% in the entire growing period **(Figure 12)**. The WFPS in all the treatments in both soils was the highest on 16th February, and generally higher in the PSB_{fresh} soils than in the PSB_{aged} soils on all sampling dates.

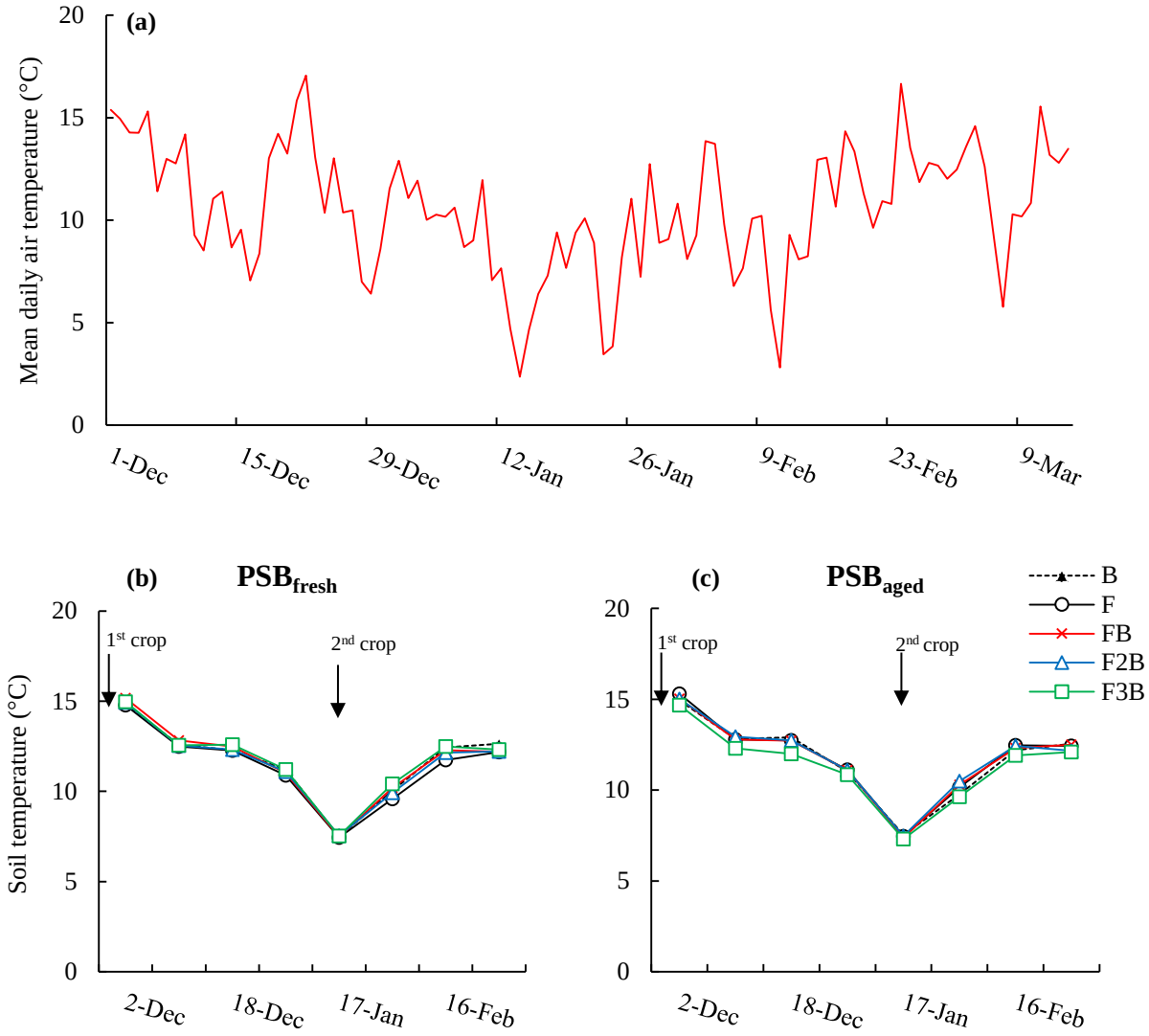

Figure 11. Variation in air temperatures inside the greenhouse throughout the two crop-growing seasons **(a)**. Changes in soil temperatures at 5 cm depth on the various gas sampling dates for PSB_{fresh} soils (**b**) and PSB_{aged} soils (**c**). PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar. PSB_{aged} represents sandy soils containing aged palm shell biochar. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Vertical arrows show the time of transplanting.

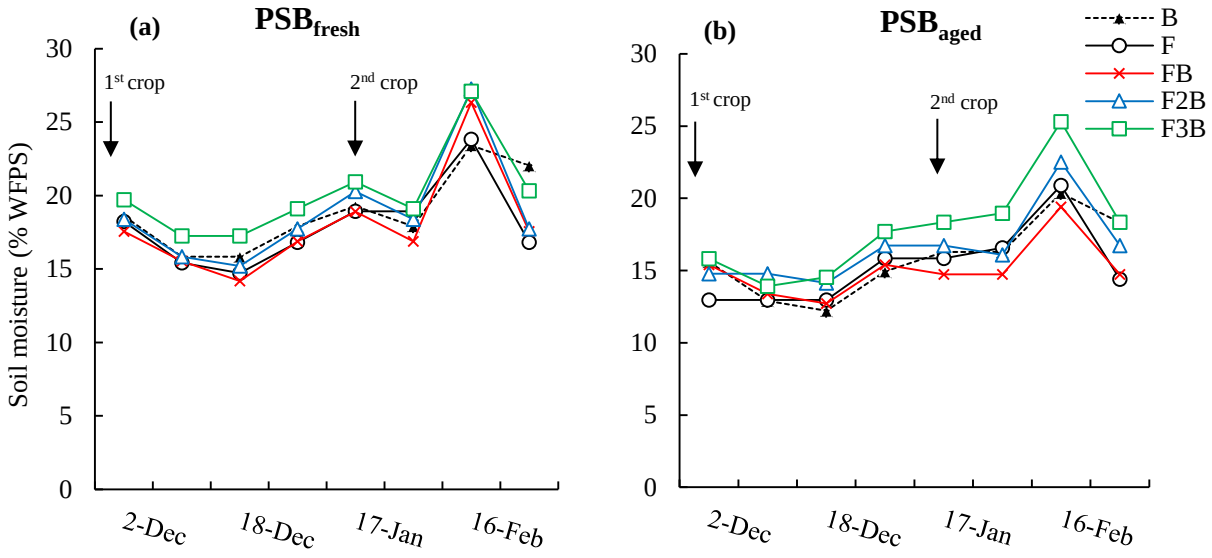

Figure 12. Changes in soil moisture content at 12 cm depth throughout the crop-growing seasons. PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar **(a)**. PSB_{aged} represents sandy soils containing aged palm shell biochar **(b)**. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Vertical arrows show the time of transplanting.

3.3.2. Soil inorganic N

3.3.2.1. Soil exchangeable NH_4^+-N

In PSB_{fresh} soils, the concentrations of exchangeable NH_4^+-N varied from 1.2 mg kg^{-1} in the B treatment to 76 mg kg^{-1} in the F2B treatment on 2nd December and 31st December respectively (**Figure 13a**). The soil exchangeable NH_4^+-N concentration for FB, F2B and F3B in the PSB_{fresh} soils gradually increased and peaked on 31st December and then declined continuously to levels below 6 mg kg^{-1} on 2nd March. Generally, biochar application showed a tendency to reduce exchangeable NH_4^+-N content in the PSB_{fresh} soils except on 31st December where exchangeable NH_4^+-N content were higher in the biochar treatments. Throughout the first crop cycle, the exchangeable NH_4^+-N concentrations were lowest in the B treatment for both PSB_{fresh} and PSB_{aged} soils. The average exchangeable NH_4^+-N concentration in PSB_{fresh} soils was generally higher than that of the PSB_{aged} soils especially during the second crop cycle.

In PSB_{aged} soils, the concentration of exchangeable NH_4^+-N content in the different treatments also changed during time with highest value (66.1 mg kg^{-1}) in the F treatment and lowest value (0.4 mg kg^{-1}) in the B treatment on 2nd December (**Figure 13b**). The exchangeable soil NH_4^+-N content for all treatments with fertilizer in the first crop cycle generally decreased over time and were significantly higher than those in the second crop cycle. Biochar showed a tendency to reduce exchangeable NH_4^+-N content in PSB_{aged} soils on all sampling dates. The soil inorganic N was mostly dominated by exchangeable NH_4^+-N.

3.3.2.2. Soil NO_3^--N

The content of NO_3^--N in PSB_{fresh} soils was generally low during the first crop cycle, ranging from 1.9 mg kg^{-1} in the B treatment to 10.2 mg kg^{-1} in the F2B treatment on 31st December and 18th December, respectively (**Figure 14a**). The NO_3^--N content gradually increased during the second crop cycle and peaked on 16th February, reaching 34.4, 13.9, 24.3 and 15.4 mg kg^{-1} in the F, FB, F2B and F3B treatments, respectively. During the first crop cycle, the soil NO_3^--N content of PSB_{fresh} soils was lower than that of the PSB_{aged} soils while in the second crop cycle, NO_3^--N content of PSB_{fresh} was higher than that of PSB_{aged} soils.

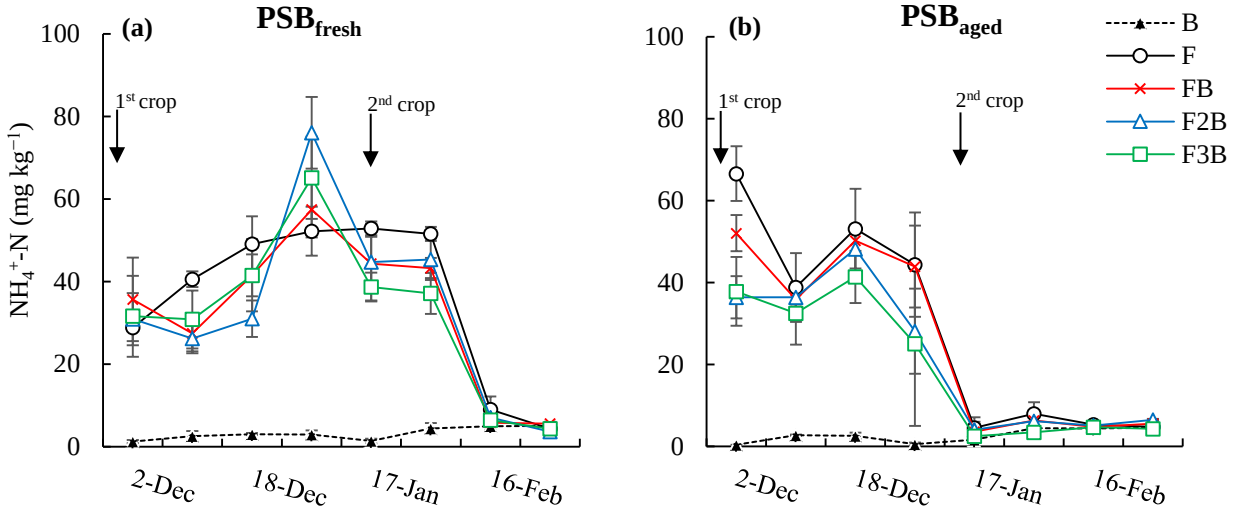

Figure 13. Variation in exchangeable NH_4^+-N content among the treatments throughout the two crop cycles. PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar **(a)**. PSB_{aged} represents sandy soils containing aged palm shell biochar **(b)**. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Vertical arrows show the time of transplanting. Data points represent mean ± standard error (n=3).

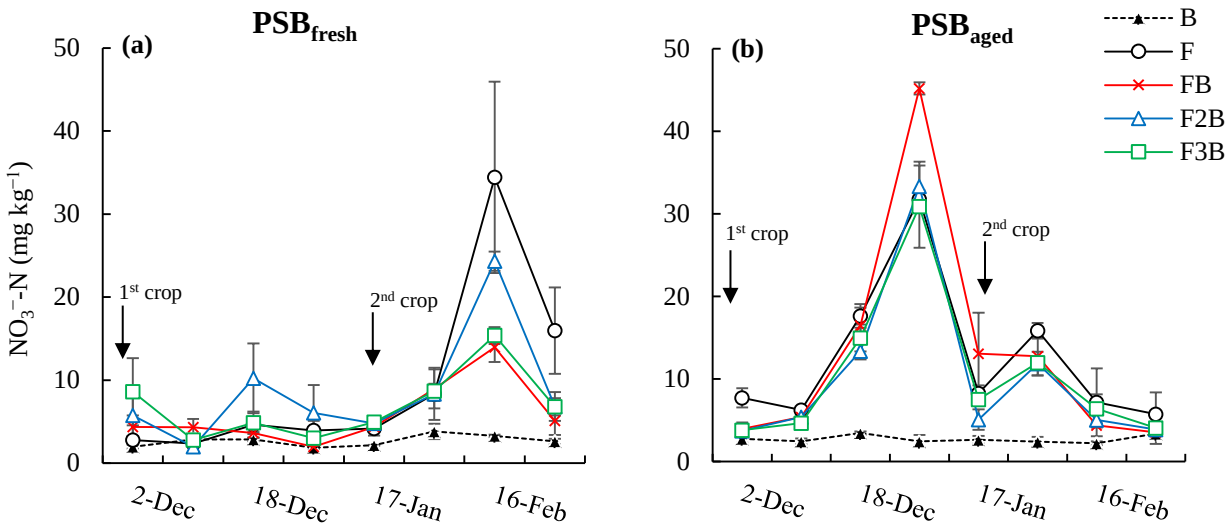

Figure 14. Variation in soil NO_3^--N content among the treatments throughout the two crop cycles. PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar **(a)**. PSB_{aged} represents sandy soils containing aged palm shell biochar **(b)**. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Vertical arrows show the time of transplanting. Data points represent mean ± standard error (n=3).

The NO_3^--N content of PSB_{aged} soils gradually increased and peaked on 31st December, reaching 31.8, 45.2, 33.3 and 30.9 mg kg^{-1} in the F, FB, F2B and F3B treatments, respectively (**Figure 14b**). The soil NO_3^--N content of the first crop cycle was higher than that of the second crop cycle. Soil NO_3^--N content of the B treatment in both PSB_{fresh} and PSB_{aged} soils remained relatively low throughout the two crop cycles at levels below 4 mg kg^{-1}. At the end of the second crop cycle, the soil NO_3^--N content of the PSB_{aged} soils was lower than in the PSB_{fresh} soils.

3.3.3. N_2O flux

The soil N_2O fluxes of the PSB_{fresh} treatments were generally low, ranging from −0.2 µg N_2O-N m^{-2} h^{-1} in the B treatment to 60.7 µg N_2O-N m^{-2} h^{-1} in the F3B treatment on 17th January and 26th January, respectively (**Figure 15a**). The N_2O fluxes of PSB_{fresh} soils were higher in the second crop cycle than in the first crop cycle. The fluxes of the B treatment were relatively low throughout the two crop cycles in both PSB_{fresh} and PSB_{aged} soils.

In the PSB_{aged} soils, the general pattern of soil N_2O fluxes was characterized by one major peak that occurred on 18th December, reaching 305.6, 130.9, 137.0, and 91.4 µg N_2O-N m^{-2} h^{-1} in the F, FB, F2B and F3B treatments, respectively (**Figure 15b**). Throughout the two crop cycles, the F treatment had the highest N_2O fluxes as compared to those in the treatments with biochar and fertilizer, and were higher in the first crop cycle than in the second crop cycle. The average N_2O fluxes for treatments in PSB_{aged} soils were significantly higher than those in the treatments in PSB_{fresh} soils.

3.3.3.1. Correlation analysis between N_2O fluxes and soil temperature, moisture and inorganic N

In PSB_{fresh} soils, the N_2O fluxes were significantly positively correlated with soil NO_3^--N content in all treatments but were weakly and not significantly correlated to soil temperature and soil exchangeable NH_4^+-N content (**Table 4**). The positive correlation between N_2O fluxes and WFPS was only significant in the F2B treatment of the PSB_{fresh} soils. In PSB_{aged} soils, the N_2O fluxes were only significantly positively correlated to the soil NO_3^--N content in the F and FB treatments, and correlated to soil exchangeable NH_4^+-N content in the F treatment.

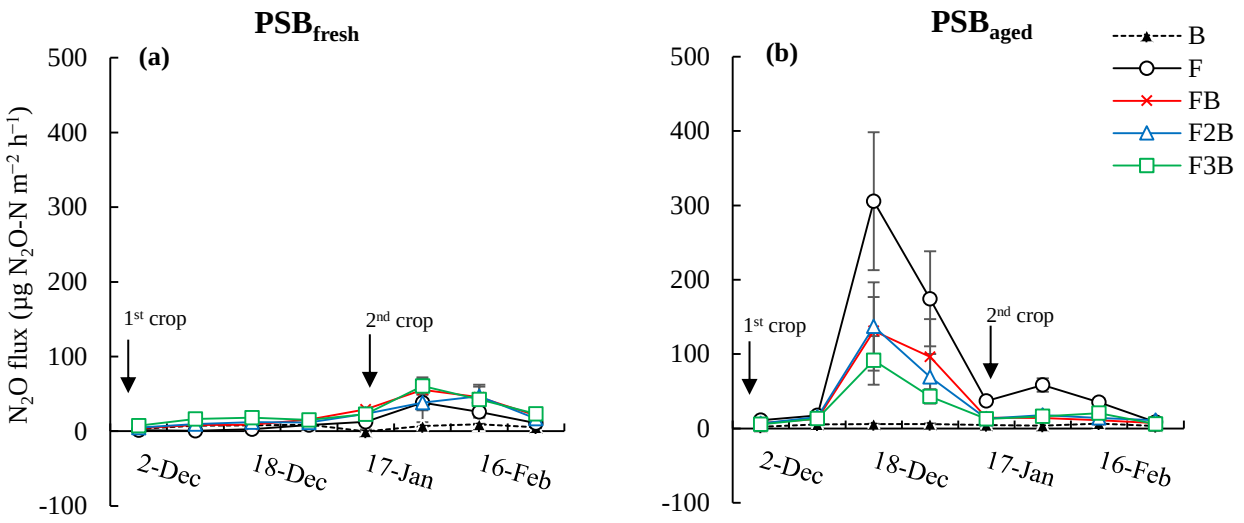

Figure 15. Variation in the N_2O fluxes from the different treatments throughout the two crop cycles. PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar **(a)**. PSB_{aged} represents sandy soils containing aged palm shell biochar **(b)**. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Vertical arrows show the time of transplanting. Data points represent mean ± standard error (n=3).

Table 4. Correlation between N_2O fluxes and soil temperature, WFPS, NH_4^+-N and NO_3^--N concentrations at the time of sampling.

Biochar type	Treatment	Soil temperature	WFPS	NH_4^+-N	NO_3^--N
PSB_{fresh}	B	0.394	−0.051	0.104	0.427*
	F	−0.313	0.293	−0.002	0.440*
	FB	−0.354	0.377	−0.126	0.573**
	F2B	−0.234	0.591**	−0.165	0.583**
	F3B	−0.076	0.303	−0.259	0.508*
PSB_{aged}	B	0.014	0.018	0.083	−0.155
	F	0.126	−0.156	0.412*	0.555**
	FB	0.215	−0.186	0.380	0.547**
	F2B	0.198	−0.150	0.341	0.377
	F3B	0.115	−0.214	0.227	0.347

PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar. PSB_{aged} represents sandy soils containing aged palm shell biochar. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. * indicates $P < 0.05$; **indicates $P < 0.01$.

3.3.4. Cumulative N_2O emissions

The total cumulative N_2O emissions significantly varied with the biochar type, treatment and the interaction between biochar type and treatment (**Figure 16**). In PSB_{fresh} soils, cumulative N_2O emissions ranged from 0.14 ± 0.02 kg N_2O-N ha^{-1} to 0.65 ± 0.04 kg N_2O-N ha^{-1} in the B and F3B treatments, respectively. Although there were no significant differences in N_2O emissions between the treatments in the PSB_{fresh} soils, the emissions in the treatments with fertilizer were higher than those without fertilizer (B treatment). The lowest N_2O emissions were observed in the B treatment of both PSB_{fresh} and PSB_{aged} soils.

In PSB_{aged} soils, cumulative N_2O emissions ranged from 0.11 ± 0.02 kg N_2O-N ha^{-1} to 1.98 ± 0.52 kg N_2O-N ha^{-1} in the B and F treatments, respectively. In addition, the highest N_2O emissions were observed in the F treatment and were significantly different from the B treatment. The N_2O emissions in the F treatment were non-significantly higher than those in the FB, F2B and F3B treatments. Furthermore, the N_2O emissions in F treatment of the PSB_{aged} soils were significantly higher than those in the F treatment of the PSB_{fresh} soils. However, in biochar amended soils with fertilizer (FB, F2B and F3B), there were no significant differences in N_2O emissions between the PSB_{fresh} and PSB_{aged} soils.

3.3.5. Soil chemical properties

A two-way ANOVA for the effect of biochar type and treatment on soil chemical properties revealed that there was a statistically significant interaction between the biochar type and treatment on soil pH, total N, available P, and exchangeable Ca and Mg but no significant interaction on soil EC, C/N ratio, and exchangeable K content **(Table 5)**. In both PSB_{fresh} and PSB_{aged} soils, biochar application significantly increased the soil pH, EC, C/N ratio, total N, available P, and exchangeable K, Ca, and Mg content when compared to the non-biochar soils (F treatment). The soil pH and total N content of the PSB_{fresh} soils were significantly higher than in the PSB_{aged} soils while available P was significantly higher in the PSB_{aged} soils than that in the PSB_{fresh} soils. The EC of PSB_{aged} soils was significantly higher than the PSB_{fresh} soils but the C/N ratio of the PSB_{fresh} soils was significantly higher than that in the PSB_{aged} soils except in the F treatment where the C/N ratio of PSB_{aged} was significantly higher than that of PSB_{fresh} soils ($P < 0.01$). Generally, there were no significant differences in exchangeable K, Ca and Mg content between the PSB_{fresh} and PSB_{aged} soils. The soil chemical properties of the B treatment in PSB_{fresh} soils did not significantly vary from those of the B treatment in PSB_{aged} soils.

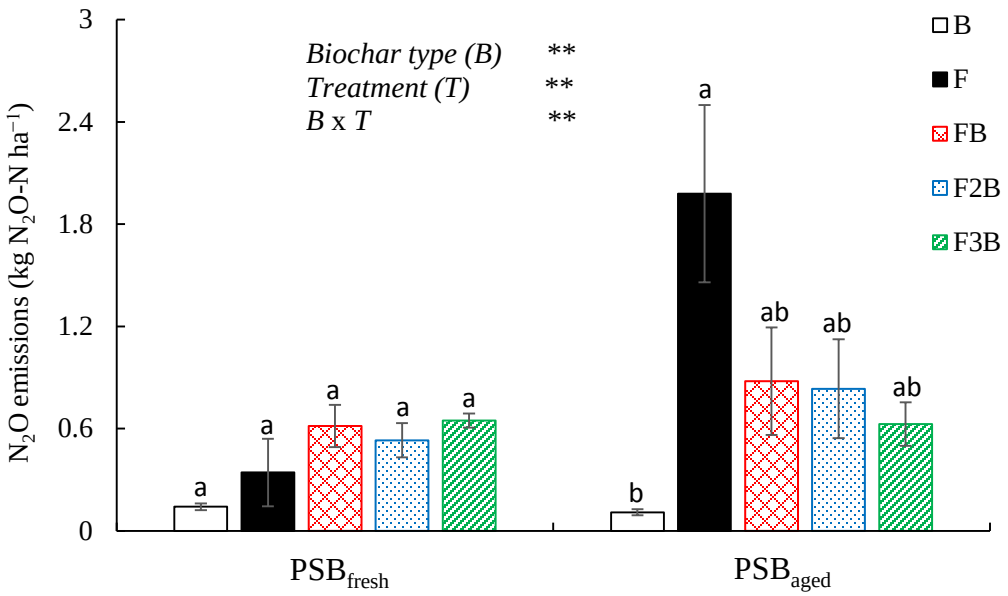

Figure 16. Cumulative N_2O emissions from the different treatments for the two crop cycles. PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar. PSB_{aged} represents sandy soils containing aged palm shell biochar. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Data points represent mean ± standard error (n=3). For each biochar type, different letters indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. ** indicates $P \leq 0.01$.

Table 5. Soil chemical properties at the end of the two crop cycles.

Biochar type	Treatment	Soil pH (H_2O)	Soil EC (dS m^{-1})	C/N	Total N (g kg^{-1})	P	K	Ca	Mg
						mg kg^{-1}			
PSB_{fresh}	B	8.45a	0.03	36.9	0.36b	9.6c	133.1	359.4b	65.3b
	F	7.05c	0.04	3.1	0.22b	5.0c	75.7	134.3c	67.8ab
	FB	8.10b	0.05	36.2	0.40b	21.5b	162.1	354.0b	62.1b
	F2B	8.02b	0.05	60.3	0.81a	30.8b	184.2	431.1b	67.7ab
	F3B	8.48a	0.08	57.0	0.85a	55.0a	275.5	801.5a	75.4a
	Mean	**8.02**	**0.05**	**38.7**	**0.53**	**24.4**	**166.1**	**416.0**	**67.7**
PSB_{aged}	B	8.57a	0.05	37.8	0.37ab	11.4c	143.0	377.5c	59.5bc
	F	5.99d	0.05	4.7	0.20b	19.0c	67.4	111.4d	50.8b
	FB	7.42c	0.05	34.4	0.39ab	39.9b	142.6	351.7c	68.1ac
	F2B	7.79b	0.07	42.0	0.55a	61.5a	202.3	521.9b	72.8a
	F3B	7.96b	0.08	39.8	0.58a	71.4a	270.5	626.2a	76.9a
	Mean	**7.55**	**0.06**	**31.8**	**0.42**	**40.6**	**165.2**	**397.8**	**65.6**
ANOVA *P* values	Biochar type (B)	***	**	*	**	***	NS	NS	NS
	Treatment (T)	***	***	***	***	***	***	***	***
	B x T	***	NS	NS	*	***	NS	**	***

PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar; PSB_{aged} represents sandy soils containing aged palm shell biochar. For each biochar type, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n=3). F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. * indicates $P < 0.05$; **indicates $P < 0.01$; *** indicates $P \leq 0.001$; NS indicates non-significant.

3.3.6. Crop growth and yield

Figure 17 shows the growth of Komatsuna in each treatment at the end of each crop cycle. The main effects of biochar type, treatment and the interaction between biochar type and treatment were significant for plant height, leaf area and shoot dry weight in the two crop cycles (**Figure 18**). The leaf chlorophyll content had a significant main effect of treatment but the main effects of biochar type were not significant for the two crop cycles. The interactions between biochar type and treatment for the leaf chlorophyll content were not significant in the first crop cycle but were significant in the second crop cycle.

In the PSB_{fresh} soils, biochar application with fertilizer significantly reduced plant height, leaf chlorophyll content, leaf area and shoot dry weight during the first crop cycle as compared to the non-biochar amended soil. During the second crop cycle, biochar application with fertilizer significantly reduced the plant height and leaf area but did not significantly affect the leaf chlorophyll content and shoot dry weight as compared to the soil without biochar. The plant height, leaf area and shoot dry weight for the treatments in the PSB_{fresh} soils were generally higher during the second crop cycle than in the first crop cycle while the leaf chlorophyll content was generally higher in the first crop cycle than in the second crop cycle.

In the PSB_{aged} soils, biochar application with fertilizer did not significantly affect plant height, leaf area and shoot dry weight but significantly reduced the leaf chlorophyll content during the two crop cycles as compared to soil without biochar. Plant height, leaf chlorophyll content and shoot dry weight of treatments in PSB_{aged} soils were generally higher in the first crop cycle than the second crop cycle. For each biochar type, the B treatment had the lowest plant height, leaf chlorophyll content, leaf area and shoot dry weight for both cycles. The crop growth and shoot dry weight of the B treatment in PSB_{fresh} soils did not significantly vary from that of the B treatment in PSB_{aged} soils. During the first crop cycle, the plant height for fertilizer treatments in the PSB_{aged} soils was significantly ($P < 0.001$) higher than those in PSB_{fresh} soils while in the second crop cycle, plant height for F and FB treatments of PSB_{fresh} soils was significantly higher than that of the F ($P = 0.001$) and FB ($P < 0.05$) treatments of the PSB_{aged} soils, respectively. The leaf area and shoot dry weight for treatments in the PSB_{aged} soils were significantly higher than those in the PSB_{fresh} soils ($P < 0.001$) during the first crop cycle while for the second crop cycle, the leaf area and shoot dry weight for treatments in the PSB_{fresh} soils were significantly ($P < 0.001$) higher than those in the PSB_{aged} soils.

Figure 17. Komatsuna growth in each of the treatments at the end of the first crop cycle **(a)** and second crop cycle **(b)**.

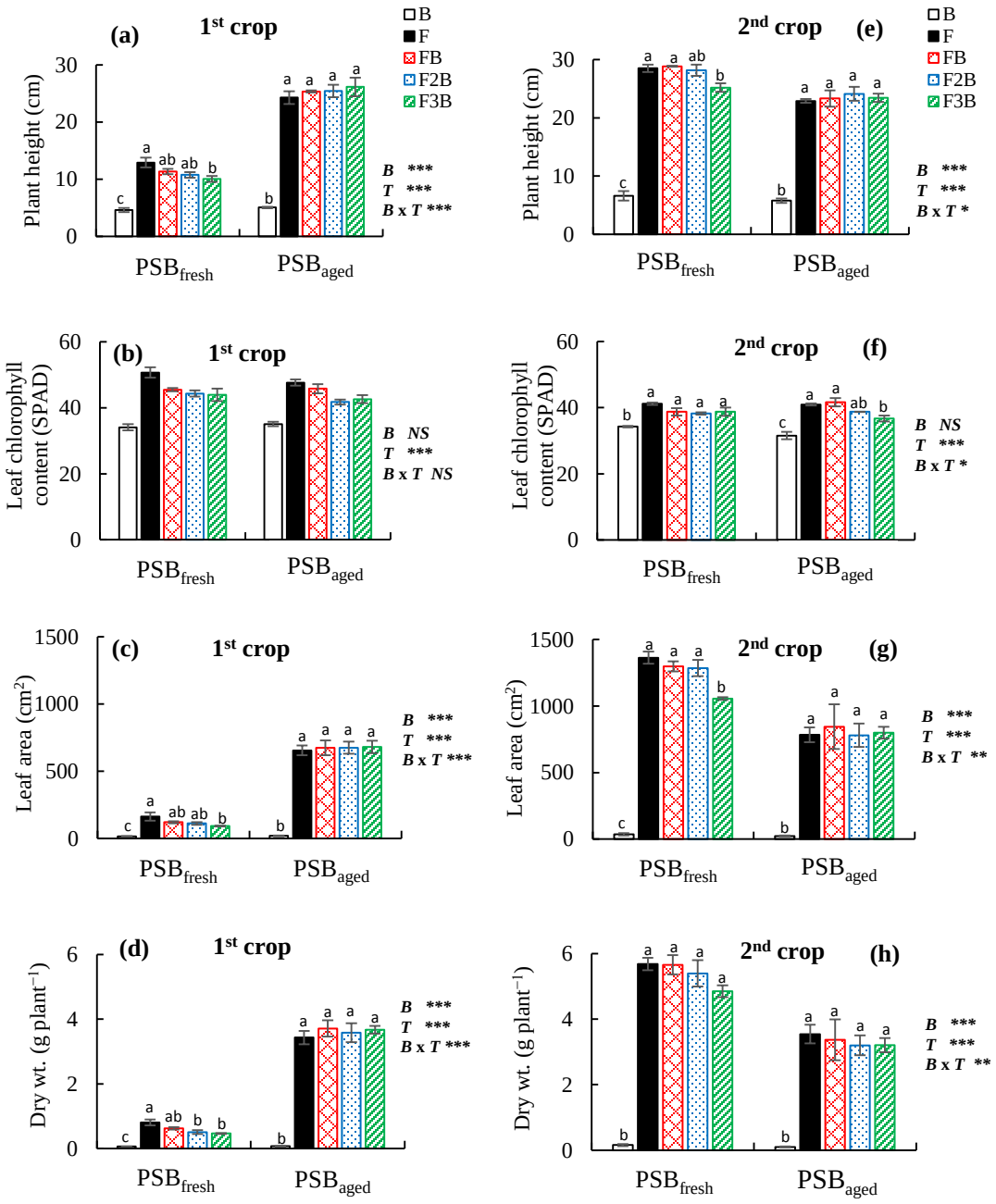

Figure 18. Crop growth and yield at the end of each crop cycle: (a), (b), (c), and (d) represent plant height, leaf chlorophyll content, leaf area and shoot dry weight, respectively, at the end of the first crop cycle; (e), (f), (g), and (h) represent plant height, leaf chlorophyll content, leaf area and shoot dry weight, respectively, at the end of the second crop cycle. F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. Data points represent mean ± standard error (n=3). *B*- Biochar type, *T*- Treatment, *B* x *T*-interaction between biochar type and treatment. For each biochar type, different letters indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar. PSB_{aged} represents sandy soils containing aged palm shell biochar. * indicates $P < 0.05$; **indicates $P < 0.01$; *** indicates $P < 0.001$; NS indicates non-significant.

3.3.7. Plant tissue nutrient concentration

Table 6 shows the changes in plant tissue nutrient concentration at the end of each crop-growing cycle. There was no significant interaction between the biochar type and treatment for plant tissue N concentration in the first crop cycle but the interaction was significant in the second crop cycle ($P < 0.001$). In the first crop cycle, biochar application with fertilizer significantly reduced plant tissue N content in PSB_{aged} soils but it was non-significantly reduced in the PSB_{fresh} soils. However, in the second crop cycle, biochar application with fertilizer significantly reduced plant tissue N content in PSB_{fresh} soils but it was not significantly affected in the PSB_{aged} soils. In the first crop cycle, plant tissue N content was significantly higher in the PSB_{fresh} than in the PSB_{aged} soils for all the treatments while in the second crop cycle, plant tissue N content was significantly higher in PSB_{aged} than in PSB_{fresh} soils for fertilizer treatments.

There was no significant interaction between biochar type and treatment for plant tissue P concentration in both crop cycles. In the first crop cycle, biochar application with fertilizer did not significantly affect plant tissue P content in PSB_{fresh} and PSB_{aged} soils but in the second crop cycle, biochar significantly reduced plant tissue P content in PSB_{fresh} and PSB_{aged} soils. In both crop cycles, plant tissue P content was significantly higher in the PSB_{fresh} than in the PSB_{aged} soils but this effect was more evident in the first crop cycle.

There were significant interactions between the biochar type and treatment for plant tissue K, Ca and Mg concentration. In both crop cycles, biochar application with fertilizer significantly increased plant tissue K and Ca contents but significantly reduced Mg content compared to the non-biochar amended soils in both the PSB_{fresh} and PSB_{aged} soils. In the absence of fertilizer (B treatment), plant tissue K, Ca and Mg contents were significantly higher in the PSB_{fresh} than in PSB_{aged} soils during both crop cycles. However, in the presence of fertilizer and biochar, plant tissue K, Ca and Mg contents were higher in the PSB_{aged} soils than in the PSB_{fresh} soils for all treatments in the first crop cycle. In the second crop cycle, no significant differences in plant tissue K concentration were observed between the PSB_{fresh} and PSB_{aged} soils for the fertilizer and biochar treatments while plant tissue Ca content was significantly higher in the PSB_{aged} than in PSB_{fresh} soils. The plant tissue Mg content for the B and F treatments was higher in PSB_{fresh} than in PSB_{aged} soils during the second crop cycle.

Table 6. Plant tissue nutrient concentration at the end of the crop growing periods.

		First crop cycle					Second crop cycle				
Biochar type	Treatment	N	P	K	Ca	Mg	N	P	K	Ca	Mg
		%					%				
PSB_{fresh}	B	4.6	0.7	4.9b	2.5a	0.8a	4.0d	0.7	5.1c	2.7a	1.0a
	F	7.4	1.2	3.9c	0.6b	0.4b	6.4a	1.0	6.4b	2.0d	1.0a
	FB	7.3	1.1	4.9b	0.7b	0.3b	6.1ab	0.9	7.6a	2.3bc	0.7b
	F2B	6.6	1.1	5.7ab	0.9b	0.3b	5.9b	0.9	8.1a	2.5ab	0.6bc
	F3B	6.7	1.2	6.2a	0.9b	0.3b	5.3c	0.8	7.9a	2.3c	0.6c
	Mean	**6.5**	**1.1**	**5.1**	**1.1**	**0.4**	**5.5**	**0.9**	**7.0**	**2.4**	**0.8**
PSB_{aged}	B	4.2	0.5	4.4d	1.9b	0.7a	2.9b	0.7	4.3c	2.1c	0.7b
	F	6.1	0.9	5.7c	1.9b	0.7a	6.6a	0.9	6.9b	2.4b	0.9a
	FB	5.9	0.8	5.9bc	2.5a	0.5b	6.4a	0.8	7.6ab	2.9a	0.8ab
	F2B	5.9	0.8	6.8ab	2.5a	0.5b	6.3a	0.8	7.8a	2.8a	0.7b
	F3B	5.7	0.8	7.0a	2.6a	0.5b	6.3a	0.8	8.0a	3.0a	0.7b
	Mean	**5.5**	**0.8**	**6.0**	**2.3**	**0.6**	**5.7**	**0.8**	**6.9**	**2.6**	**0.7**
ANOVA *P* values	Biochar type (B)	***	***	***	***	***	**	**	NS	***	*
	Treatment (T)	***	***	***	***	***	***	***	***	***	***
	B x T	NS	NS	***	***	***	***	NS	**	***	***

PSB_{fresh} represents sandy soils mixed with fresh palm shell biochar; PSB_{aged} represents sandy soils containing aged palm shell biochar. For each biochar type, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n=3). F represents fertilizer; B, 2B, and 3B represent biochar application at 6%, 12%, and 18% (w/w) respectively. *indicates $P < 0.05$; **indicates $P \leq 0.01$; *** indicates $P \leq 0.001$; NS indicates non-significant.

3.4. Discussion

3.4.1. Nitrous oxide emissions

In the current study, the potential of 1-year aged palm shell biochar to reduce N_2O emissions from soils continuously cropped with Komatsuna was observed under greenhouse conditions. Fresh and aged biochar may interact with different soils to influence N transformation rates hence affecting the related N_2O production (Duan et al., 2018). Research that has focused on the effects of both fresh and aged biochar on N_2O emissions still reports contradicting results. For instance, Wu et al. (2018a) reported that both fresh and 3-year field-aged biochar significantly reduced the N_2O emissions in the wheat-growing season while in another study, Wu et al. (2018b) reported that both fresh and 3-year field-aged wheat straw biochar did not affect N_2O emissions as compared to the control in an acidic (pH 5.7) silty clay loam soil. Furthermore, using wheat straw biochar, Zhang et al. (2019a) showed non-significant effects of fresh biochar on N_2O emissions in acidic (pH 4.9) and alkaline (pH 8.0) soils while 5-year field-aged biochar application increased N_2O production in similar soils in an incubation experiment. The differences in the results comparing the simultaneous effects of fresh and aged biochar on N_2O emissions may vary with the biochar type and aging conditions, fertilizer management programs, crop type, and crop seasons.

The significantly higher N_2O emissions in the F treatment of PSB_{aged} soils compared to the F treatment of PSB_{fresh} soils showed that in the absence of biochar, addition of more fertilizers in soils previously cropped with Komatsuna significantly increases N_2O emissions. The interaction between the experimental factors was significant because the cumulative N_2O emissions in the F treatment of PSB_{aged} soils were significantly higher than that in the F treatment of PSB_{fresh} soils. This could be attributed to the higher total N and mineral N content of the F treatment in PSB_{aged} soils which remained after the previous croppings (**Table 3**). At the start of the present experiment in November 2016, the same amount of N was applied in both PSB_{fresh} and PSB_{aged} soils. This means that the mineral N in the F treatment of PSB_{aged} soils was higher compared to the F treatment of PSB_{fresh} soils at the initial time of this experiment which might explain why N_2O emissions in PSB_{aged} soils were generally higher than that in PSB_{fresh} soils especially in the treatment without biochar. Moreover, **Table 3** shows that soil NO_3^--N content increased but exchangeable NH_4^+-N content decreased with increasing amounts of applied biochar which may indicate that biochar inhibited plant NO_3^--N uptake but adsorbed NH_4^+-N. Gai et al. (2014) showed the potential of biochar to adsorb substantial amounts of NH_4^+-N and attributed it to the high CEC of biochar,

while biochar did not adsorb NO_3^--N but instead released it into the solution in proportion to the total N of biochar. Therefore, most of the N (NH_4^+-N) in FB, F2B, and F3B in the PSB_{aged} soils at the start of this experiment might have been fixed on biochar. Considering the above discussion, the higher N_2O emission in PSB_{aged} treatment might not be from the applied fertilizer N but from the residual N in the previous experiment.

The lower N_2O emission in FB, F2B and F3B compared to F treatment of the PSB_{aged} soils might be attributed to N immobilization and adsorption on the surface of biochar. The mechanisms for the reduction in N_2O emissions following biochar application may partly be explained by; i) the reduction in N pools available for nitrifiers and denitrifiers through N immobilization due to the high C/N ratios of biochar, NH_4^+/NO_3^- sorption by biochar, and increased plant N uptake that competes with microbes for available N (Saarnio et al., 2013; Clough et al., 2013) although the latter did not occur in this study most likely due to high biochar application rates; ii) liming effects of biochar in soil and the ability of biochar to facilitate the transfer of electrons to denitrifying microorganisms hence reducing N_2O to N_2 (Cayuela et al., 2013, 2014). However, in this study, the reducing tendency of N_2O emissions could be attributed to the reduction in N pools mainly by NH_4^+ adsorption as previously explained. Although the liming ability of the biochar was observed, its contribution to N_2O mitigation might be low since denitrification was not the major N_2O production process in the present study.

The lack of a significant correlation between soil exchangeable NH_4^+-N and soil N_2O fluxes, together with the significant correlation between soil NO_3^--N content and soil N_2O fluxes for PSB_{fresh} soils, and the FB treatment in PSB_{aged} soils (**Table 4**) could indicate the importance of NO_3^--N on N_2O emissions in this study. This may suggest the possibility of denitrification-derived N_2O production which is in agreement with Gelfand et al. (2016) who reported denitrification as the main source of N_2O under similar scenarios. A possible mechanism for denitrification under aerobic conditions is that NO_2^- or its decomposition products such as NO which are toxic may be produced in soil at high concentrations and denitrification is induced as a protection mechanism (Mørkved et al., 2007). However, since the soils used in this experiment were sandy soils which are known for their high aeration (which may also increase with biochar application) and low water-holding capacity, these conditions could have limited denitrification to some extent and therefore, this process should not be the main source for N_2O production in this study. Moreover, the low water-filled pore spaces (WFPS) observed in this study were below 30% **(Figure 12)**

which favors nitrification rather than denitrification. Furthermore, Bateman and Baggs (2005) reported that nitrification was the main N_2O producing process at 35–60% WFPS while denitrification was predominant above 70% WFPS. Therefore, it is likely that nitrification was the dominant process of N_2O production in the present study.

Nitrification generally requires CO_2 as a source of carbon for autotrophic nitrifying bacteria (Weil and Brady, 2016), but because dolomite was applied in this experiment, it might have absorbed the CO_2 at the early stage of the experiment (Wang et al., 2014; Xiao et al., 2016) thereby limiting this process in the PSB_{fresh} soils. This was further evidenced by the high levels of exchangeable NH_4^+-N and low levels of NO_3^--N in the first crop cycle as compared to the second crop cycle where an opposite trend occurred **(Figures 13 and 14)**. Moreover, Baggs et al. (2000a) reported that a correlation between N_2O fluxes and the rise in soil NO_3^--N coupled with a decrease in soil NH_4^+-N concentrations suggested that nitrification contributed to N_2O production. In addition, fresh soil with a lower C content was used in the PSB_{fresh} soil treatments implying that CO_2 production through the decomposition of soil organic matter may have been low due to the lower microbial activity and C source. This could also explain the lower N_2O emissions in the PSB_{fresh} soils than in the PSB_{aged} soils. Furthermore, since the time was too short to release and diffuse N from the applied fertilizer, biochar could not adsorb N hence resulting in non-significant effects of biochar on N_2O emissions for the treatments in PSB_{fresh} soils. The non-significant differences between treatments in the PSB_{fresh} soils implied that under conditions of this study, the initial one time seasonal fertilizer application with biochar may not cause significant effects on N_2O emissions in Komatsuna production during winter conditions.

In all treatments, soil temperatures did not significantly influence N_2O emissions mainly because this experiment was conducted during the winter to early spring seasons when the soil temperatures are relatively low. The cumulative N_2O emissions from this study are lower than those of Jia et al. (2012a) under vegetable production. This could be due to the lower N fertilizer application rate (225 kg N ha^{-1}) used in the present study compared to 400 kg N ha^{-1} used in their pot experiment, and also, due to the variation in weather conditions. The very low N_2O emissions in the B treatments were attributed to the low levels of available N (**Figures 13 and 14**) because the N from biochar did not result in increased emissions since it was in recalcitrant forms. This further clarifies the role of inorganic N fertilizers in accounting for most of the N_2O emissions from sandy soils.

3.4.2. Soil chemical properties

Although it was hypothesized that both fresh and aged biochar increases soil chemical properties, the results from this study revealed that the specific levels of each biochar may have varying responses on some soil nutrients. For instance, the interaction between experimental factors for soil pH and Ca was caused by the non-significant difference between the FB and F2B treatment of PSB_{fresh} soils while for total N, it was due to the non-significant difference among the FB, F2B and F3B treatments of PSB_{aged} soils. For soil Mg, it was due to the non-significant differences between the F and FB treatment of PSB_{fresh} soil. Furthermore, interactions for soil P were caused by the non-significantly higher P content in the F treatment than in the B treatment of PSB_{aged} soils as compared to the opposite trend observed in the F and B treatments of PSB_{fresh} soils. The results obtained from this study could not explicitly explain these interactions. However, they could be attributed to the changes in plant nutrient uptake due to the variations in plant growth among the treatments during the previous crop seasons which affected the soil nutrient status at the specific levels of biochar.

A number of studies have shown that biochar application increases soil pH (Major et al., 2010; Chintala et al., 2013; Zhao et al., 2014) as observed in this study. Although the non-biochar amended soils had a lower soil pH than all the biochar treated soils, the soil pH of the F treatment of PSB_{aged} soils was lower than that of the F treatment of PSB_{fresh} soils and this could most likely be attributed to plant effects in soil rather than from the direct acidification effects from N fertilizer application in soil. The extent to which the soil pH changes in soil depends on plant species and nitrogen source such as NO_3^--N or NH_4^+-N (Marschner and Römheld, 1983; Marschner, 2012). Komatsuna has a high preference for NO_3^--N over NH_4^+-N (Ikeda and Osawa, 1981; Xu et al., 2017). During the uptake of NO_3^--N, other anions such as HCO_3^-, OH^-, and organic anions are excreted or protons are taken up by the plant roots to maintain the cation and anion balance (Marschner, 2012; Weil and Brady, 2016), which results in the alkalinizing effect of the rhizosphere and the pH of the surrounding soil (Blossfeld et al., 2010). However, since the conditions in the present study favored nitrification which generates two protons per NH_4^+ molecule; while only one OH^- will be excreted by roots during uptake of one molecule of NO_3^-, it is therefore possible that this net balance could still cause soil acidification especially if nitrification and NO_3^- uptake processes are predominant in the rhizosphere (Blossfeld et al., 2011). This was further evidenced by the fact that the soil NO_3^--N content of the F treatment at the end

of the three crop cycles was lower than in biochar treatments (**Table 3**) due to the higher N uptake in the plants in the absence of biochar (**Table 2**) which may have caused soil acidification in the F treatment of the PSB_{aged} soils (**Table 5**). These results could imply that under nitrification conditions, continuous cropping of Komatsuna might gradually result in a reduction in soil pH especially in sandy soils which are known to have a low buffering capacity (ability to resist a drop or rise in soil pH) due to their low soil organic matter content but longer-term studies are still needed to clarify this phenomenon.

The results also revealed that once biochar was applied during the initial cultivation season, it could still mitigate soil acidification during the following crop-growing seasons **(Table 5)**. These results are similar to those of Wang et al. (2017) who reported soil acidification in soils with fertilizer and the role of biochar to increase soil pH due to its liming ability as a result of the inherent alkalinity of biochar. The non-significant effects of biochar on soil pH between the B treatment of PSB_{fresh} soil and the B treatment of PSB_{aged} soil showed that without fertilizer application, the effects of biochar aging did not significantly alter soil pH. This meant that the changes in soil pH among biochar treatments with fertilizer in PSB_{fresh} and PSB_{aged} soils might not be attributed to biochar aging but rather to acidification resulting from the synergistic effects of plant NO_3^--N uptake and nitrification processes as explained above.

The high inherent nutrients in the biochar explained the increase in soil EC in the PSB_{fresh} and PSB_{aged} soils. The high soil EC in the F3B treatments was a result of higher amounts of biochar which resulted in extra soil nutrients due to the increased base cations with biochar addition (Ajayi and Horn, 2016). In addition, the higher soil EC of the PSB_{aged} soils is accounted for by the additional basal fertilizer applied in these soils and the nutrients that remained after harvesting the preceding crops. The non-significant differences in soil N, P, K, Ca and Mg between the B treatment of PSB_{fresh} and the B treatment of PSB_{aged} soils showed that even after 1 year, these nutrients did not vary in absence of chemical fertilizer. Therefore, the changes in the nutrient status in the PSB_{fresh} and PSB_{aged} soils were due to fertilizer application and to a less extent biochar. N release and N benefits from the decomposition of stable biochar are likely to be minimal over time periods relevant to plant growth (Lehmann and Joseph, 2009).

Although the total N in the B treatments was generally higher than that in the F treatments of both PSB_{fresh} and PSB_{aged} soils, the inorganic N was significantly lower in the B treatments than in the F treatments (**Figures 13 and 14**). This implied that for plant available N, the N from this

biochar could not be relied on to estimate crop yield because it was not beneficial to the plants. Probably, this biochar could enhance N uptake indirectly through increasing soil pH. However, soil pH increases N availability in soils with a higher fertility status which was not the case with soils used in this study. This also explains why lowest crop growth and yield were observed in the non-fertilizer amended soils. The reduction in soil NH_4^+-N and NO_3^--N in the PSB_{fresh} soils following biochar application during the crop growth periods was possibly due to NH_4^+ adsorption and N immobilization in biochar amended soils (Clough et al., 2013). However, in the PSB_{aged} soils, with additional basal fertilizer application, biochar still reduced the NO_3^--N except in the FB treatment and this implied that the negative effects of biochar application on soil NO_3^--N content are likely to be minimized as biochar ages to result into increased N mineralization especially at lower biochar rates. However, further research is still needed on how biochar addition affects inorganic soil N contents in long-term periods.

3.4.3. Crop growth and yield

The significant interaction between experimental factors for both crop cycles is attributed to the lower plant height, leaf area and crop yield in the F3B treatment of PSB_{fresh} soils as compared to the other treatments with fertilizer. This implies that high biochar application rates for fresh biochar may result in the reduction in crop yield but when it ages in soil, the effects could be neutralized due to the similar variation in plant nutrient N uptake. The decreasing tendency in crop yield for PSB_{fresh} soils could be attributed to the reduction in soil NH_4^+-N and NO_3^--N as evidenced by a higher soil NH_4^+-N and NO_3^--N amounts in the F treatment of PSB_{fresh} soils and the reduction in plant tissue N content especially during the second crop cycle.

The significantly higher plant height and crop yield for the treatments in the PSB_{aged} soils during the first crop cycle could be attributed to the residual effects from the fertilizer applied during the previous year and from additional basal fertilizer applied. Moreover, soil NH_4^+-N and NO_3^--N were high in the PSB_{aged} soils during the first crop cycle **(Figures 13 and 14)** due to the inorganic N that remained from the previous cropping (**Table 3**), which also explains the higher crop yield in the PSB_{aged} soils as compared to the PSB_{fresh} soils. As earlier discussed, vegetables in the brassica family have a higher preference for NO_3^--N than NH_4^+-N (Ikeda and Osawa, 1981; Xu et al., 2017). Komatsuna belongs to the brassica family which prefers NO_3^--N and hence the high NO_3^--N concentrations in the PSB_{aged} soils than in PSB_{fresh} soils during the growing season may explain the high crop growth and yield in the PSB_{aged} soils during the first crop cycle.

Contrary, the very low dry weight, height and leaf area of Komatsuna in the PSB_{fresh} soils during the first crop cycle were due to the slower growth rates as compared to those in the PSB_{aged} soils resulting from the lower levels of nitrification as earlier discussed. Therefore, the data for PSB_{fresh} soils in the first crop cycle can only be used for experimental purposes since the plants did not reach normal market size.

The significantly higher crop yield in PSB_{fresh} than in PSB_{aged} soils during the second crop cycle could also have been due to the higher inorganic N contents in PSB_{fresh} soils as compared to the PSB_{aged} soils **(Figures 13 and 14)**. In addition, the lower crop growth in the treatments with PSB_{aged} soils during the second crop cycle could also have been the higher nutrient depletion by the previous crop which had higher biomass compared to that in the PSB_{fresh} soils. These results are similar to those obtained in the previous study, where there was nutrient depletion from the preceding crops and where biochar hindered N availability to plants resulting in negative effects on crop growth and yield during the second and third crop cycles (**Figure 8**).

The very low crop growth and yield in the B treatments were due to the biochar N being in recalcitrant forms which were unavailable for plant uptake as evidenced by the low levels of soil inorganic N in these treatments. In this study, the growth of Komatsuna was not affected significantly by biochar because the nutrients were not limiting factors. Moreover, biochar is not able to further increase biomass yields in an optimized system, i.e., an ecosystem where plant growth is not limited by nutrients (Hagemann et al., 2017). During the second crop cycle, the interaction between the experimental factors was significant because the leaf chlorophyll content of the F3B treatment of PSB_{aged} soils was lower than that of the other treatments with fertilizer. This interaction could not be thoroughly explained by the results in this study. However, the reduced chlorophyll content is attributed to the reduction in N and Mg uptake with increasing biochar rates which was also observed in the previous study (**Figure 8b and Table 2**). To further clarify the importance of N on chlorophyll content, Marks et al. (2016) explained the higher average leaf chlorophyll content in the 100%-N treatment to be as a result of higher N application rate.

3.4.4. Plant tissue nutrient concentrations

In the present study, biochar either decreased or did not have any significant effects on plant tissue N concentration and this was evidenced by the significant interaction between biochar type and treatment during the second crop cycle. The interaction was caused by the significantly lower

tissue N concentration in the F3B treatment of PSB_{fresh} soils as compared to the other treatments which had fertilizer. The decrease in plant tissue N content was a result of high biochar application rates which reduced the soil inorganic N especially in the PSB_{fresh} soils during the second crop cycle. These results are consistent with the findings of Syuhada et al. (2016) who reported lower N concentration in the tissue of corn plants grown at higher biochar application rates (15 g kg^{-1}) in the presence of inorganic fertilizers. The higher plant tissue N and P concentration in the PSB_{fresh} than in PSB_{aged} soils during the first crop cycle could be attributed to the growth stage of plants at the time of sampling. Since plants in the PSB_{fresh} soils in the first crop cycle were harvested before maturity, their tissues were younger and hence had higher concentrations of N and P as compared to the mature plants in the PSB_{aged} soils. This is consistent with Walworth and Sumner (1987) who reported that as plants age, foliar concentrations of N, P, K and S tend to decrease while Ca and Mg concentrations tend to increase. Yan et al. (2016) also showed that leaf N and P concentrations of *Arabidopsis thaliana* decreased in older plant tissue. However, it can be speculated that the lower K concentrations in young leaves (from plants grown in PSB_{fresh} soils) as compared to the old leaves (from plants grown in PSB_{aged} soils) during the first crop cycle could possibly be due to the aging effects of biochar which might have increased soil K content. In comparison with the PSB_{aged} soils, the reduction in tissue N concentration of plants grown in PSB_{fresh} soils during the second crop cycle could imply that fresh biochar may reduce N uptake but as it ages in soil, the negative effects of biochar on N availability would be offset hence increasing N availability if N fertilizer is applied.

The interaction between the experimental factors was significant because in PSB_{fresh} soils, the plant tissue K and Mg content in the B treatment was higher than that in the F treatment during the first crop cycle and this also occurred for plant tissue Ca content in both crop cycles. This showed the potential of biochar to increase K, Ca, and Mg uptake in plants especially when it has just been applied in soil during the first crop season. The fresh biochar used in the study had high amounts of K, Ca, and Mg, and this could partly explain the increased uptake of these cations especially K. Moreover, biochar amendments are better than fertilizer at increasing plant K and P tissue concentration (Biederman and Harpole, 2013). During the second crop cycle, the interaction was significant because in the PSB_{fresh} soils, the plant tissue K content in the F3B treatment was non-significantly lower than that in the F2B treatment while for plant tissue Mg content, the significant interaction resulted from the non-significant difference between the F and B treatments.

The high plant tissue K, Ca and Mg content during the second crop cycle was as a result of the continuous release of these cations from biochar surfaces. Nigussie et al. (2012) reported increased N, P and K uptake with biochar addition and attributed it to the high nutrient content in the maize stalk biochar and highest nutrient concentration in biochar amended soils. The higher K, Ca and Mg concentration in the PSB_{aged} soils than in the PSB_{fresh} soils especially in the first crop cycle could have been a result of the additional basal fertilizer. The high amounts of K in biochar lead to its luxury consumption thereby having adverse impacts on Ca and Mg nutrition (Butnan et al., 2015; Wacal et al., 2019). This explains the negative relationship between plant tissue K and Mg content under the biochar amendment.

3.5. Conclusion

Even after 1 year of application in the soil, palm shell biochar still showed a potential to reduce N_2O emissions following additional basal chemical fertilizer. The liming ability of biochar still existed and was able to offset soil acidification in biochar amended soils. Generally, biochar application with fertilizer significantly increased plant K and Ca content but decreased N, P and Mg content. At higher application rates, biochar had negative effects on crop yield but as it aged, the negative effects were offset. Therefore, since seasonal N fertilizer application seems to be inevitable in Komatsuna cultivation, addition of biochar could be a possible way of counteracting the effects of excessive fertilizer use. Although the relatively high biochar rates (equivalent 85–250 t ha^{-1}) used in this study could somewhat mitigate N_2O emissions and reduce soil acidification, they might not be economically feasible under large-scale field conditions because of the enormous quantities of feedstock (palm shells) that could be needed to make the biochar. Nevertheless, this work could be a springboard for further research exploring the aging effects of different biochars at varying application rates in different biochar types and crops before recommending it for use. More research is still needed on the optimal biochar application rates that would increase or maintain the yield of Komatsuna with less effects on the environment.

CHAPTER FOUR

Assessment of crop residue and palm shell biochar incorporation on greenhouse gas emissions during the fallow and crop growing seasons of broccoli (*Brassica oleracea* var. *italica*)

4.1. Introduction

Agriculture is among the main anthropogenic sources of greenhouse gas emissions (IPCC, 2013). The anthropogenic N inputs in agricultural systems include N from chemical fertilizer, animal wastes, increased biological N-fixation, cultivation resulting in enhanced organic matter mineralization, and mineralization of crop residues in the field (Mosier et al., 1998). Crop residue incorporation in the soil is an important strategy to maintain soil fertility but its influence on greenhouse gas (GHG) emissions should be considered (Lehtinen et al., 2014). A number of studies have shown that crop residue incorporation in soil results in an increase in N_2O and CO_2 emissions (Neeteson and Carton, 2001; Velthof et al., 2002; Huang et al., 2004; Nett et al., 2015; Gao et al., 2016; Nguyen et al., 2016; Badagliacca et al., 2017; Scheer et al., 2017; Pugesgaard et al., 2017), but has no significant effects on CH_4 emissions in upland fields (Nguyen et al., 2016).

Vegetables in the brassica family leave large amounts of highly moist and nitrogenous rich residues in the soil which through decomposition, result into a substantial increase in N_2O emission (Rahn et al., 2003; Nett et al., 2016). For instance, cauliflower residues contain 80 to 120 kg N ha^{-1}, white cabbage and brussel sprout residues contain 150 to 250 kg N ha^{-1} (Neeteson and Carton, 2001) while broccoli residues contain 76 to 304 kg N ha^{-1} (Bakker et al., 2009). In addition, vegetable crops are characterized with low C/N ratios which are mostly less than 20 (Velthof et al., 2002; Shan and Yan, 2013; Rezaei Rashti et al., 2016). Therefore, the low C/N ratios, high water and N contents of the crop residues facilitate rapid mineralization resulting into the release of N_2O, and CO_2 emissions during microbial respiration. Moreover, Baggs et al. (2000a) reported large emissions from crop residues and attributed them to the low C/N ratio of 7.5:1 which may have promoted mineralization and creating anaerobic microsites associated with high water content of the residues.

Broccoli is one of the brassica vegetables that accumulates large amounts of nitrogen in the above ground biomass which are often left in the soil as crop residues after harvest. The fate of this nitrogen most likely depends on post-harvest management strategies of the crop residues

such as incorporation in soil. Incorporation of crop residues by ploughing in soil results in the highest N_2O emissions in a wide range of soils (Nett et al., 2016). Once incorporated, crop residues influence soil biological activities as well as the availability of nutrients (Koulibaly et al., 2017). The mineralization rates after incorporation of plant material are dependent on the quality of organic material, soil type, temperature, moisture, timing and method of incorporation in relation to environmental parameters (Baggs et al., 2000b). Furthermore, the emissions of N_2O and CO_2 vary with different soil and crop residue types, and amount of inorganic N added (Velthof et al., 2002; Novoa and Tejeda, 2006). Therefore, further studies should examine the effects of crop residues in other various soil types and climatic conditions (Shan and Yan, 2013; Pugesgaard et al., 2017). In sub-tropical Australia, Scheer et al. (2014) showed that N_2O and CO_2 emissions from a broccoli field during the fallow period accounted for 70.8% and 55.1% of the total emissions respectively under conventional fertilizer application. This implies that the strategies required to reduce CO_2 and N_2O emission in broccoli fields should focus more on the post-harvest GHG emissions which occur after crop residue incorporation in the soil. Many studies have shown various methods of mitigating GHG emissions from crop residue incorporation through reduction in the available soil nitrogen content during the post-harvest or fallow seasons. These could include; removal of crop residues from the fields, use of nitrification inhibitors (Scheer et al., 2014; Scheer et al., 2017; Rezaei Rashti et al., 2017), co-incorporation of crop residues with materials such as straw (De Neve et al., 2004) and biochar (Nguyen et al., 2016) that have higher C/N ratios. The investigation of the effects of plant residues with various biochemical characteristics while assessing fertilizer and plant residue management strategies may improve the understanding of systems underlying the various GHG mitigation strategies (Rezaei Rashti et al., 2017).

The application of biochar, a product from the pyrolysis of crop residues under limited oxygen, has been proposed to reduce GHG emissions, enhance C sequestration and improve crop productivity (Sohi et al., 2009; Lehmann and Joseph, 2015). Several studies have demonstrated that biochar can reduce (Schimmelpfennig et al., 2014; Thomazini et al., 2015), increase (Petter et al., 2016; Zhou et al., 2017) or have no significant effects (Suddick and Six, 2013; Lin et al., 2015) on N_2O emissions. In addition, biochar application in soil can reduce (Nguyen et al., 2016) or increase (Troy et al., 2013; Hawthorne et al., 2017) CO_2 emissions. These contradicting functions of biochar in soil are due to the different feedstocks used, pyrolysis methods and varying soil types in which biochar is applied (Hussain et al., 2017). The reduction in N_2O emissions following

biochar application in soil has partly been explained by the high C/N ratios of biochar which result in N immobilization, increase in soil pH and soil aeration (Clough et al., 2013; Cayuela et al., 2014). Improved plant growth following biochar application has been attributed to the improved soil N retention, nutrient uptake, water holding capacity and its inherent ability to supply nutrients such as N, P and K required for crop growth (Schimmelpfennig et al., 2014; Zhou et al., 2017; Griffin et al., 2017; Saarnio et al., 2018). The application of biochar in high in-put agricultural systems may not result in significant soil quality and crop production improvement (Boersma et al., 2017) but there is a high possibility that it could reduce GHG emissions from broccoli crop residues incorporated in the soil. Palm shell biochar (PSB) is one of the plant material derived biochars whose effects in soil are poorly known. Moreover, the combined effects of PSB and crop residues in soil under broccoli cultivation, with emphasis on GHG emissions during the fallow and crop growing seasons under field conditions are still unclear.

The objectives of this study were to; (1) evaluate the effects of different broccoli crop residue management strategies (removal vs incorporation with or without biochar) on GHG emissions; (2) compare GHG emissions from fallow (post-harvest) and crop growing seasons of broccoli; (3) evaluate the effects of PSB on broccoli residue biomass and N uptake; (4) evaluate the effects of PSB on soil chemical properties. The underlying hypotheses were; (a) PSB incorporation in soil may reduce GHG emissions from broccoli residues; (b) the fallow season would have higher GHG emissions than the crop growing season; (c) PSB would increase broccoli residue biomass, N uptake, and improve on soil chemical properties.

4.2. Materials and methods

4.2.1. Experimental site and design

The field experiment was established in a farmer's broccoli field in Kotoura, Tottori prefecture, Japan (35°30' 34" N 133°38' 27"E) from May 2017 to April 2018. This region is known for intensive broccoli cultivation where by crop residues (**Figure 19a**) are normally incorporated in soil after the crop growing season. It experiences very hot and humid summers and moderately cold winters with an average annual precipitation of 1840 mm and annual average air temperature of 15.3°C. The soil at this site is classified as a Cambisol (FAO/IIASA/ISRIC/ISSCAS/JRC, 2009) and its characteristics are shown in **Table 7.**

Table 7. Selected characteristics of soil and biochar used in this study.

Properties	**Units**	**Soil**	**Biochar**
pH (H_2O)		5.54	7.99
EC	dS m^{-1}	0.07	0.39
Total C	g kg^{-1}	27.5	350
Total N	g kg^{-1}	2.50	5.20
C/N		11.0	67.4
Available P	mg kg^{-1}	147	135
Exchangeable K	mg kg^{-1}	285	1540
Exchangeable Ca	mg kg^{-1}	1316	3103
Exchangeable Mg	mg kg^{-1}	141	185
Ash	%	-	13.1
CEC	cmol (+) kg^{-1}	13.5	12.8
Bulk density	g cm^{-3}	1.20	0.78
Sand	%	26.8	-
Silt	%	35.2	-
Clay	%	38.0	-
Texture		Clay loam	-

EC-Electrical conductivity, CEC- Cation exchange capacity

On 8th May 2017, the broccoli crop residues were ploughed into the soil using a tractor and palm shell biochar (PSB) was mixed in the selected plots (**Figure 19b**). In the biochar treated plots, PSB was uniformly spread over the soil surface and then gently incorporated manually up to 15 cm soil depth using a spade. **Table 7** shows the characteristics of the palm shell biochar (pyrolyzed at 400–550°C) which was purchased from a commercial company (King Coal Co. Ltd, Tokyo, Japan). The treatments were established in plots measuring 2 m x 2 m (4 m^2) with 1m wide buffer zones between plots and they included; No-residues (NR), Residues (R), Residues + 10 t ha^{-1} PSB (R10), Residues + 20 t ha^{-1} PSB (R20) and Residues + 40 t ha^{-1} PSB (R40), arranged in a completely randomized block design with three replications. To establish the No-residue (NR) treatment, all the broccoli residues were thoroughly handpicked out of the soil. Biochar was not applied in the NR and R treatments. The application rate of the crop residues was estimated prior to residue incorporation by sampling and weighing 10 randomly selected plants which were used to for plant analysis, and calculation of the amount of crop residues in 1 ha which was 57.0 t ha^{-1} with a total N content of 248 kg N ha^{-1} and a C/N ratio of 15.8. The experiment was conducted in two phases; the first phase (fallow season) was conducted from 8th May to 26th September 2017 while the second phase (crop growing season) was conducted from 11th October 2017 to 10th April 2018.

After crop residue decomposition at the end of the fallow season, the entire field was ploughed and ridges were made but before ploughing and fertilizer application, the PVC bases used during gas sampling (details explained in the next section) and pegs were removed and later re-established in their respective plots after transplanting. On 11th October 2017, broccoli seedlings of cultivar 'Okumidori' were transplanted on ridges in single rows at a spacing of 65 cm between rows and 35 cm with in rows. All the crop agronomic practices were done by the farmer. Before transplanting, 2 t ha^{-1} chicken manure were applied containing 60 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1}. In addition to the chicken manure, chemical fertilizer was applied at 112 kg N ha^{-1}, 164 kg P_2O_5 ha^{-1}, 96 kg K_2O ha^{-1}, 362 kg CaO ha^{-1} and 84 Kg MgO ha^{-1}. The second fertilizer application was done on 13th November 2017 at a rate of 56 kg N ha^{-1}, 32 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1}.

Figure 19. Broccoli crop residues left after harvest (**a**) and PSB applied to soil before mixing (**b**).

4.2.2. Gas sampling and analysis

The manually closed chambers were used to collect air samples from the field throughout the period of study from 11th May 2017 to 10th April 2018 between 9:00 am to 12:00 pm on each sampling day (**Figure 20**). Within each plot, one circular PVC base frame with a height of 13 cm was inserted permanently into the soil to 8 cm depth and later used to fit the chambers during gas sampling. The frustum shaped sampling chambers described in section 3.2.2 were used for gas sampling. Gas samples were drawn from the headspace at 0, 20 and 40 minutes after fitting the chambers onto the bases and 30 ml were immediately injected into 15 ml pre-evacuated vials (Nichiden-Rika Glass Co. Ltd, Kobe, Japan) fitted with butyl rubber stoppers and then stored in the laboratory until analysis. The air temperature inside each chamber was simultaneously measured by a digital thermo recorder (TR-71U*i*, T & D Corporation, Nagano, Japan). N_2O was analyzed using a gas chromatograph (GC-14B Shimadzu, Kyoto, Japan) equipped with an electron capture detector (ECD) while concentrations of CO_2 and CH_4 were analyzed using gas chromatographs (GC-8A and GC-14A, Shimadzu, Kyoto, Japan) equipped with the thermal conductivity detector (TCD) and flame ionization detector (FID) respectively. Standard gas concentrations were provided by the Institute for Agro-Environmental Sciences (NIAES), National Agriculture and Food Research Organization, Tsukuba, Japan. The Gas fluxes were calculated by considering the linear increase in gas concentrations in chamber headspace over the 40 minutes (Minamikawa et al., 2015). The total emissions of N_2O, CO_2 and CH_4 were calculated directly from the gas fluxes by summing up all the average daily emissions obtained from every two adjacent sampling dates.

4.2.3. Crop residue N_2O emission factors (EF_R)

Emission factors (EF_R), which is a percentage of N in residues emitted as N_2O-N was calculated from the following equation (Huang et al., 2004);

$$EF_R\ (\%) = \frac{[N2O{-}N\ (with\ residues)]-[N2O{-}N\ (without\ residues)]}{[Total\ N\ of\ residues\ applied]}\ X\ 100$$

Figure 20. Gas sampling during the fallow season (**a**) and the crop growing season (**b**).

4.2.4. Auxiliary measurements

Digital thermo recorders (TR-71wf, T & D Corporation, Nagano, Japan) were inserted at 5 cm soil depth to monitor the mean daily soil temperature throughout the experiment period. At the end of the experiment, soil bulk density from each treatment was calculated after oven drying soil in 100 cm^3 cores at 105°C for 24 h. On each day of gas sampling, soil moisture at 12 cm depth was measured using time domain reflectometry (TDR) probes and then expressed as water-filled pore space (WFPS) using the equation described in section 3.2.2.

In addition, soil samples were taken on each sampling day to determine the inorganic N (exchangeable NH_4^+-N and NO_3^--N). In each plot, three soil samples were taken at 15 cm depth using a garden trowel and thoroughly mixed in a bucket. Plant debris were removed and the soil subsamples were stored in an ice cooler box up to the laboratory where they were later stored at −80ºC until further analysis. To measure exchangeable NH_4^+-N, 5 g of field moist soil were extracted with 50 ml of 10% potassium chloride (KCl) solution by shaking the soil slurry for 1 hour using an orbital shaker at 250 rpm. The samples were then filtered and stored at 4°C before analysis which was done within one week. Exchangeable NH_4^+-N was determined by the Indophenol blue method (Smith and Cresser, 2004) at 693 nm using a spectrophotometer (U-5100, Hitachi, Tokyo, Japan). To measure soil NO_3^--N, 1 g of field moist soil was extracted with 100 ml of 0.01% $AlCl_3 \cdot 6H_2O$ solution by shaking for 30 minutes using an orbital shaker at 250 rpm. The filtered samples were analyzed on the same day of extraction by the ultraviolet absorption method (Yamaki, 2003) at 210 nm using a spectrophotometer (U-5100, Hitachi, Tokyo, Japan).

4.2.5. Soil, biochar and plant analysis

At the end of the crop growing season, soil samples from each plot were obtained at 15 cm depth, air-dried and sieved using a 2 mm sieve. Soil pH, EC, total C, total N and available P were measured following similar procedures described in section 2.2.3. To measure exchangeable K, Ca and Mg, 2 g of soil were extracted with 30 ml of 1N ammonium acetate (NH_4OAC) buffered at pH 7.1 by shaking the soil slurry using a mechanical shaker for 15 minutes. The samples were then centrifuged at 3,000 rpm for 3 minutes and the supernatant filtered into 100 ml flasks. Again, 30 ml NH_4OAC were added and centrifugation was repeated twice, and supernatants collected into flasks. After filtration, the remaining ammonium saturated soils were kept for CEC determination. The final solution was diluted with NH_4OAC to 100 ml, then filtered through 0.45 µm nylon syringe filters and measured by the atomic absorption spectrophotometer (Z-2300; Hitachi, Tokyo,

Japan). To determine CEC, the ammonium saturated soils were washed three times with 20 ml of 80% methanol while centrifuging resulting in a total volume of 60 ml. 30 ml of 10% KCl were added, shaken for 15 minutes, centrifuged and filtered into 100 ml flasks. Centrifugation with the same volume of 10% KCl was repeated twice and diluted to 100 ml. To 20 ml of filtrate, one spatula full of MgO was added and later used for determination of NH_4^+ by Kjeldahl distillation. The ammonia liberated was collected in 4% boric acid (with indicator), titrated with standard 0.05M H_2SO_4 (Chapman, 1965) and the titre values used for calculating the CEC. Soil texture was determined using the pipette method. The chemical analysis of biochar was done following similar procedures used in soil analysis. The bulk density of the biochar was determined by averaging three values of density obtained from the weight of compacted biochar in 100 cm^3 soil cores. The ash content of biochar was measured by heating it at 550°C in a muffle furnace for 4 h.

To determine the N uptake in crop residues, two plants adjacent to the PVC bases in each plot were uprooted and separated into leaves, heads, stems and roots. Each plant part from the two sampled plants was mixed and ground into powder by use of a stainless steel wonder blender and later analyzed for total N, C and C/N by the dry combustion method using the C/N corder described above. The total N uptake in the different parts was determined by multiplying dry weight of every plant part with their corresponding N contents and then expressed in kg ha^{-1}. Total N uptake was calculated by adding up the N uptake in all plant parts.

4.2.6. Statistical analysis

All data was analyzed by analysis of variance (ANOVA) using IBM SPSS software (Version 20.0). The statistical differences between treatment means were tested using Tukey's HSD test at 5% level of significance unless otherwise specified. Data are presented as mean ± standard error.

4.3. Results

4.3.1. Weather conditions, soil temperature and moisture

The total rainfall received during the experimental period was 1640.5 mm **(Figure 21)**. The amount of rainfall received during the fallow period was 586.5 mm while that of the crop growing season was 1054 mm. The highest amount of rainfall (351.5 mm) occurred during October 2017 while the lowest (25 mm) occurred in May 2017. The maximum daily rainfall was 132.5 mm and it was received on 22nd October 2017. This rainfall peak coincided with the time of transplanting broccoli seedlings which was done in October 2017. The maximum daily air temperature was 36.3°C while the minimum daily temperature was −5.7°C recorded on 6th August 2017 and 9th

February 2018 respectively **(Figure 21)**. The hottest months were July and August with daily mean temperatures of 26.9 and 26.7°C respectively while the coldest month was February, with a daily mean temperature of 0.3°C.

Soil temperature followed a trend similar to that of air temperature (**Figure 22a)**. Biochar application slightly increased soil temperature especially in the months of May and June which had low rainfall and moderately high air temperatures, as well as in December, January and February which had low air temperatures. Soil moisture ranged between 17.5% and 60.3% WFPS in the NR and R40 treatments on 15th June 2017 and 6th July 2017 respectively **(Figure 22b).** Soil WFPS were generally higher in the R40 treatments than in the NR and R treatments especially after periods of heavy rainfall. For example, during October, the month with the highest rainfall, plots with 40 t ha^{-1} of biochar had higher WFPS than the other treatments.

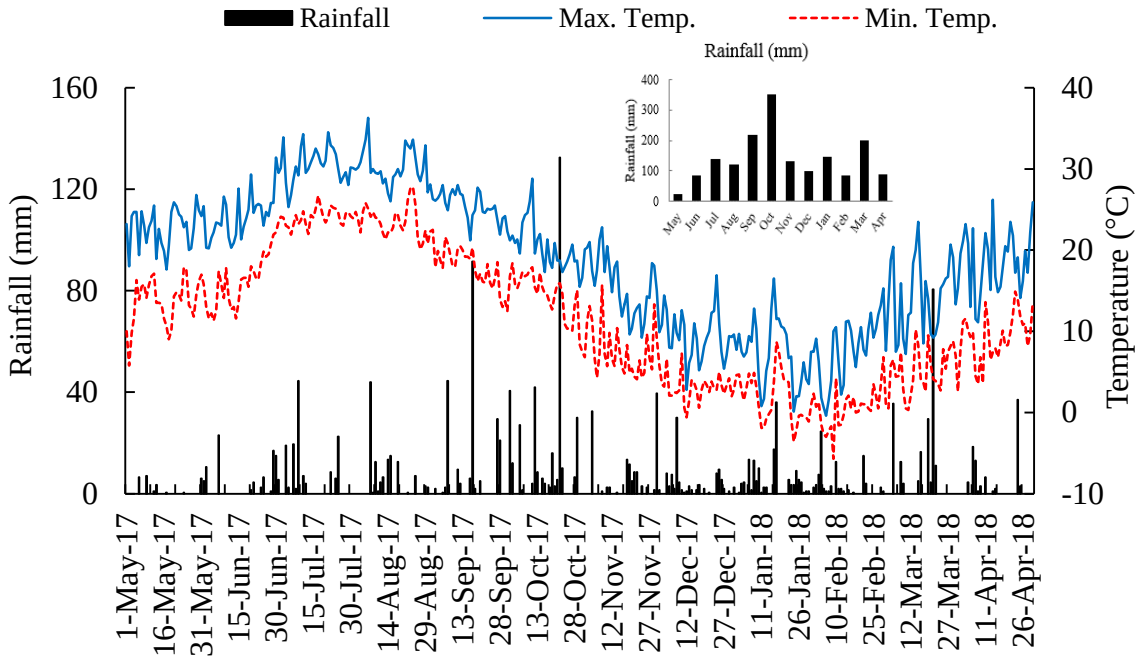

Figure 21. Daily average rainfall, maximum and minimum temperature around the experiment site from May 2017 until April 2018. Insert shows the average monthly rainfall throughout the period of study.

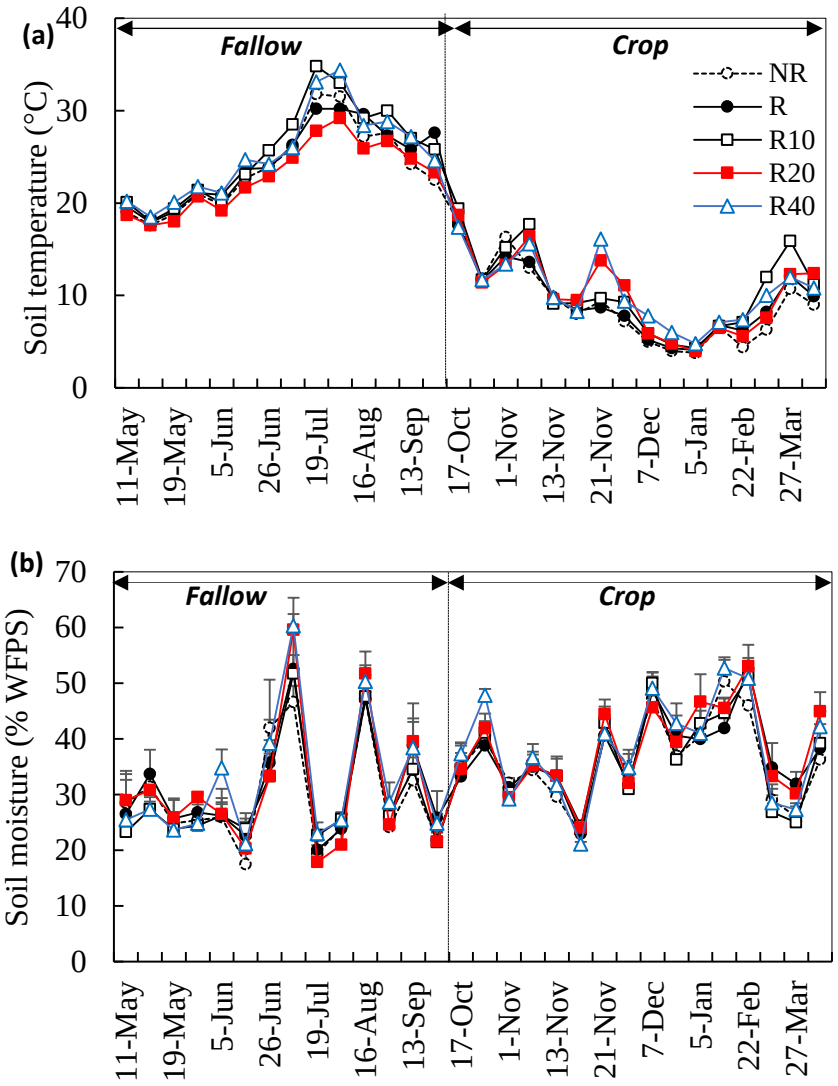

Figure 22. Changes in soil temperature at 5 cm soil depth **(a)** and soil moisture content at 12 cm depth **(b)** throughout the experimental period. NR-No residues, R-Residues, R10-Residues + 10 t ha^{-1} biochar, R20-Residues + 20 t ha^{-1} biochar and R40-Residues + 40 t ha^{-1} biochar. Data points represent mean ± standard error (n=3).

4.3.2. Soil inorganic N

The concentrations of exchangeable NH_4^+-N in the different treatments significantly varied across the experiment period. The exchangeable NH_4^+-N content of soil during the fallow season ranged from 0.67 to 71.8 mg kg^{-1} in the R and R10 treatments respectively while in the crop growing season, soil exchangeable NH_4^+-N content ranged from 1.1 to 94.3 mg kg^{-1} in NR and R10 treatment respectively (**Figure 23a**). Following the incorporation of crop residues, the content of soil exchangeable NH_4^+-N in the treatments with residues were generally high but gradually decreased until 19th July and later remained low until 26th September at levels below 5 mg kg^{-1}. During the first two months after crop residue incorporation, the content of exchangeable NH_4^+-N in the NR treatment was significantly lower than that of the other treatments. Following fertilizer application at the start of the crop growing season, there was a significant increase in the concentration of NH_4^+-N with the highest levels occurring on 17th October and thereafter gradually decreasing until 10th April, to levels below 5 mg kg^{-1} in all the treatments. During the crop growing season, the concentrations of exchangeable NH_4^+-N in the NR and R10 treatments were generally higher than those of R, R20 and R40 treatments. Soil NH_4^+-N was the dominant form of inorganic N during the crop growing season while NO_3^--N was most dominant during the fallow season on most sampling dates.

The concentrations of soil NO_3^--N also varied significantly and they ranged from 0.8 mg kg^{-1} in NR to 140.8 mg kg^{-1} in R10 during the fallow season while for the crop growing season, the NO_3^--N concentrations ranged from 2.2 mg kg^{-1} to 48.1 mg kg^{-1} in the NR treatment (**Figure 23b**). The variation in soil NO_3^--N concentrations was characterized by two peaks. The major peak occurred during the fallow period on 26th June in NR (46.3 mg kg^{-1}), R (117.7 mg kg^{-1}), R10 (140.8 mg kg^{-1}), R20 (90.1 mg kg^{-1}) and R40 (111.9 mg kg^{-1}) treatments. The minor peak occurred during the crop growing season on 17th November in NR (48.1 mg kg^{-1}), R10 (38.0 mg kg^{-1}), R20 (31.7 mg kg^{-1}) and R40 (38.4 mg kg^{-1}) treatments. During the fallow season, the NO_3^--N content of the NR treatment was significantly lower than that of the other treatments.

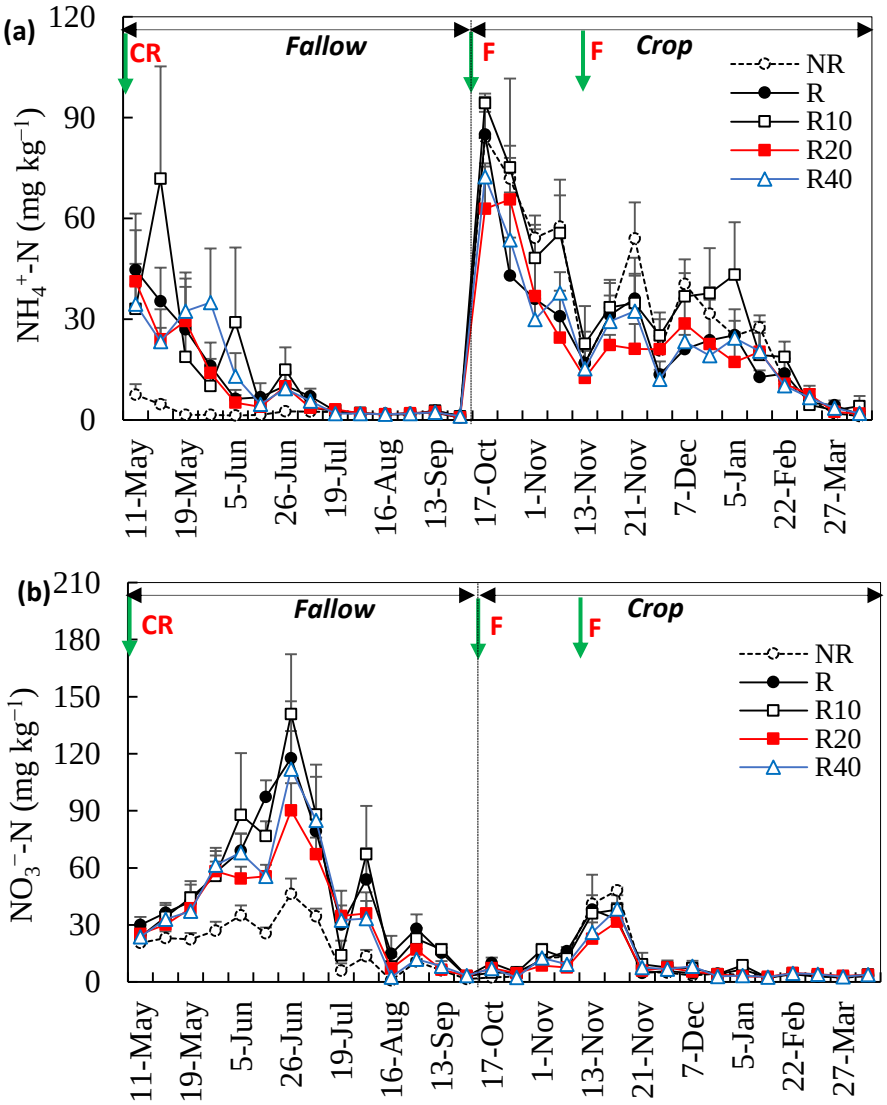

Figure 23. Variation in NH_4^+-N **(a)** and NO_3^--N **(b)** among the treatments throughout the experiment period. NR-No residues, R-Residues, R10-Residues + 10 t ha^{-1} biochar, R20-Residues + 20 t ha^{-1} biochar and R40-Residues + 40 t ha^{-1} biochar. Data points represent mean ± standard error (n=3). Arrows indicate the time for crop residue (CR) incorporation and fertilizer (F) application.

4.3.3. Greenhouse gas emissions

4.3.3.1. Nitrous oxide emissions and emission factors

N_2O fluxes varied significantly ranging from 1.4 to 650.3 µg N m^{-2} h^{-1} in NR and R10 treatments respectively during the fallow season and from 7.0 to 102.3 µg N m^{-2} h^{-1} in the R20 and R treatments respectively during the crop growing season (**Figure 24a**). Most N_2O fluxes occurred during the fallow season after the incorporation of broccoli crop residues into the soil and they were significantly higher than those of the crop growing season. During the first two months after residue incorporation, the fluxes in the R, R10, R20 and R40 treatments were significantly higher than those in the NR treatment but those of the R40 treatment were lower than the R20, R10 and R treatments. At the start of the crop growing period, there was a rise in N_2O fluxes on 17th October, reaching 52.6, 102.3, 63.8, 68.6 and 65.8 µg N m^{-2} h^{-1} in the NR, R, R10, R20 and R40 treatments respectively. From 28th November to 5th January, the N_2O fluxes in NR treatment were higher than those of the other treatments. However, from 5th January to 10th April, there was no much variation in N_2O fluxes among the treatments.

Analysis of variance revealed no significant main factor effects and interaction between season and treatments for cumulative N_2O emissions **(Table 8)**. Nitrous oxide emissions from the fallow period accounted for 8.7, 77.3, 81.5, 70.4 and 54.0% of the total N_2O emissions in the NR, R, R10, R20 and R40 treatments respectively. In the NR treatment, the N_2O emissions from the crop growing season were significantly higher than those of the fallow period ($P < 0.05$). Crop residue incorporation during the fallow period significantly increased N_2O emissions by 4.06 kg N ha^{-1} in R treatment and by 1.47 kg N ha^{-1} in the R40 treatment. Biochar application tended to decrease N_2O emissions from crop residues in the R20 and R40 treatments but the reduction was not significant. During the crop growing season, the cumulative N_2O emissions in the NR treatment were higher than in the R, R10, R20 and R40 treatments but there were no significant differences among the treatments. The total emissions from the fallow and crop seasons for the NR treatment were lower than those of the residue treatments but did not significantly vary among the treatments. Calculations of EF_R showed that the cumulative N_2O emissions accounted for 1.64 ± 0.87 % of the broccoli residue-N in the R treatment over the fallow period. The EF_R for crop residues incorporated with biochar were; 2.18 ± 0.97, 1.22 ± 0.81 and 0.59 ± 0.13% in the R10, R20 and R40 treatments respectively. Although not significant, biochar application at 20 and 40 t ha^{-1} showed a tendency to decrease N_2O emission factors from crop residues.

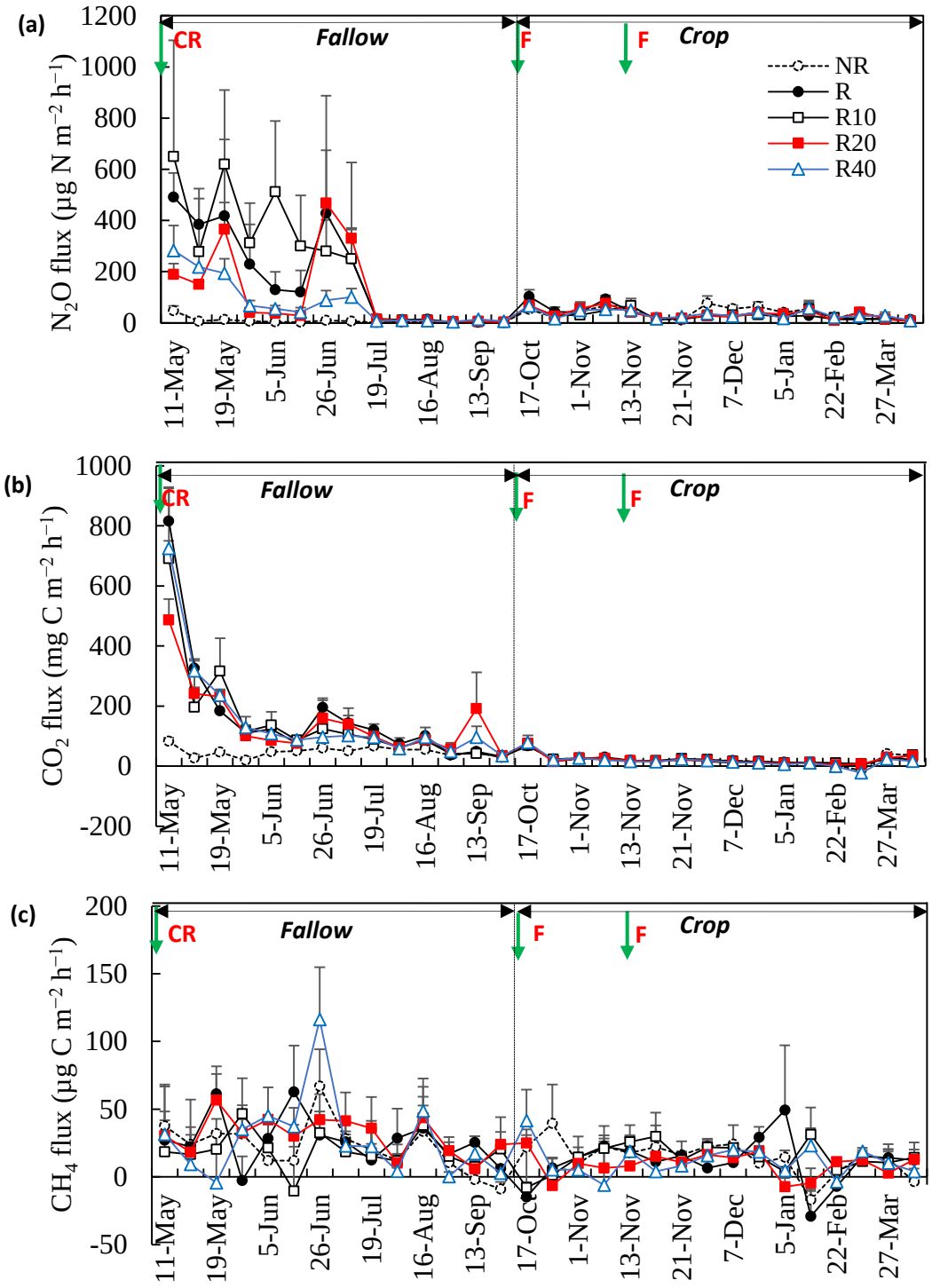

Figure 24. Temporal dynamics N_2O fluxes (**a**), CO_2 fluxes (**b**) and CH_4 fluxes (**c**) during the fallow and crop growing seasons. NR-No residues, R-Residues, R10-Residues + 10 t ha^{-1} biochar, R20-Residues + 20 t ha^{-1} biochar and R40-Residues + 40 t ha^{-1} biochar. Data points represent mean ± standard error (n=3). Arrows indicate the time for crop residue (CR) incorporation and fertilizer (F) application.

Table 8. Effect of crop residues and biochar on cumulative N_2O, CO_2 and CH_4 emissions for the fallow season, crop season and total emissions for the fallow and crop seasons.

Season	Treatment	N_2O emissions (kg N ha^{-1})	CO_2 emissions (t C ha^{-1})	CH_4 emissions (kg C ha^{-1})
Fallow	NR	0.16 ± 0.01b	1.59 ± 0.11b	0.68 ± 0.08a
(8th May–26th	R	4.22 ± 2.15a	3.83 ± 0.34a	0.89 ± 0.20a
September 2017)	R10	5.56 ± 2.40a	3.43 ± 0.61ab	0.63 ± 0.07a
	R20	3.19 ± 2.01a	3.82 ± 0.55a	0.99 ± 0.06a
	R40	1.63 ± 0.31ab	3.57 ± 0.16a	0.96 ± 0.09a
Crop	NR	1.67 ± 0.34a	0.67 ± 0.09a	0.43 ± 0.01a
(11th October	R	1.23 ± 0.08a	0.70 ± 0.07a	0.32 ± 0.08a
2017–10th April	R10	1.27 ± 0.15a	0.76 ± 0.11a	0.59 ± 0.23a
2018)	R20	1.34 ± 0.57a	0.69 ± 0.19a	0.32 ± 0.05a
	R40	1.39 ± 0.44a	0.49 ± 0.06a	0.50 ± 0.17a
Total†	NR	1.83 ± 0.33a	2.25 ± 0.04b	1.12 ± 0.07a
(8th May 2017–10th	R	5.46 ± 2.11a	4.54 ± 0.40a	1.21 ± 0.28a
April 2018)	R10	6.82 ± 2.36a	4.19 ± 0.51ab	1.22 ± 0.20a
	R20	4.53 ± 2.57a	4.51 ± 0.67a	1.31 ± 0.11a
	R40	3.02 ± 0.73a	4.06 ± 0.11ab	1.46 ± 0.14a
ANOVA *P* values	Season (S)	0.058	<0.001	<0.001
	Treatment (T)	0.313	0.006	0.706
	S x T	0.188	0.005	0.122

†Represents the total gas emissions for the fallow and crop seasons.

NR- No-residue, R- Residues, R10-Residues + 10 t ha^{-1} biochar, R20-Residues + 20 t ha^{-1} biochar and R40-Residues + 40 t ha^{-1} biochar. For each season, different letters within a column indicate significant differences between treatments at $P < 0.05$ using Tukey's HSD test. Numbers in the table represent mean ± standard error (n=3).

4.3.3.2. Carbon dioxide emissions

The CO_2 fluxes were initially high after the incorporation of crop residues in soil but they gradually declined until the end of the crop growing season (**Figure 24b**). The fluxes generally ranged from 29.2 to 815.5 mg C m^{-2} h^{-1} in the NR and R treatments respectively during the fallow season and from −22.0 to 78.9 mg C m^{-2} h^{-1} in the R40 treatment during the crop growing season. The CO_2 fluxes for the fallow season were higher than those of the crop growing season and the fluxes for the NR treatment were significantly lower than those of the other treatments. On 17th October, CO_2 fluxes increased up to 73.8, 67.6, 69.1, 75.8 and 78.9 mg C m^{-2} h^{-1} in NR, R, R10, R20 and R40 treatments respectively. During the crop growing season, there were no significant variations in CO_2 fluxes among the treatments.

There were significant differences in cumulative CO_2 emissions between the seasons, treatments and interaction between seasons and treatments **(Table 8)**. The cumulative CO_2 emissions during the fallow period ranged from 1.59 to 3.83 t C ha^{-1} in the NR and R treatments respectively. Crop residue incorporation in soil significantly increased CO_2 emissions in the R treatment by 140.9% as compared to the NR treatment. However, application of biochar did not significantly influence CO_2 emissions during the fallow period as evidenced by the non-significant differences among the R, R10, R20 and R40 treatments. The CO_2 emissions from the fallow period accounted for 70.6, 84.4, 81.7, 84.7 and 87.9% of the total emissions in the NR, R, R10, R20 and R40 treatments respectively. During the crop growing season, there were no significant variations in cumulative CO_2 emissions among the treatments. In all treatments, the emissions that occurred during the fallow season were significantly higher than those of the crop growing season. The total emissions from the fallow and crop growing seasons did not significantly vary among the R, R10, R20 and R40 treatments.

4.3.3.3. Methane emissions

Soil CH_4 fluxes ranged from −8.99 to 116.0 µg C m^{-2} h^{-1} in the NR and R40 treatments respectively during the fallow period while in the crop growing seasons, they ranged from −29.0 to 49.3 µg C m^{-2} h^{-1} in the R treatment **(Figure 24c)**. The CH_4 fluxes during the fallow period were slightly higher than those of the crop growing season. Crop residue and biochar incorporation did not have any significant influence on CH_4 fluxes on all the sampling days.

There were significant differences in cumulative CH_4 emissions between the seasons but no significant difference among the treatments and interaction between seasons and treatments

(Table 8). Methane emissions ranged from 0.63 kg C ha^{-1} in the R10 treatment to 0.99 kg C ha^{-1} in the R20 treatment during the fallow period and from 0.32 to 0.59 kg C ha^{-1} in the R and R10 treatments respectively during the crop growing season. Cumulative CH_4 emissions in the fallow period were higher than those of the crop growing seasons in all the treatments but they were only significant in the NR ($P < 0.05$) and R20 ($P = 0.001$) treatments. The CH_4 emissions from the fallow period accounted for 60.7, 73.6, 51.6, 75.6 and 65.8% of the total methane emissions in the NR, R, R10, R20 and R40 treatments respectively.

4.3.4. N uptake, C/N ratio, biomass and water content of crop residues after harvest

In all the treatments, N uptake in the different plant parts varied as follows; leaves >stems> heads> roots while C/N ratio varied as follows; stems> roots >leaves> heads (**Table 9**). However, the N uptake and C/N ratios of the individual plant parts and total N uptake did not differ significantly among the treatments. Crop residue biomass (fresh weight) after harvest was 27.3 ± 2.1 t ha^{-1}, 24.6 ± 0.1 t ha^{-1}, 26.2 ± 2.3 t ha^{-1}, 27.0 ± 2.8 t ha^{-1}, and 25.9 ± 2.4 t ha^{-1} in the NR, R, R10, R20 and R40 treatments respectively. The percentage water content of the crop residues was 88.7 ± 0.4 in NR, 90.4 ± 1.2 in R, 88.4 ± 0.7 in R10, 89.1 ± 0.3 in R20 and 89.3 ± 0.3 in R40 treatments. Biochar application did not have any significant effect on biomass and water content of the broccoli crop residues remaining in the field.

4.3.5. Soil chemical properties

Table 10 shows the changes in soil chemical properties at the end of the crop growing season. In comparison to the control (NR treatment), biochar application at 40 t ha^{-1} significantly increased the total N, total C, C/N ratio and exchangeable K by 5.51, 55.6, 46.8 and 45.7% respectively ($P < 0.05$). However, soil pH, EC, available P, Exchangeable Ca and Mg, and CEC did not significantly vary between the treatments.

Table 9. Effect of biochar on nutrient content of broccoli residue parts at harvest under biochar treatments.

Treatment	Broccoli residue N uptake (kg N ha^{-1})					C/N			
	Leaves	Heads	Stems	Roots	Total plant N uptake	Leaves	Heads	Stems	Roots
NR	74.1 ± 2.1a	8.2 ± 1.7a	18.9 ± 1.1a	4.6 ± 0.9a	105.9 ± 2.6a	9.2 ± 0.4a	6.1 ± 0.1a	18.5 ± 1.1a	10.9 ± 0.6a
R	54.0 ± 6.6a	6.2 ± 1.7a	15.7 ± 1.9a	4.7 ± 0.3a	80.6 ± 10.4a	9.2 ± 0.1a	6.3 ± 0.1a	18.6 ± 0.2a	9.9 ± 0.6a
R10	65.9 ± 9.0a	11.2 ± 2.1a	19.3 ± 2.5a	5.9 ± 0.3a	102.2 ± 11.5a	10.1 ± 1.1a	6.4 ± 0.1a	17.8 ± 0.2a	10.1 ± 0.7a
R20	68.7 ± 8.7a	10.2 ± 1.4a	21.5 ± 3.0a	5.0 ± 1.2a	105.4 ± 13.4a	9.5 ± 0.2a	6.5 ± 0.2a	16.2 ± 1.1a	10.6 ± 0.7a
R40	59.8 ± 3.8a	9.7 ± 1.8a	18.4 ± 1.3a	4.4 ± 0.4a	92.4 ± 6.9a	9.7 ± 0.1a	6.5 ± 0.1a	18.4 ± 0.1a	10.6 ± 1.0a

NR- No-residue, R- Residues, R10- Residues + 10 t ha^{-1} biochar, R20-Residues + 20 t ha^{-1} biochar and R40-Residues + 40 t ha^{-1} biochar. Within a column, means followed by the same letter are not significantly different ($P > 0.05$) among treatments using Tukey's HSD test. Numbers in the table represent mean ± standard error (n=3).

Table 10. Effect of biochar on soil chemical properties at the end of the crop growing season.

	Soil pH (H_2O)	Soil EC (mS m^{-1})	C/N	Total C	Total N	Avail. P	Exch. K	Exch. Ca	Exch. Mg	CEC
				g kg^{-1}		mg kg^{-1}				cmol (+) kg^{-1}
NR	5.6 ± 0.1a	7.9 ± 0.4a	11.1 ± 0.2b	27.0 ± 0.3b	2.36 ± 0.02b	151.2 ± 2.4a	304.1 ± 12.5b	1265.5 ± 41.7a	150.9 ± 10.9a	16.2 ± 0.5a
R	5.6 ± 0.1a	8.1 ± 0.1a	10.9 ± 0.2b	27.2 ± 0.4b	2.42 ± 0.03ab	161.8 ± 8.8a	340.8 ± 27.2b	1294.9 ± 92.6a	142.2 ± 6.0a	17.3 ± 1.6a
R10	5.6 ± 0.1a	9.3 ± 0.7a	12.7 ± 0.7ab	32.1 ± 1.6ab	2.45 ± 0.01ab	155.4 ± 6.7a	387.3 ± 14.8ab	1339.4 ± 51.6a	149.6 ± 2.1a	16.1 ± 2.2a
R20	5.7 ± 0.1a	7.2 ± 0.3a	13.9 ± 1.3ab	35.6 ± 4.0ab	2.46 ± 0.04ab	159.0 ± 2.9a	387.8 ± 17.5ab	1384.0 ± 42.7a	153.7 ± 2.6a	15.5 ± 1.9a
R40	5.7 ± 0.1a	8.4 ± 0.1a	16.3 ± 1.7a	42.0 ± 4.6a	2.49 ± 0.02a	160.8 ± 4.1a	443.2 ± 16.9a	1460.9 ± 71.7a	161.6 ± 1.9a	13.6 ± 1.6a

NR- No-residue, R- Residues, R10- Residues + 10 t ha^{-1} biochar, R20-Residues + 20 t ha^{-1} biochar and R40-Residues + 40 t ha^{-1} biochar. Different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. Avail. P: Available P; Exch. K: Exchangeable K; Exch. Ca: Exchangeable Ca; Exch. Mg: Exchangeable Mg. Numbers in the table represent mean ± standard error (n=3).

4.4. Discussion

4.4.1. N_2O and CO_2 emission from crop residues

Crop residues are important sources of GHG emissions in agricultural soils but the conversion of these residues to biochar as a soil amendment has a climate change mitigation potential beyond biochar C storage in soils (Nguyen et al., 2016). In the present study, broccoli residues were incorporated together with palm shell biochar (PSB) in the soil at the beginning of the fallow period in broccoli production. The results confirmed the hypothesis that crop residue incorporation increases GHG emissions during the fallow phase especially during the first two months after crop residue incorporation as compared to the crop growing phase. The results in this study were consistent with those in other reports (Velthof et al., 2002; Scheer et al., 2014; Nett et al., 2015; Nett et al., 2016; Rezaei Rashti et al., 2016; Scheer et al., 2017) which showed increase in GHG emissions following incorporation of vegetable crop residues in soil. The reason for the increased N_2O emissions was mainly attributed to the high N content (248 kg N ha^{-1}), low C/N ratio (15.8) and the high moisture content (>85%) of the broccoli residues used in the present study. The high CO_2 emission occurring after broccoli residue incorporation provides evidence that high N_2O emissions are caused by the rapid decomposition of crop residues that creates anaerobic microsites in soil thereby favoring denitrification (Scheer et al., 2017). This is because in addition to N, crop residue incorporation increases available organic C which stimulates soil heterotrophic respiration hence lowering oxygen partial pressures in soils, thereby creating anaerobic conditions for denitrifiers (Giles et al., 2012). In addition, the concurrent reduction in NH_4^+-N and increase in NO_3^-- N (**Figure 23**) shows that nitrification was possibly the main process for N_2O emissions in this study. Baggs et al. (2000a) also reported that the strong correlation between N_2O fluxes and the rise in soil nitrate concentration coupled with a decrease in ammonium concentration suggested that nitrification significantly contributed to N_2O production in their study. Also, nitrification is the main source of N_2O in soils at 35–60% WFPS (Bateman and Baggs, 2005) as observed in the present study. The increased oxygen availability at low WFPS inhibits activity of denitrifiers, with nitrifiers being mainly responsible for N_2O emissions (Cayuela et al., 2014). The low N_2O emissions in the NR treatment is attributed to the low levels of inorganic N (**Figure 23**) and low carbon which occurred after crop residue removal from the soils.

The non-significant effects of biochar on N_2O emissions from crop residues could most likely be attributed to the differences between replication plots of the same treatment. Suddick and

Six (2013), and Scheer et al. (2017) also accounted for the non-significant differences to the high spatial differences between replication plots. Another reason could be the relatively low cation exchange capacity (CEC) of the PSB as compared to the soil (**Table 7**) that might have had little capacity to adsorb NH_4^+ ions. Moreover, studies that have reported significant reductions in N_2O emissions have CECs of biochar which are higher than 12.8 cmol (+) kg^{-1} (Zhang et al., 2013; Li et al., 2015b). The crop residue induced N_2O emission factors (EF_R) in this study is within the range of 0.3–2.2 % which was reported in cauliflower residues (Nett et al., 2016). Although there were non-significant effects of biochar on EF_R, the present study also revealed that higher biochar rates (>20 t ha^{-1}) may play an important role in reducing N_2O emission factors of broccoli crop residues. The N_2O reduction tendency of biochar at higher application rates used in this study may have resulted from the increased soil aeration which inhibits the rates of denitrification, and also reduction in the inorganic N pools available for nitrifiers and/or denitrifiers in soil, thereby reducing the substrate availability for N_2O production (Clough et al., 2013; Cayuela et al., 2014; Nguyen et al., 2016).

Although biochar did not significantly affect CO_2 emissions in the present study, elevated CO_2 emissions following biochar application have been shown in previous studies (Troy et al., 2013; Case et al., 2015; Oo et al., 2018). The increase in CO_2 emissions following biochar application is attributed to the increased rates of C mineralization of labile C added with biochar (Troy et al., 2013). Contrary, Nguyen et al. (2016) reported that biochar incorporation with or without maize crop residues significantly reduced CO_2 emissions. The authors attributed it to the ability of biochar to reduce the bioavailability of soluble organic substrate by organic matter sorption to biochar and physical protection which slowed down mineralization and decomposition of soil organic matter. However, it is difficult to compare the above results in their studies to those in this study since their experiments were conducted in different soil types, biochar types and environmental conditions.

The higher N_2O and CO_2 emissions in the fallow season as compared to the crop growing season showed that crop residue induced emissions had more impact than the chicken manure and chemical fertilizer induced emissions. This result is in conformity with Scheer et al. (2014) who reported that higher N_2O and CO_2 emissions occurred during the post-harvest season after the incorporation of broccoli crop residues in soils. Whereas the GHG emissions reported in this study during the crop growing season were from a combined effect of both chicken manure and chemical

fertilizer, future studies should also focus on the individual contribution of each N source to the total GHG emissions. The lower N_2O emissions in the crop growing season than the fallow season could have been due to the low microbial activities as a result of the reduction in soil temperatures which could not favor microbial activity hence lower levels of nitrification and/or denitrification **(Figure 22)**. This was evidenced by the fact that in all the treatments, the exchangeable NH_4^+-N concentration was generally higher than NO_3^--N concentration for most of the crop growing season **(Figure 23)**. The low microbial activity could also be due to the low soil respiration rates resulting from the low C source (lack of crop residues), as evidenced by the very low CO_2 emissions during the crop growing season **(Figure 24b).** The negative soil CO_2 fluxes could not be thoroughly explained by the results obtained in this study. However, soil CO_2 fluxes are bidirectional depending on the predominance of various biological and non-biological components in soils; with soils acting as a net sink of CO_2 when non-biological components outcompete the biological ones (Cueva et al., 2019).

4.4.2. CH_4 emission from crop residues

This study showed little effects of incorporation of crop residues and biochar on CH_4 emissions and these results are similar to those from previous studies which reported no significant effects of biochar and/or crop residues on CH_4 emissions in upland fields (Troy et al., 2013; Li et al., 2015b; Lin et al., 2015; Nguyen et al., 2016). This is because this study was conducted on an upland field which was mostly aerobic and the addition of biochar further increased soil aeration (Karhu et al., 2011). However, methane producing bacteria (methanogens) are extremely sensitive to oxygen, and methane production (methanogenesis) in soil only occurs under anaerobic highly reducing conditions in the absence of other potential electron acceptors like NO_3^- and SO_4^{2-} (Topp and Pattey, 1997). The higher CH_4 emissions during the fallow season were attributed to the high soil temperature **(Figure 22a)** and high amounts of crop residues that resulted to higher C pools. Similarly, Oo et al. (2013) attributed the high methane emissions in rice fields to the high soil temperature and the increased availability of substrates that favored methanogenic activities to decompose soil organic matter. The results from the present study could imply that under conditions of high soil temperature, broccoli crop residue incorporation with or without biochar in soil may be a potential source of CH_4 emissions.

4.4.3. N uptake, C/N ratio, biomass and water content of broccoli residues after harvest

It is assumed that biochar did not affect broccoli residue biomass possibly due to the adequate supply of N from chicken manure and chemical fertilizer which was further evidenced by the non-significant influence of biochar on N uptake of broccoli residues (**Table 9**). Nguyen et al. (2016) also reported a non-significant effect of rice husk biochar on maize biomass and attributed it to the adequate supply of mineral N fertilizer. Different plant parts i.e. stems, leaves and roots show specific patterns of decomposition related to their biochemical composition (Agneessens et al., 2014). Plant residues with a higher quality (high N contents and low C/N ratios) often show high decomposition and N mineralization rates (Kamkar et al., 2014). This implies that the rate of decomposition and GHG emissions from the broccoli residue plant parts might be in the order; heads >leaves> roots> stem, but further studies involving the incorporation of different plant parts in soil are still needed to clarify this phenomenon. Therefore, the rate of GHG emissions would depend majorly on the proportions of these residues left in the field after final harvest and the time from harvest to residue incorporation. The longer the time from harvest to residue incorporation, the higher the biomass and remaining total N content in these residues. For instance, the total biomass of the remaining crop residues ranged from 24.6–27.3 t ha^{-1} with total N of 80.6–105.9 kg N ha^{-1} **(Table 9)** which was approximately two times lower than the fresh biomass (57.0 t ha^{-1}) and N content (248 kg N ha^{-1}) used at the start of the experiment. The water contents of broccoli residues ranged from 88.4–90.4%, which is characteristic of most vegetables in the brassica family. These results are in agreement with those of Nett et al. (2016) who reported water content of 88% in cauliflower residues.

4.4.4. Soil chemical properties

Many studies have shown that application of biochar in soil improves soil quality through improving soil chemical properties (Chan et al., 2007; Quilliam et al., 2012; Suddick and Six, 2013; Subedi et al., 2016), and also neutralizing soil acidity, as well as enhancing CEC of soils (Cha et al., 2016). However, the effect of biochar on soil chemical properties may vary with biochar characteristics, soil type and the type of crop grown among others. Lin et al. (2015) reported that application of maize stalk biochar to a loamy sand soil did not affect soil pH, Ca^{2+} and Mg^{2+} but significantly increased exchangeable K^{+} as observed in this study. The significant increase in exchangeable K was attributed to the release of mineral elements in biochar (Lehmann and Joseph, 2015). The nutrient content of the biochar varies with feedstock type and pyrolysis

temperatures (Ding et al., 2016; Gunarathne et al., 2017) which results in biochars with different effects in soil. For instance, Martinsen et al. (2015) reported that application of 2% (about 60 t ha^{-1}) oil palm shell biochar resulted in smaller increment in soil exchangeable Ca, Mg and K as compared to cacao shell biochar which had higher increments of these base cations. Although the palm shell biochar used in this study had a high content of exchangeable Ca (**Table 7**), the reasons for the non-significant increase in exchangeable Ca in soil after biochar addition could most likely be attributed to soil factors rather than plant factors. Regarding soil factors, there exists differences in the tenacity with which several colloids hold specific cations and in the ease with which they exchange the cations (Weil and Brady, 2016). Therefore, it may be possible that the soil used in this study had a low capacity to hold exchangeable Ca which could have been lost by leaching and erosion losses. The potential of Ca water leaching from biochar is due to the emergence of calcium bicarbonate, a salt existing in aqueous solution which has much higher solubility than calcium carbonate during water leaching (Wu et al., 2011).

In addition, the magnitude of changes in soil chemical properties is proportional to the biochar application rates, with significant differences observed at higher application rates >50 t ha^{-1} (Chan et al., 2007). Therefore, the non-significant effects of biochar on soil pH, EC, available P, exchangeable Ca, Mg, and CEC in our study may also be attributed to the moderately lower biochar application rates which were up to 40 t ha^{-1}. Biochar contains high concentrations of N, P, Ca and K which may directly provide soils with nutrients (Cha et al., 2016). The authors added that when biochar is applied to soil, basic cations are discharged in the soils, thereby replacing Al and H^+ hence enhancing soil CEC, which generally increases with increasing pH. In the present study, the non-significant changes in soil CEC may also partly be explained by the little variation in soil pH and basic cations (Ca and Mg) in biochar amended soils as compared to soils without biochar. Moreover, among important soil properties, soil exchangeable Ca^{2+} content is the primary factor controlling the direction of biochar induced change in soil CEC and exchangeable Ca^{2+} content (Hailegnaw et al., 2019). Furthermore, the non-significant changes in soil CEC could be attributed to the reduction of the CEC of biochar (pH-dependent binding sites at the biochar surfaces) when added to acidic soils (Martinsen et al., 2015). The authors also reported an increase in soil CEC after adding oil palm shell biochar (11–20 cmol (+) kg^{-1}) to soils with very low CEC (5.62 cmol (+) kg^{-1}) which was not the case in this study where the values for biochar and soil

CEC were in the same range (**Table 7**). This could explain why the biochar used in this study had a low capacity to increase the soil CEC even after applying 40 t ha^{-1}.

The increase in pH associated with adding biochar to acidic soils is due to the increased concentration of Ca^{2+}, Mg^{2+}, K^{+}, and a reduction in soil Al^{3+} concentration (Steiner et al., 2007). The effectiveness of biochar addition for increasing soil pH is greater in soils with low pH, CEC, exchangeable Ca^{2+} content, and clay fraction, and in soils with a higher sand fraction (Hailegnaw et al., 2019). For instance, Streubel et al. (2011) reported that the sandy soil (3.3 cmol (+) kg^{-1}) exhibited the greatest and most rapid increase in soil pH compared to the silty loam soils (15.4–16.6 cmol (+) kg^{-1}) after biochar addition up to 39 t ha^{-1} due to the inherently lower buffering capacity of sand compared to silty loam soils. Therefore, in the present study, the non-significant increase in pH in response to biochar application may also be attributed to the CEC of the soil (13.5 cmol (+) kg^{-1}).

The tendency of biochar to reduce inorganic N at higher biochar application rates on some soil sampling dates could be attributed to adsorption of NH_4^+-N and /or NO_3^--N on biochar surfaces (Clough et al., 2013). The adsorption of NO_3^--N could be further explained by the anion exchange capacity (AEC) of biochar (Lawrinenko, 2014). In addition, Ippolito et al. (2016) reported significant reductions in soil NO_3^--N with increasing biochar rates and attributed it to microbial immobilization. Contrary, Quilliam et al. (2012) showed that biochar application increases soil exchangeable NH_4^+-N as observed in the R10 treatment on some of the soil sampling dates (**Figure 23**). This could be attributed to the increase in organic matter mineralization (Suddick and Six, 2013).

4.4.5. Mitigation strategies for N_2O emissions from broccoli crop residues

The control strategies for N_2O emissions from broccoli fields are most likely to vary with the season of broccoli incorporation in soil which depends on the time of transplanting. In a given farming community like the one used in this study, farmers have different broccoli cropping calendars. Therefore, if the time of broccoli incorporation in soil coincides with the time when the environmental conditions are favorable for nitrification and denitrification processes, higher GHG emissions are expected to occur. Also, if the proportion of crop residues contains more plant parts with lower C/N ratios, higher amounts of GHG emissions are likely to occur. Moreover, other studies (Huang et al., 2004; Toma and Hatano, 2007) reported an increase in cumulative N_2O and CO_2 emissions as the C/N ratio of crop residues decreased, and that applying residues with a high

C/N ratio causes the immobilization of soil mineral N. Therefore, farmers should consider thorough harvesting of the broccoli heads since they are most likely to emit more GHG than other plant parts. The high N content of broccoli implies higher GHG emissions which may necessitate higher biochar application rates than those used in this study. However, since higher biochar application rates might not be feasible under field conditions, alternative GHG mitigation strategies are needed. Niu et al. (2018) showed that the combined application of biochar and nitrification inhibitors in a sandy loam soil has a potential to reduce N_2O emissions from N fertilizers. Therefore, further research should also explore the effects of simultaneous biochar incorporation with nitrification inhibitors on mitigating N_2O emissions from broccoli crop residues in soil.

4.5. Conclusion

After final harvest, broccoli plants leave high amounts of crop residues with high N_2O emission factors. In the present study, the incorporation of broccoli crop residues in soil during the fallow season significantly increased N_2O and CO_2 emissions but did not affect CH_4 emissions. The application of palm shell biochar did not significantly affect the seasonal GHG emissions, plant residue biomass and N uptake after harvest. The study highlights the potential for palm shell biochar (at application rates $\geq$40 t ha^{-1}) to improve on the soil nutrient status in soils under vegetable production. These findings imply that instead of removing broccoli crop residues after harvest, they should rather be incorporated with biochar as it could be beneficial to soil through maintaining a high soil nutrient status which may improve productivity of the subsequent crops. However, this study did not look at the effect of biochar on the crop growth and yield of broccoli. Therefore, future work will be needed to assess if biochar can improve the productivity of broccoli as well as the further evaluation of the effects of combined incorporation of broccoli crop residues with biochar on GHG emissions and soil nutrient status in different soils under various tillage systems.

CHAPTER FIVE

Effect of activated carbon on greenhouse gas emissions, seed yield, soil chemical properties and isoflavone content of soybean genotypes with varying nodulation capacities under sandy soil conditions

5.1. Introduction

The agriculture sector significantly contributes to global anthropogenic greenhouse gas (GHG) emissions (IPCC, 2013) mainly from excessive use of chemical fertilizer, animal wastes, crop residues and biological nitrogen fixation (BNF) (Mosier et al., 1998). Nitrous oxide (N_2O) emissions from BNF are much lower than those from other nitrogen (N) sources hence this process could make significant contributions to lowering GHG emissions from the agriculture sector (Syakila and Kroeze, 2011; Skiba and Rees, 2014). BNF minimizes GHG emissions during the manufacture, transportation and application of N fertilizers in the legume fields (Sant'Anna et al., 2018). However, the benefits from BNF could vary with the type of leguminous crop under consideration.

Soybean (*Glycine max* (L.) Merr.) is one of the leguminous crops grown mainly for its health benefits, with a high potential to fix large amounts of N from the atmosphere. For instance, in 2014, estimates of N fixation from selected legumes showed that soybeans alone contributed 23.4 Tg N, which represented 81% of total N fixed by the legumes reported (Islam and Adjesiwor, 2018). Soybean crops can fix up to 450 kg N ha^{-1} from atmospheric N through BNF (Hungria and Mendes, 2015), which occurs in the root nodules. The nodules develop on roots through signal exchange between plant roots and rhizobia (Hassan and Mathesius, 2012; Flynn et al., 2014), and the number of root nodules is often used as an indicator of BNF (Mete et al., 2015). Higher N fixation capacity can also be achieved by planting super-nodulating soybean genotypes that have nodules 11–14 times more than the normal soybean types or the parent lines (Gremaud and Harper, 1989; Takahashi et al., 2003). An upland field experiment conducted on an Andisol, involving three soybean genotypes of varying nodulation i.e. super-nodulating, normal nodulating and non-nodulating genotypes revealed that the N_2O emissions were highest in the super-nodulating genotype especially during the full bloom (R2) and full pod stages (R4) but insignificant during the seed filling stage (Kim et al., 2005). Although super-nodulation is an important trait in terms of the abundant supply of fixed atmospheric N, super-nodulating genotypes have been regarded as

inferior in growth and seed yield due to their high energy requirements which requires a high consumption of carbohydrates to form the root nodules (Takahashi et al., 2003; Ferguson, 2013). However, there could be a potential for improved super-nodulating genotypes to perform better in soils with low fertility such as sandy soils that might need abundunt inputs especially synthetic N fertilizers to improve their productivity in the absence of BNF.

The process of symbiotic N fixation does not stimulate N_2O production or emission, but rather the senescence and decomposition of the roots and nodules during the late stages of soybean growth (Yang and Cai, 2005; Yang and Cai, 2006; Shah, 2014), and after crop harvest. Little work has been done to investigate the GHG emissions from legume fields but an understanding of the mechanism through which N_2O emissions occur may aid in their mitigation (Flynn et al., 2014). High soil N_2O emissions in legume crops may mostly be attributed to N release from root exudates during the growing season, and from decomposition of crop residues after harvest, rather than from BNF (Rochette and Janzen, 2005). Moreover, inoculated and non-inoculated legumes showed no significant differences in N_2O emissions (Zhong et al., 2009) but degraded nodules were a source of N_2O in the soybean rhizosphere due to the microbial mediated processes such as nitrification and denitrification (Inaba et al., 2009). Root exudates in soybean plants include flavonoids such as isoflavones (Cesco et al., 2010; Duressa et al., 2010; Sugiyama, 2019).

Isoflavones are important secondary metabolites found in all the plant parts and rhizosphere of soybean. They mainly exist as aglycone (daidzein, glycitein and genistein) and glycoside (daidzin, glycitin and genistin) forms which can be found in root exudates and soil, but aglycones are the most active forms mediating legume-rhizobial interactions as well as defenses against pathogens in the rhizosphere (Sugiyama and Yazaki, 2014). Root nodule formation in soybeans is aided mainly by daidzein and genistein secreted by the host plant roots to act as signaling compounds which activate the production of nod factors by homologous rhizobia, that are perceived by the plant to allow symbiotic infection of the root, leading to nodulation (Guo et al., 2011; Liu and Murray, 2016; Sugiyama et al., 2017). However, environmental conditions, genotype, year of cropping, planting location and soil chemical properties affect isoflavone content and composition of soybeans (Lee et al., 2003; Tepavčević et al., 2011; Kim et al., 2014**)**. Therefore, any manipulation in soil properties may affect the concentration of the isoflavones in the soybean rhizosphere which could directly affect the nodulation capacity of the plants and indirectly affect soil GHG emission as well as plant agronomic traits and seed quality. Moreover,

there is scarce information on the actual flavonoid content in soil and how they change over space and time (Hassan and Mathesius, 2012).

Pyrogenic carbonaceous soil amendments such as biochar have been reported to adsorb signaling compounds in soils, mitigate GHG emissions and increase crop productivity (Lehmann et al., 2011; Hussain et al., 2017). However, the adsorption performance of biochar is limited by its low surface area; hence the physical or chemical activation of biochar to form activated carbon (AC) increases its surface area and forms functional groups that may improve its adsorption properties (Zhang et al., 2019b). The activation of biochar involves treatment with steam and chemicals such as $ZnCl_2$ and KOH at temperatures more than 700°C (Ahmed et al., 2019). The effects of commercially produced AC are reported to be similar to those of natural-occurring fire produced charcoal (Pietikäinen et al., 2000; Berglund et al., 2004).

AC can reduce the concentrations of main secondary metabolites and phenolics in soil (Miao et al., 2013; Yuan et al., 2014). It can also affect crop growth through altering nutrient availability, minimizing negative growth effects of allelochemicals and changes in the soil microbial compositions (Pietikäinen et al., 2000; Lau et al., 2008). The alterations in nutrient availability include changes in the soil nutrient status by increasing the soil chemical properties through reduced N, Ca and Mg leaching (Lehmann et al., 2003). Moreover, charcoal exhibits important characteristics that affect regulating steps in the transformation and cycling of N (Berglund et al., 2004). The action of AC in soil may affect N transformations in soybean cropping systems by adsorbing signaling molecules such as daidzein and genistein. This could decrease root nodulation thereby reducing GHG emissions from BNF and from the decomposition of root nodules but the subsequent effect of AC on crop growth and yield should not be ignored. In addition, AC can enhance nitrification by eliminating inhibitory compounds such as phenolics which have a negative effect on soil nitrifying bacteria (Paavolainen et al., 1998; DeLuca et al., 2006).

Although studies have been done on the effects of AC in agriculture, there are no reports on the combined effect of AC on yield, GHG emissions, isoflavone content and soil properties in soybean cropping systems. Therefore, in this study, a 2-year pot experiment was conducted to assess the effect of AC on (1) GHG emissions; (2) nodulation and agronomic traits of soybean; (3) soil chemical properties; (4) Seed protein and isoflavone content; (5) root and soil isoflavone content of soybean genotypes with varying nodulation capacities under sandy soil conditions. It

was hypothesized that AC would reduce GHG emissions, reduce nodulation, increase seed yield, soil chemical properties and seed protein content, and also reduce isoflavone content in the seeds, roots and soil. The effect of AC on the studied variables would also vary depending on the genotypes.

5.2. Materials and methods

5.2.1. Establishment of the pot experiment

This experiment was conducted in a greenhouse (vinyl house) at Tottori University, Tottori, Japan (35°30' 52"N 134°10'13"E) from June to October of 2017 and 2018 in Wagner pots (29.3 cm in height, 25.6 cm outer diameter and 24.0 cm inner diameter) filled with sandy soil. The sandy soil was collected from Tottori sand dunes, Tottori, Japan and its properties are described in **Table 1**. Prior to the experiment, the soil was air dried and passed through a 2 mm sieve to remove weeds and other debris. Wood activated carbon (AC) was purchased in powder form from a commercial company (Ajinomoto Fine-Techno Co., Japan) and its physicochemical properties are shown in **Table 11**. The AC was applied once at the start of the 2017 season at rates equivalent to 0, 2.4, 4.8 and 9.6 t ha^{-1} (0, 12, 24, and 48 g per pot) herein referred to as CTR, AC1, AC2 and AC3 treatments respectively, with twelve replications per treatment arranged as a randomized complete block design. Chemical fertilizers were applied each year before transplanting at rates equivalent to 45 kg N ha^{-1}, 150 kg P ha^{-1}, 150 kg K ha^{-1} as NPK, TSP, and K_2SO_4, with dolomite at 500 kg ha^{-1}. The Fertilizer and AC were thoroughly mixed in soil at 10 cm depth (7.2 kg air dry basis).

Three different soybean genotypes were TnVRSN4, Tachinagaha and TnVRNN4; selected based on their nodulation capacities i.e. High, normal and low nodulation capacities respectively (**Figure 25**). **Figure 26** shows a pictorial summary of the how this experiment was established. Each of the soybean genotypes was grown at the four levels of AC. In the first year, seeds were sown in seedling trays on 30th June 2017 for TnVRSN4 and TnVRNN4, while those of Tachinagaha were sown on 7th July 2017. One seedling of each genotype was transplanted in each pot on 12th July 2017 for TnVRSN4 and TnVRNN4, and on 19th July 2017 for Tachinagaha when the seedlings were at the V1 stage (first trifoliate stage). In the second year, the seeds of the three soybean genotypes were sown in seedling trays on 29th June 2018. One seedling (V1 stage) of each genotype was transplanted on 11th July 2018 in the same pots used in 2017.

Table 11. Selected characteristics of the activated carbon (AC) used in this study.

Properties	Units	AC
pH (H_2O)		9.9
EC	dS m^{-1}	4.3
Total C	g kg^{-1}	620
Total N	g kg^{-1}	2.9
C/N		215
Available P	mg kg^{-1}	412
Exchangeable K	mg kg^{-1}	8157
Exchangeable Ca	mg kg^{-1}	12329
Exchangeable Mg	mg kg^{-1}	1035
Specific surface area	m^2 g^{-1}	1038
Pore diameter	mm	0.709
Total pore volume	cc g^{-1}	0.628
Micro pore surface area	m^2 g^{-1}	762
Micro pore volume	cc g^{-1}	0.320
Meso pore surface area	m^2 g^{-1}	223
Meso pore volume	cc g^{-1}	0.280
Total meso + macro pore surface area	m^2 g^{-1}	276

EC-Electrical conductivity

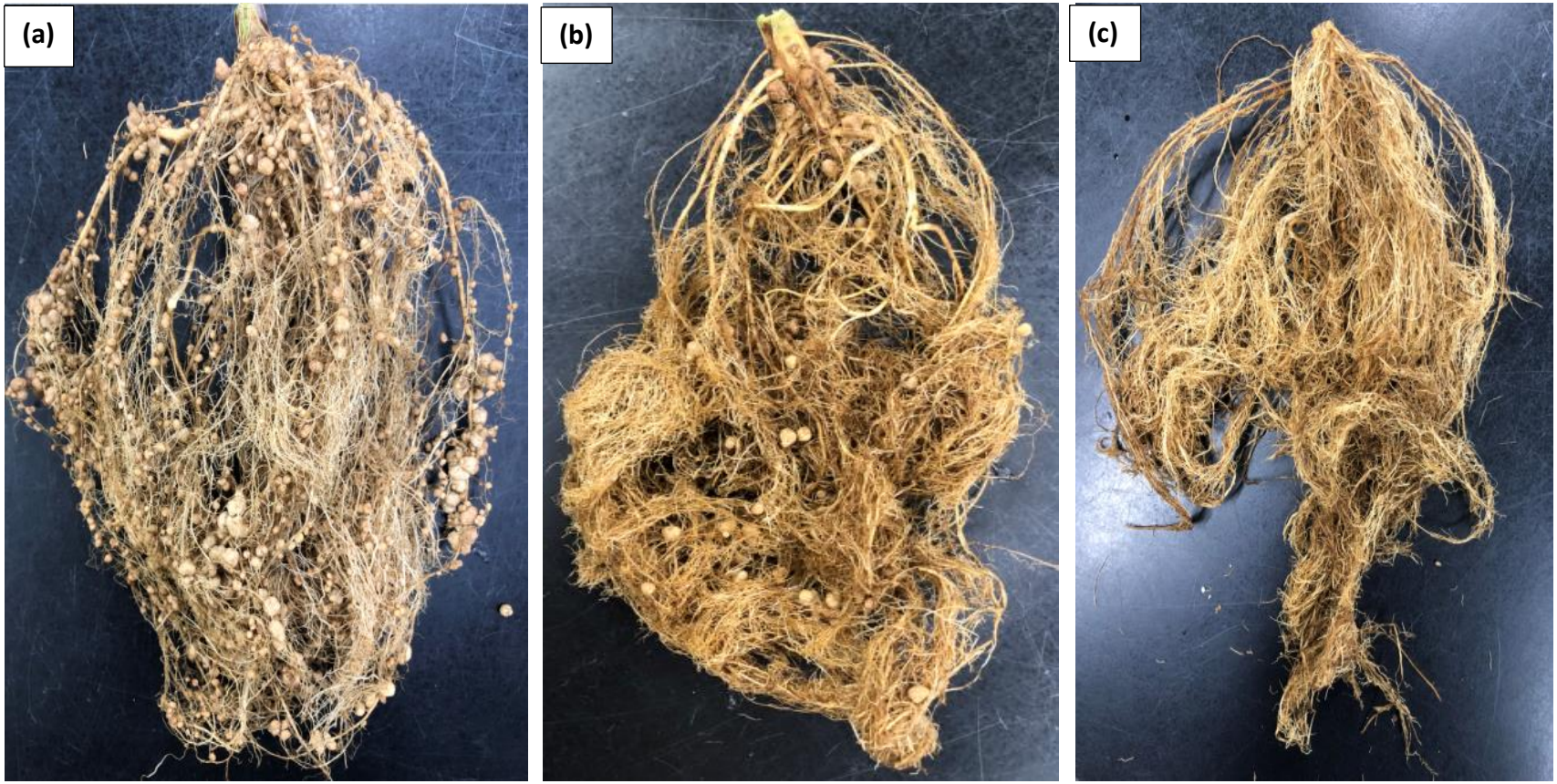

Figure 25. Distribution of root nodules on the roots of the soybean genotypes; TnVRSN4 (**a**), Tachinagaha (**b**) and TnVRNN4 (**c**).

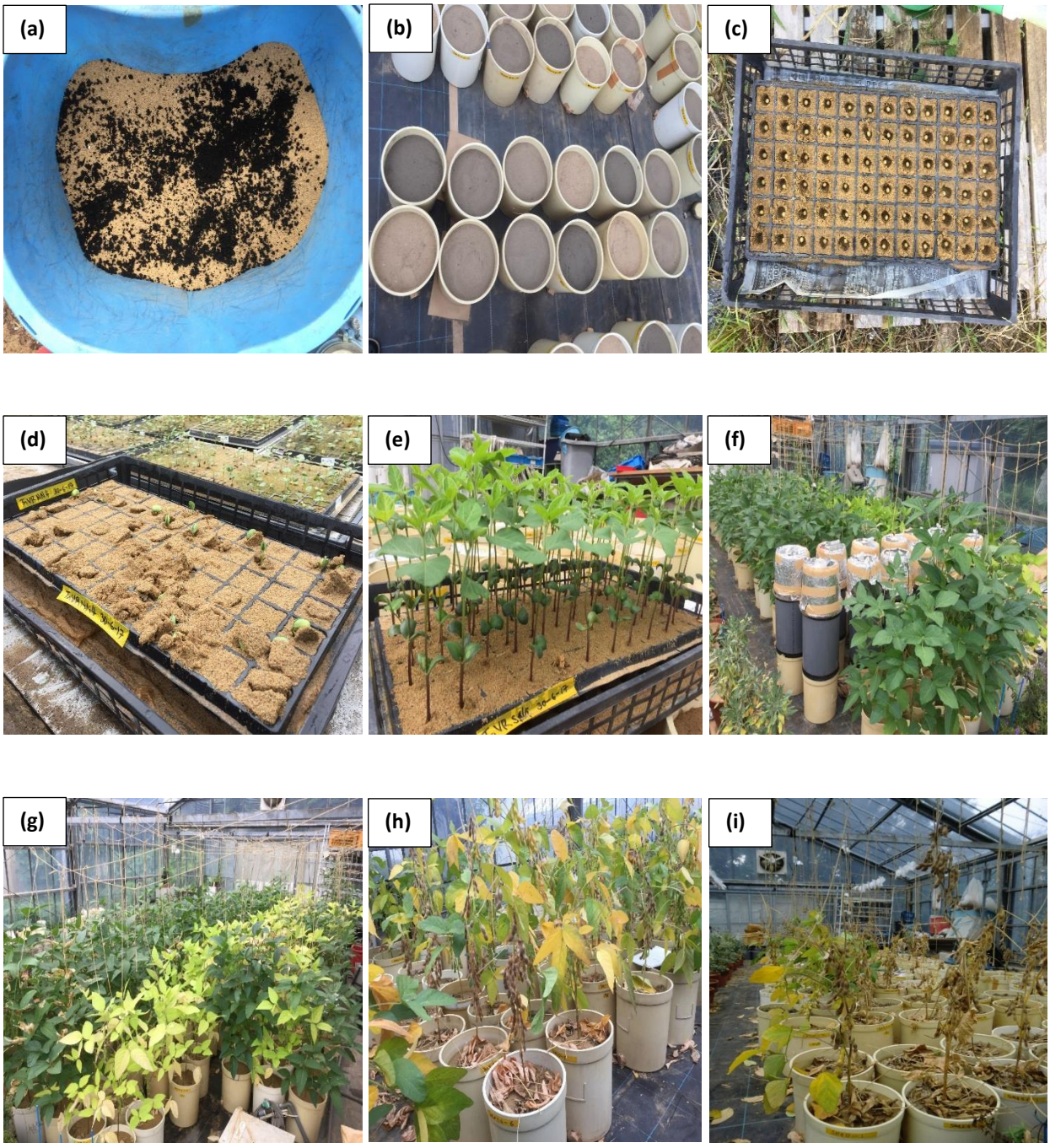

Figure 26. Establishment of the experiment. Mixing AC and fertilizer (**a**), AC and fertilizer mixed in pots (**b**), seeds sown in a seedling tray (**c**), seedlings at emergence stage (**d**), seedlings at V1 stage (**e**), during gas sampling (**f**), yellowing of TnVRNN4 plants (**g**), at physiological maturity (**h**), at harvest (**i**).

Before transplanting, the soil in the pots was moistened to 60% water-filled pore space (WFPS). Thereafter, irrigation was done using a watering can by adding 200–300 ml of water to each pot once or twice a day depending on the daily temperatures and crop growth stage; water did not leach out of the pots. Soil moisture at 12 cm depth was monitored using time-domain reflectometry (TDR) probes and then expressed as WFPS as described above in section 3.2.2.

In the first year, TnVRNN4, Tachinagaha and TnVRSN4 soybeans were harvested on 6th October, 12th October and 28th October 2017 respectively while in the second year, they were harvested on 4th October, 10th October and 26th October 2018 respectively. The yield in the second year was obtained from the nine replications where the roots and soil had not been removed (for analysis) at the end of the first season in 2017. Thermo recorders (TR-71wf, T & D Corporation, Nagano, Japan) were inserted at 5 cm soil depth to monitor the soil temperature in each treatment while air temperature inside the greenhouse was monitored by a thermo recorder (RTR-500B1, T & D Corporation, Nagano, Japan) for the entire periods of crop growth.

5.2.2. Gas sampling and analysis

Manually closed chambers were used to collect air samples from the pots (three replicates) throughout the period of study from 14th July to 27th September 2017 and from 12th July to 28th September 2018 between 6:00 am to 10:00 am on each sampling day. The chambers had an outer radius R of 13.5 cm, inner radius r of 11.9 cm and height of 26 cm (for the first two weeks after transplanting), and each had three ports; one for sampling, the other for measuring air temperature inside the chamber and the other fitted with an air buffer bag to compensate for the pressure differences. Due to the increasing plant growth, the chamber height was increased to 66 cm (from the third week after transplanting) by tightly connecting PVC pipes (height of 40 cm) on the pots during sampling. Gas samples were drawn from the headspace at 0, 20 and 40 min after fitting the chambers onto the pots. 30 ml of gas were immediately injected into 15 ml pre-evacuated vials (Nichiden-Rika Glass Co. Ltd, Kobe, Japan) fitted with butyl rubber stoppers and then stored in the laboratory until analysis. The air temperature inside each chamber was simultaneously measured by a digital thermo recorder (TR-71U*i*, T & D Corporation, Nagano, Japan). N_2O concentration was analyzed using a gas chromatograph (GC-14B Shimadzu, Kyoto, Japan) equipped with an electron capture detector (ECD) while CO_2 and CH_4 concentrations were analyzed using gas chromatographs (GC-8A and GC-14A, Shimadzu, Kyoto, Japan) equipped with the thermal conductivity detector (TCD) and flame ionization detector (FID) respectively.

Gas analysis was done at the Institute for Agro-Environmental Sciences (NIAES), Tsukuba, Japan. The Gas fluxes were calculated by considering the linear increase in gas concentrations in chamber headspace over the 40 minutes of closure (Minamikawa et al., 2015) while the cumulative gas emissions were calculated directly from the gas fluxes by adding up all average daily emissions obtained from every two adjacent sampling dates.

5.2.3. Soil and activated carbon analysis

At the end of each crop season, soil was sampled from pots in the three replications used during gas analysis and analyzed for soil inorganic N (exchangeable NH_4^+-N, and NO_3^--N), pH, EC, total N, C/N ratio, available P, exchangeable K, Ca and Mg. In brief, soil samples at 0–10 cm depth were taken, mixed homogeneously, air-dried and sieved (< 2mm). Fresh soil samples were also collected, uniformly mixed and kept in a cooler box and later stored at −80°C until further analysis for inorganic N. Soil exchangeable NH_4^+-N was extracted from fresh soil samples using 10% potassium chloride, KCl (1:10, w/v) that was shaken for 1 h on a mechanical shaker, and then analyzed using the indophenol blue method (Smith and Cresser, 2004) at 693 nm on a spectrophotometer (U-5100, Hitachi, Tokyo, Japan). Soil NO_3^--N was also extracted from fresh soil using 0.01% $AlCl_3 \cdot 6H_2O$ (1:100, w/v) that was shaken for 30 min, and analyzed using the ultraviolet absorption method (Yamaki, 2003) at 210 nm on a spectrophotometer (U-5100, Hitachi, Tokyo, Japan). Soil pH and EC were measured at a 1:5 (w/v) soil to water ratio using pH and EC electrodes (F-74 pH/ION/COND meter, Horiba Ltd, Kyoto, Japan). Soil total C and total N contents were determined by dry combustion using the C/N corder (JM1000CN, J-SCIENCE LAB, Kyoto, Japan). The soil available P was determined using 0.002N H_2SO_4 buffered with $(NH_4)_2SO_4$ solution (Truog, 1930) and the P in the soil filtrate determined by the phosphomolybdate blue method (Murphy and Riley, 1962) using a spectrophotometer at 710 nm (U-5100, Hitachi, Tokyo, Japan). Soil exchangeable K, Ca, and Mg contents were determined by extraction with 1N ammonium acetate (pH 7.1), followed by analysis using the atomic absorption spectrophotometer (Z-2300; Hitachi, Tokyo, Japan). The chemical analysis of AC was done following similar procedures used in soil analysis.

5.2.4. Growth and yield of soybeans

At harvest, the pods were removed from the dry plants and stored in paper bags for further air drying. Plant height, stem diameter, number of branches, number of filled pods were determined for each plant. The dried pods were threshed and the number of seeds per pod, number of seeds per plant, seed weight per plant and average weight per seed were recorded. The average weight per seed was used to extrapolate the 100-seed weight. Data for all the agronomic traits of soybeans were obtained from nine plants. The roots from the three replications used during gas sampling were thoroughly washed to remove soil particles and the nodules were removed from the roots and later counted and weighed. The roots were air dried and later ground into a fine powder which was used for isoflavone analysis.

5.2.5. Isoflavone and protein analysis

After harvest and removal of plant roots, the soil was sampled and passed through a 2 mm sieve to remove visible roots particles and then stored in plastic bags at −80°C until analysis for isoflavones. Briefly, 40 g of fresh soil were extracted with 20 ml of 70% (v/v) ethanol, shaken and then centrifuged for 15 min at 15,000 rpm at 10°C and the supernatant collected in a flask. Again, 20 ml of 70% (v/v) ethanol were added to the residue and centrifugation repeated twice. Extraction was repeated one more time making a total of 80 g of soil extracted per sample. The supernatants were all collected in the same flask and then filtered through 0.22 µm nylon syringe filters. The filtered samples were evaporated to dryness under vacuum using a rotary evaporator (Rotavapor RII series, BUCHI, Flawil, Switzerland) and the dry residue was dissolved in 5 ml of 70% ethanol and stored at −20°C until analysis.

The seeds were ground to fine powder and analyzed for protein and isoflavone content. Protein content was determined by multiplying the seed total N (%) by 5.51 (Fujihara et al., 2010). The seed total N was determined by dry combustion using the C/N corder as previously described. To determine the root and seed isoflavone content, 1 g of powder was extracted using 70% (v/v) ethanol and sonicated at room temperature for 15 min. The mixtures were centrifuged for 15 min at 15,000 rpm at 10°C. After centrifugation, the supernatants were put in 25 ml flasks and again ethanol was added to the residue, sonicated and centrifuged for two more times. Supernatants were collected into the flask and finally diluted to 25 ml with 70% ethanol and thereafter filtered through the 0.22 µm nylon syringe filters for analysis.

Seed, root and soil isoflavone analysis was performed by the High Performance Liquid Chromatography (HPLC) apparatus consisting of the following components; the pump (L-2130, Hitachi, Tokyo, Japan), column oven (L-2350, Hitachi, Tokyo, Japan), UV detector (L-2400 Hitachi, Tokyo, Japan) and an auto sampler (Chromaster 5210, Hitachi, Tokyo, Japan). The GL Science Inertsil® ODS-3 column (5 µm, 4.6 x 150 mm) was used for separation. The binary mobile phase consisted of 100% acetonitrile (solvent A) and solvent B, containing 10 ml acetic acid and 7.7 g of ammonium acetate mixed in 1000 ml of Milli-Q water. A linear gradient program was used as follows: 0 min, A:B of 12:88; 3 min, A:B of 15:85; 5 min, A:B of 15:85; 7 min, A:B of 20:80; 15 min, A:B of 25:75; 30 min, A:B of 45:55; 45 min, A:B of 75:25; 48 min, A:B of 75:25; 50 min, A:B of 12:88; 60 min, A:B of 12:88. The solvent flow rate was 1.0 ml min^{-1} and injection volume of 10 µl for all the standards and samples. Analysis was performed at UV absorption of 260 nm and column temperature of 40°C. Daidzein was purchased from Toronto Research Chemicals (Toronto, ON, Canada); daidzin and genistin were purchased from ChromaDex, Inc (Irvine, CA, USA) while glycitin, glycitein and genistein were purchased from Wako Pure Chemical industries, Ltd, Osaka, Japan. Isoflavone standards for preparing the standard calibration curves were dissolved in 99.5% ethanol. Isoflavones were identified from chromatograms by comparing their retention times to those of the standards and peak areas used to quantify them. Individual isoflavone contents were calculated from equations obtained from the standard curves and expressed as mg $100g^{-1}$ for seeds, mg g^{-1} for roots and mg kg^{-1} for soil. Isoflavone and protein analysis was only done for the three replications used during gas sampling. Total isoflavone content in seeds was obtained by adding up the values obtained for the six individual isoflavones.

5.2.6. Statistical analysis

All data was analyzed by analysis of variance (ANOVA) using IBM SPSS software (Version 20.0) to examine the main effects and the interactions between year of cropping, soybean genotype and treatment. The statistical differences between treatment means were tested using Tukey's HSD test at 5% level of significance unless otherwise specified.

5.3. Results

5.3.1. Air temperature, soil temperature and soil moisture

The air temperatures inside the greenhouse generally decreased across the crop growing seasons and they ranged from 19.0 to 36.0°C in 2017 and from 18.5 to 35.4°C in 2018 (**Figure 27a**). Generally, the air temperatures during 2018 were slightly higher than in 2017. Soil temperatures showed similar patterns to that of air temperatures and did not differ among the treatments during 2017 (**Figure 27b**) but in 2018, soil temperatures in the AC3 treatment were higher than in the other treatments (**Figure 27c**). Soil moisture varied between 10 and 40% WFPS during the growing seasons (**Figure 28**). There were no much variations in soil moisture among genotypes during each year of cropping. However, the soil moisture in the AC2 and AC3 treatments was generally higher than the CTR and AC1 treatments.

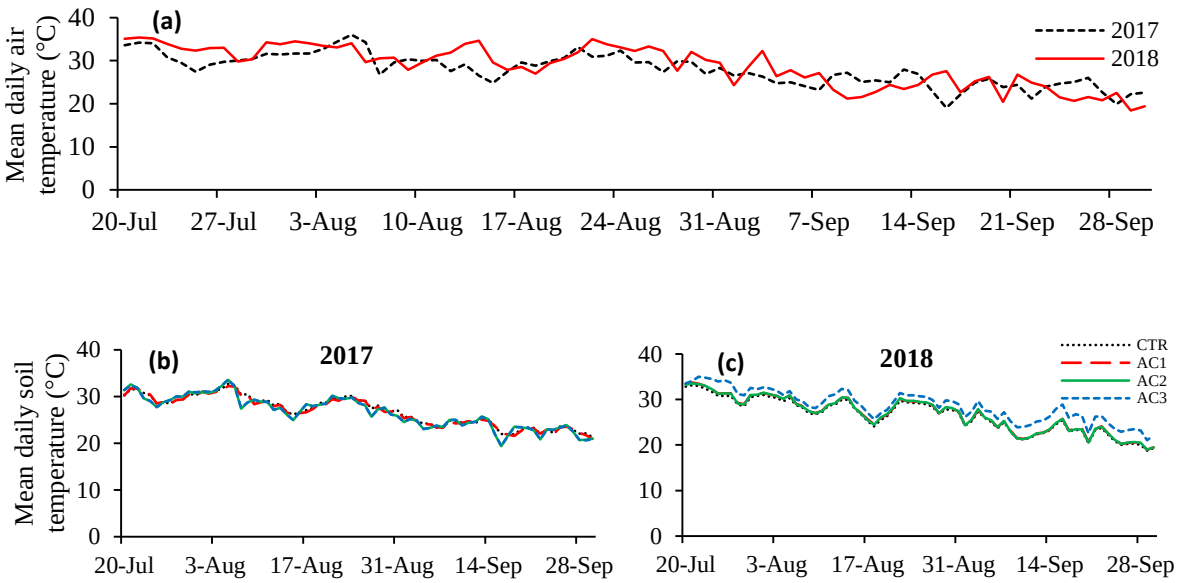

Figure 27. Variation in air temperatures inside the greenhouse during the crop-growing seasons **(a)**. Variation in average soil temperatures at 5 cm depth for the three genotypes across the crop-growing period in each treatment during 2017 **(b)** and 2018 **(c)**. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively.

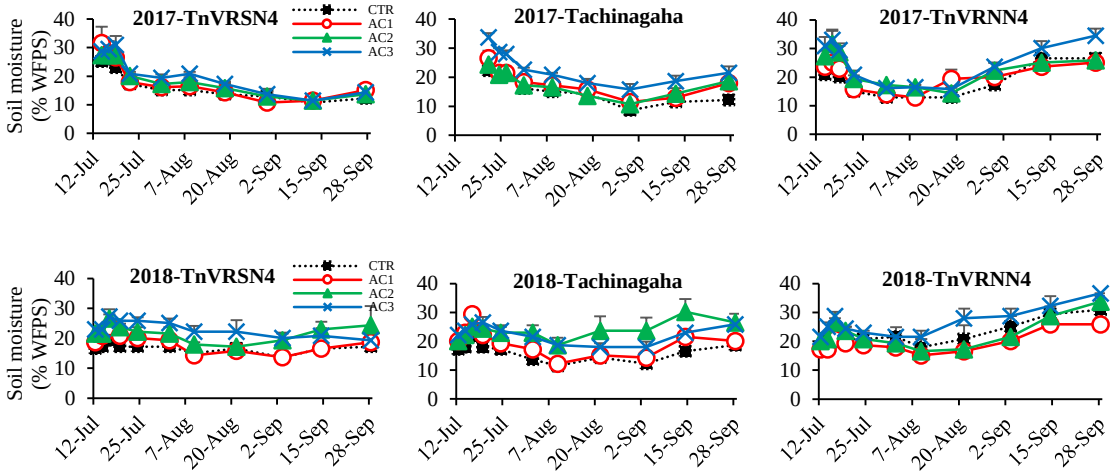

Figure 28. Variation in soil moisture content at 12 cm depth for the soybean genotypes over the crop-growing seasons. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Data points represent mean ± standard error (n = 3).

5.3.2. Greenhouse gas emissions

5.3.2.1. Nitrous oxide emissions

The N_2O fluxes did not significantly vary among the treatments, and followed similar patterns for all the soybean genotypes but these patterns were different in each year of cropping (**Figure 29**). In 2017, the N_2O fluxes ranged from −16.3 to 27.0, −12.4 to 18.6 and −17.6 to 24.9 μg N_2O-N m^{-2} h^{-1} while in 2018, the fluxes ranged from −23.3 to 96.1, −22.1 to 80.9 and −21.0 to 90.5 μg N_2O-N m^{-2} h^{-1} in soils of the high, normal and low nodulating genotypes respectively. The cumulative N_2O emissions were significantly higher in 2018 than in 2017 (**Table 12**). The genotype and AC treatments did not significantly affect N_2O emissions. However, N_2O emissions from the soils of the high nodulating genotype were generally higher than in the other genotypes especially in the absence of AC which tended to reduce N_2O emissions in this genotype during the two cropping seasons.

5.3.2.2. Carbon dioxide and methane emissions

The CO_2 fluxes in all the soils were relatively low at the start of the crop growing season but later increased during the crop growing season and then decreased during the late stages of crop growth (**Figure 30**). CO_2 fluxes varied with soybean genotype and were significantly higher in the 2018 than in the 2017 crop season. In 2017, the CO_2 fluxes ranged from −5.4 to 389.2, −9.2 to 251.5 and −3.1 to 179.5 mg CO_2-C m^{-2} h^{-1} while in 2018, the fluxes ranged from 18.9 to 761.5, 8.1 to 527.5 and 19.9 to 255.6 mg CO_2-C m^{-2} h^{-1} in soils of the high, normal and low nodulating genotypes respectively. In both years, highest cumulative CO_2 emissions occurred in the soils of the high nodulating genotype while the lowest emissions occurred in soils of the low nodulating genotype but this was more evident in 2018 (**Table 12**). AC did not significantly affect cumulative CO_2 emissions as compared to the control in all the soybean genotypes.

The soil CH_4 fluxes did not vary significantly among the treatments in both years of cropping (**Figure 31**). There were no significant differences in cumulative CH_4 emissions among the treatments, genotype, year of cropping, and their interactions (**Table 12**).

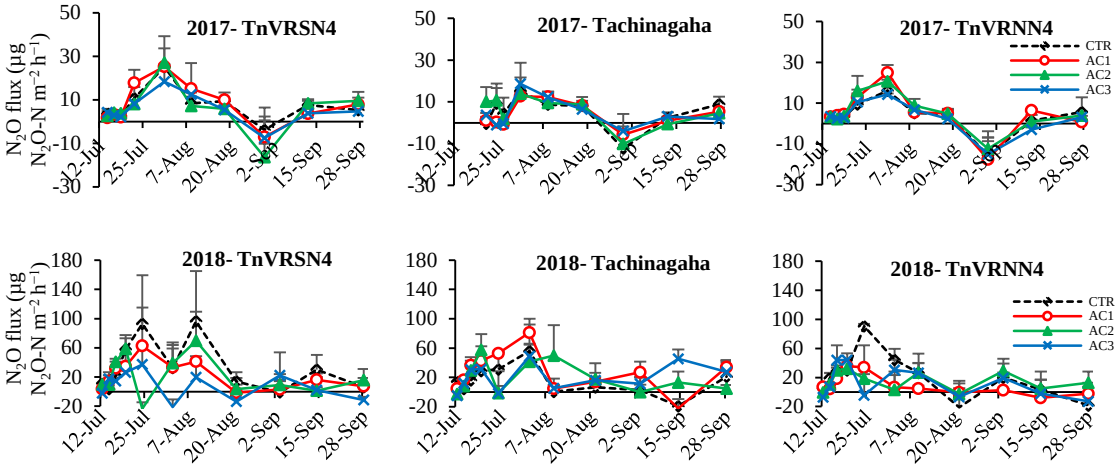

Figure 29. Temporal variation in N_2O fluxes in soils of the soybean genotypes over the crop-growing seasons. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Data points represent mean ± standard error (n = 3).

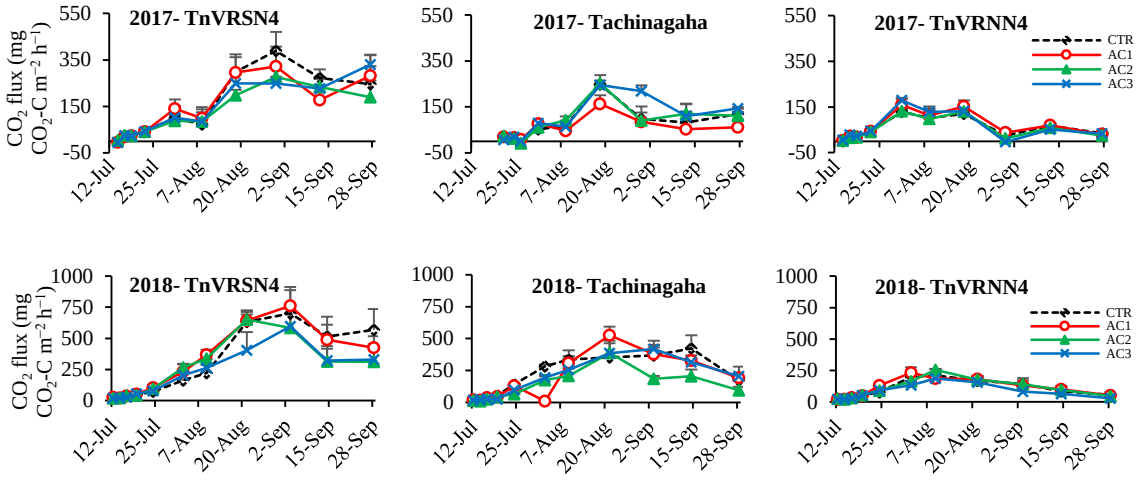

Figure 30. Temporal variation in CO_2 fluxes in soils of the soybean genotypes over the crop-growing seasons. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Data points represent mean ± standard error (n = 3).

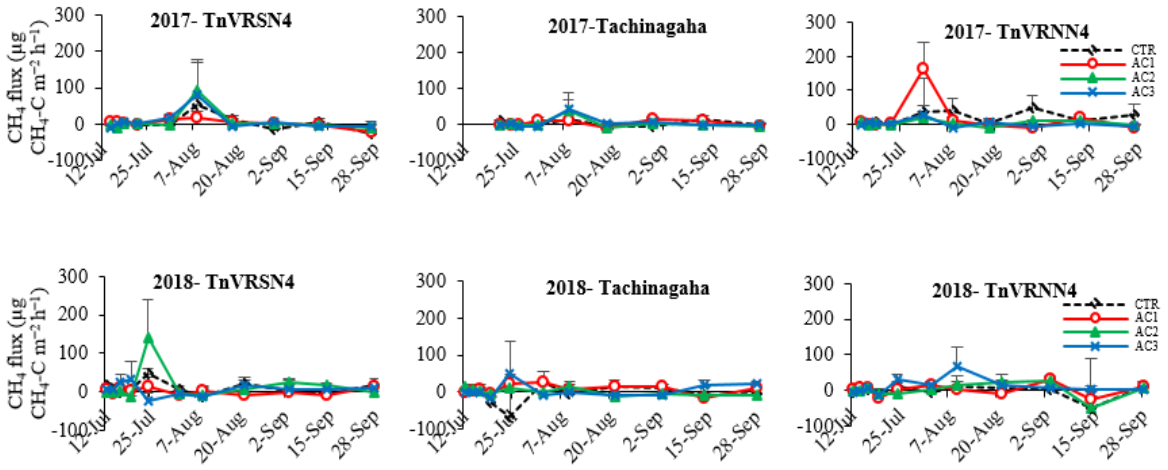

Figure 31. Temporal variation in CH_4 fluxes in soils of the soybean genotypes over the two growing seasons. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Data points represent mean ± standard error (n = 3).

Table 12. Effect of AC on cumulative soil greenhouse gas emissions for the soybean genotypes over the two years of cropping.

Year of cropping (Y)	Soybean Genotype (G)	Treatment (T)	N_2O emissions (kg N ha^{-1})	CO_2 emissions (t C ha^{-1})	CH_4 emissions (kg C ha^{-1})
2017	TnVRSN4	CTR	0.14a	3.77a	0.08a
		AC1	0.15a	3.44a	0.04a
		AC2	0.10a	2.94a	0.19a
		AC3	0.10a	3.24a	0.20a
	Tachinagaha	CTR	0.06a	1.70ab	0.05a
		AC1	0.07a	1.22b	0.08a
		AC2	0.06a	1.85ab	0.04a
		AC3	0.08a	2.22a	0.09a
	TnVRNN4	CTR	0.04a	1.26a	0.40a
		AC1	0.07a	1.46a	0.38a
		AC2	0.08a	1.18a	0.08b
		AC3	0.02a	1.31a	0.03b
2018	TnVRSN4	CTR	0.65a	7.43a	0.16a
		AC1	0.39a	7.85a	-0.02a
		AC2	0.35a	6.62a	0.34a
		AC3	0.12a	5.66a	0.07a
	Tachinagaha	CTR	0.21a	5.32a	-0.04a
		AC1	0.44a	4.99a	0.15a
		AC2	0.37a	3.46a	-0.06a
		AC3	0.42a	4.82a	0.07a
	TnVRNN4	CTR	0.33a	2.35a	-0.08a
		AC1	0.11a	2.48a	0.03a
		AC2	0.26a	2.43a	0.01a
		AC3	0.19a	1.87a	0.29a
ANOVA *P* values		Y	***	***	NS
		G	NS	***	NS
		T	NS	NS	NS
		Y x G	NS	**	NS
		Y x T	NS	NS	NS
		G x T	NS	NS	NS
		Y x G x T	NS	NS	NS

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n = 3). ** indicates $P < 0.01$; *** indicates $P < 0.001$; NS-non-significant.

5.3.3. Nodulation and agronomic traits

The fresh weight and number of nodules were significantly higher in 2018 than in 2017 and were highest in high nodulating genotype and lowest in low nodulating genotype (**Figure 32 and Table 13**). AC generally reduced the number and fresh weight of the root nodules in the high nodulating genotype, but the effects were more prominent in 2018. In the normal nodulating genotype, AC significantly increased the number and fresh weight of root nodules in 2017 but the fresh weight was non-significantly increased during 2018. Furthermore, during 2017, AC significantly increased the number of nodules in the low nodulating genotype but non-significantly increased the fresh weight of the nodules while in 2018, AC significantly reduced the fresh weight of the nodules.

The main effect of genotype was significant for all the agronomic traits but treatment effects were not significant for all the traits except stem diameter and number of branches (**Table 13**). Plant height, number of branches, 100-seed weight and seed weight per plant were all significantly affected by the interactions between year and genotype. The agronomic traits of the three genotypes were better in the high nodulating genotype than in the low nodulating genotype. In 2017, the seed weight per plant for the high and normal nodulating genotypes were not significantly affected by AC but it was significantly reduced in the low nodulating genotype (**Figure 33**). In 2018, AC significantly reduced the seeds per plant and seed weight per plant in the normal nodulating genotype but were not significantly affected in the other genotypes.

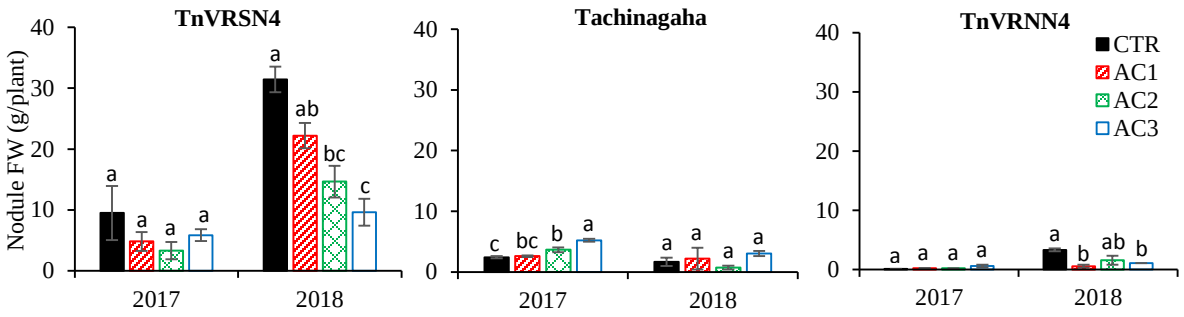

Figure 32. Root nodule fresh weight for the soybean genotypes. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Different letters indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. Data points represent mean ± standard error (n = 3).

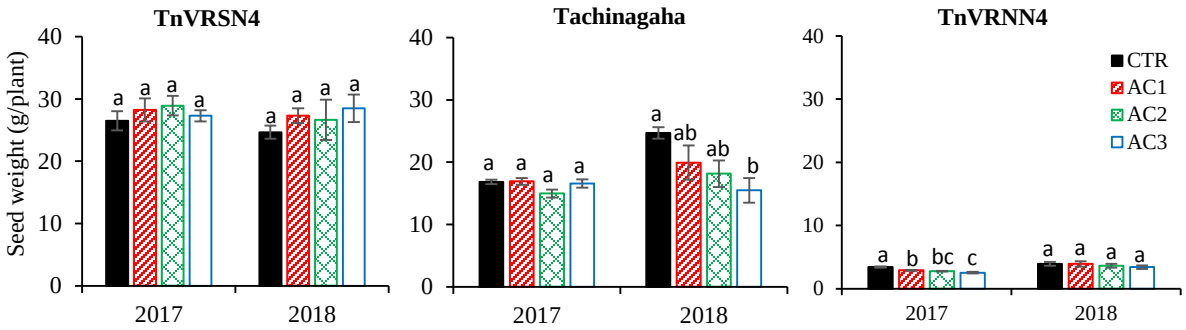

Figure 33. Seed weight per plant of the soybean genotypes. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Different letters indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. Data points represent mean ± standard error (n = 9).

Table 13. Effect of AC on agronomic traits of the soybean genotypes over the two years of cropping.

Year of cropping (Y)	Soybean Genotype (G)	Treatment (T)	Plant height (cm)	Stem diameter (mm)	No. of branches	No. of Nodules/ plant	No. of filled pods	100-seed weight (g)	Seeds/ pod	seeds/ plant
2017	TnVRSN4	CTR	77.6a	7.2a	5.7a	330.3a	34.9a	38.9a	2.0a	69.8a
		AC1	71.8a	7.0a	5.1a	147.7a	40.3a	37.4a	2.0a	83.7a
		AC2	68.1a	6.4a	4.4a	171.0a	40.2a	38.2a	2.2a	77.4a
		AC3	74.2a	6.6a	4.4a	189.3a	35.9a	39.1a	2.1a	72.3a
	Tachinagaha	CTR	71.6a	5.1b	3.3a	63.3b	32.2a	26.8b	2.2a	65.3a
		AC1	70.3a	5.1b	3.6a	76.7ab	27.4a	28.2ab	2.2a	62.1a
		AC2	70.1a	5.4ab	3.0a	90.7a	26.9a	26.7b	2.2a	62.0a
		AC3	65.8a	5.5a	3.2a	95.3a	26.2a	30.5a	2.1a	61.3a
	TnVRNN4	CTR	69.7a	5.6a	4.3a	5.0b	9.4a	18.8a	1.9a	18.7a
		AC1	64.8a	5.4ab	4.3a	6.0b	8.8a	19.0a	1.9a	17.4a
		AC2	69.4a	5.2b	3.1a	9.0ab	9.7a	18.2a	1.8a	17.0a
		AC3	68.1a	5.4ab	4.3a	19.0a	8.6a	17.5a	1.9a	17.0a
2018	TnVRSN4	CTR	77.8a	7.5a	7.4a	1270.0a	43.7a	38.0a	1.7b	65.3a
		AC1	73.8a	7.3a	7.8a	1045.7ab	44.3a	39.0a	2.0a	70.6a
		AC2	81.8a	7.3a	6.8a	894.5ab	40.9a	41.5a	1.8ab	66.1a
		AC3	78.2a	7.1a	7.7a	509.7b	44.6a	39.2a	2.1a	74.1a
	Tachinagaha	CTR	66.6a	6.6a	4.1a	n.d.	39.8a	34.4a	2.1a	71.9a
		AC1	65.4a	5.9a	4.0a	n.d.	36.8a	30.8c	2.0ab	64.0ab
		AC2	62.6a	6.2a	3.8a	n.d.	31.7a	34.3ab	1.9ab	52.6ab
		AC3	62.9a	5.5a	2.9a	n.d.	29.1a	31.5bc	1.7b	48.8b
	TnVRNN4	CTR	63.2a	6.4a	4.6a	n.d.	10.3a	23.1a	1.9a	17.7a
		AC1	61.1a	5.9ab	3.8ab	n.d.	10.4a	22.4a	1.6a	18.4a
		AC2	57.3a	5.4ab	3.8ab	n.d.	10.0a	21.3ab	1.8a	17.7a
		AC3	62.3a	5.0b	2.9b	n.d.	11.1a	17.9b	1.8a	19.4a
ANOVA *P* values		Y	*	***	***	N/A	***	***	***	NS
		G	***	***	***	N/A	***	***	***	***
		T	NS	**	**	N/A	NS	NS	NS	NS
		Y x G	***	NS	***	N/A	NS	**	NS	NS
		Y x T	NS	*	NS	N/A	NS	*	NS	NS
		G x T	NS	NS	NS	N/A	NS	NS	**	*
		Y x G x T	*	NS	NS	N/A	NS	NS	*	NS

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n = 9). For nodules per plant, n = 3. * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$; NS-non-significant; n.d. - not determined. N/A-not applicable due to insufficient data because some nodules had decomposed.

5.3.4. Soil chemical properties

The main effects of year, genotype and treatment were significant for soil pH but not for soil EC (**Table 14**). There was a significant interaction between genotype and treatment for soil pH. AC generally increased soil pH in the normal and low nodulating genotypes in 2017 and 2018 respectively but was not significantly affected in the other genotypes in each year of cropping. Soil EC, was affected by the interaction between year and genotype.

The main effects of genotype and treatment on total N, total C and C/N ratio were significant but the main effect of year was significant for only total N and C/N ratio (**Table 14**). Total N was higher in 2018 than in 2017 season, and for all genotypes, AC generally increased total N, total C and C/N ratio. In both years, the total N was significantly highest in the soils of the high nodulating genotype and lowest in the soils of the other genotypes. The main effect of treatment was significant for soil NO_3^--N but not soil NH_4^+-N. The average soil NO_3^--N for the control treatments was significantly higher than that in AC amended soils.

AC did not significantly affect soil available P, exchangeable K and Ca in the high nodulating genotype during the two years (**Table 14**). In the normal nodulating genotype, AC significantly increased soil available P, exchangeable K and Ca during 2017 while in 2018, AC did not significantly affect soil available P and exchangeable K but significantly increased exchangeable Ca. In the low nodulating genotype, AC non-significantly increased soil available P and exchangeable K but significantly increased exchangeable Ca during 2017 while in 2018, AC significantly increased available P, exchangeable K and Ca. In addition, soil available P was significantly affected by year and genotype interactions and was lowest in the high nodulating genotype. There were also significant year x genotype x treatment interaction effects on soil exchangeable Mg. In 2017, AC addition significantly reduced soil exchangeable Mg in the high nodulating genotype but was not affected in the other genotypes while in 2018, AC significantly increased soil Mg content in the normal nodulating genotype.

Table 14. Effect of AC on soil properties for the soybean genotypes over the two years of cropping.

Year of cropping (Y)	Soybean Genotype (G)	Treatment (T)	pH (H_2O)	EC (mS m^{-1})	Total N	Total C	C/N	NH_4^+-N	NO_3^--N	P	K	Ca	Mg
					g kg^{-1}			mg kg^{-1}					
2017	TnVRSN4	CTR	6.71a	2.34a	0.13a	0.64d	5.0d	1.63a	1.73a	9.5a	36.4a	116.1a	47.8a
		AC1	6.63a	2.76a	0.13a	1.33c	10.2c	1.51a	1.82a	10.0a	20.5a	115.7a	33.8b
		AC2	6.59a	2.17a	0.14a	2.13b	15.7b	1.67a	1.23a	10.0a	22.7a	121.9a	34.4b
		AC3	6.69a	2.59a	0.14a	3.63a	26.2a	1.30a	1.35a	9.0a	33.6a	158.4a	32.9b
	Tachinagaha	CTR	6.74bc	2.52a	0.12b	0.57d	5.0d	1.62a	1.57a	10.9b	24.0b	111.9b	45.9a
		AC1	6.69c	2.59a	0.12b	1.28c	11.0c	2.79a	1.75a	11.5b	38.7ab	117.9b	43.2a
		AC2	6.93ab	2.64a	0.12ab	1.98b	15.9b	1.67a	1.51a	17.6a	31.1ab	147.8a	47.5a
		AC3	6.97a	3.47a	0.13a	3.52a	27.0a	1.18a	1.52a	17.2a	46.2a	159.8a	44.2a
	TnVRNN4	CTR	6.90a	2.48b	0.11a	0.54d	4.9d	2.69a	2.22a	11.4a	40.8a	131.8b	46.1a
		AC1	6.85a	3.89ab	0.12a	1.42c	11.7c	2.33a	1.37a	12.3a	70.8a	126.1b	46.4a
		AC2	6.80a	4.28a	0.12a	2.10b	17.9b	2.06a	1.35a	16.8a	73.2a	154.7ab	50.5a
		AC3	6.96a	4.63a	0.12a	3.50a	28.2a	1.32a	1.31a	19.4a	93.6a	172.6a	45.4a
2018	TnVRSN4	CTR	6.70a	3.31a	0.14a	0.79d	5.8d	2.01a	2.53a	30.0a	27.8a	134.3a	24.0a
		AC1	6.36a	3.39a	0.16a	1.59c	10.2c	1.51a	3.09a	26.9a	22.3a	143.9a	22.2a
		AC2	6.38a	3.22a	0.15a	2.07b	13.8b	2.01a	1.84a	28.4a	34.3a	128.4a	20.8a
		AC3	6.52a	3.37a	0.16a	3.54a	21.9a	2.21a	1.52a	29.2a	30.6a	146.1a	23.2a
	Tachinagaha	CTR	6.55a	3.63a	0.12b	0.66d	5.4d	1.65a	2.02a	37.2a	39.4a	138.4c	28.6bc
		AC1	6.51a	3.68a	0.13ab	1.34c	10.3c	1.98a	1.71a	37.0a	47.7a	148.6bc	27.5c
		AC2	6.97a	3.10a	0.13ab	1.90b	14.4b	2.21a	1.28a	37.4a	61.3a	166.9ab	34.2a
		AC3	6.89a	3.43a	0.14a	3.42a	23.6a	1.76a	1.61a	35.6a	60.8a	172.4a	33.4ab
	TnVRNN4	CTR	6.75b	2.49b	0.12b	0.66d	5.3d	1.94a	2.14a	37.2b	65.6b	146.0b	37.4a
		AC1	6.81ab	2.89b	0.13ab	1.40c	10.8c	1.76a	1.89a	33.1b	77.6ab	147.7b	31.2a
		AC2	6.69b	3.55a	0.13ab	2.16b	16.3b	1.63a	1.40a	36.4b	92.5a	156.6b	28.3a
		AC3	7.08a	2.53b	0.14a	3.45a	24.4a	1.52a	1.54a	56.8a	76.2b	208.6a	37.8a
ANOVA *P* values		Y	*	NS	***	NS	***	NS	NS	***	**	***	***
		G	***	NS	***	*	***	NS	NS	***	***	***	***
		T	*	NS	***	***	***	NS	*	**	***	***	*
		Y x G	NS	***	NS	NS	NS	NS	NS	*	NS	NS	NS
		Y x T	NS	NS	NS	NS	***	NS	NS	NS	NS	NS	NS
		G x T	*	NS	NS	NS	NS	NS	NS	**	*	*	*
		Y x G x T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n = 3). * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$; NS-non-significant.

5.3.5. Seed isoflavone and protein content

Seed isoflavone content was mostly dominated by isoflavone glycosides (daidzin, glycitin and genistin), with daidzin being the most abundant isoflavone (**Table 15**). The concentrations of the individual aglycone isoflavones in seeds were generally low and below 2.5 mg $100g^{-1}$. In 2018, glycitein was not detected in the seeds of the three soybean genotypes. The individual isoflavones and total isoflavones were significantly affected by the interactions between year and genotype. In 2017, the highest content of glycitin and aglycone isoflavones were observed in the seeds of the low nodulating genotype. In the same year, the highest content of daidzin was observed in seeds of the high and normal nodulating genotypes but highest content of genistin was observed in the seeds of the normal nodulating genotype. In 2018, seeds of the high nodulating genotype had the highest daidzin and daidzein content while seeds of the low nodulating genotype had the highest glycitin, genistin and genistein contents. In 2017, the total isoflavone content of the seeds were generally highest in the normal nodulating genotype while in 2018, it was highest in the seeds of the high and low nodulating genotypes. The average protein content for seeds in 2018 was higher than in 2017. For both years, the seed protein content was highest in the high nodulating genotype and lowest in the low nodulating genotype. The main effect of treatment (AC) was not significant for the isoflavone and protein content of soybean seeds.

5.3.6. Root and soil isoflavone content

In both years of cropping, AC did not significantly affect the isoflavones in the roots of all the soybean genotypes and the number and content of isoflavones detected in the roots during 2018 were generally higher than in 2017 (**Figure 34**). The soil isoflavones varied with the year of cropping, genotype and treatment (**Figure 35**). Daidzin and daidzein were the most dominant isoflavones in the soils of the soybean genotypes especially in the low nodulating genotype which had the highest concentration of isoflavones during the two years of cropping. The soils for the normal nodulating genotype had the lowest concentration of isoflavones. AC showed a potential to adsorb isoflavones especially daidzin and daidzein. However, the potential of AC to adsorb glycitin and glycitein was generally low. In 2017, genistin, glycitein and genistein were not detected in any of the treatments for all the soybean genotypes while in 2018, daidzin, genistin and genistein were not detected in the soils of the normal nodulating genotype.

Table 15. Effect of AC on seed isoflavone and protein content of the soybean genotypes over the two years of cropping.

Year of cropping (Y)	Soybean Genotype (G)	Treatment (T)	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein	Total isoflavone	Protein content (%)
			mg $100g^{-1}$							
2017	TnVRSN4	CTR	59.8a	30.7a	26.3a	1.21a	0.86a	1.05a	119.9a	34.4a
		AC1	71.1a	34.1a	27.3a	1.02a	0.81a	0.83a	135.1a	33.5a
		AC2	53.3a	30.2a	23.8a	1.05a	0.77a	0.87a	109.9a	33.8a
		AC3	52.5a	31.1a	19.6a	0.82a	0.73a	0.67a	105.5a	35.0a
	Tachinagaha	CTR	58.9a	36.4a	28.4a	1.27a	1.04a	1.06a	127.1a	31.5a
		AC1	62.5a	36.7a	31.7a	1.01ab	1.04a	0.79b	133.7a	31.2a
		AC2	51.6a	37.0a	25.4a	1.00ab	0.97a	0.85ab	116.8a	31.3a
		AC3	54.6a	37.1a	24.0a	0.91b	0.93a	0.65b	118.2a	32.2a
	TnVRNN4	CTR	46.4a	48.6a	20.6a	1.57a	1.12a	1.89ab	120.2a	21.5ab
		AC1	43.7a	49.0a	20.2a	1.93a	1.31a	2.48a	118.7a	20.9b
		AC2	46.3a	47.4a	19.2a	1.57a	1.16a	1.72b	117.3a	22.4ab
		AC3	48.9a	43.6a	19.0a	1.62a	1.13a	1.75ab	115.9a	23.8a
2018	TnVRSN4	CTR	52.1b	26.3a	13.0b	0.78a	n.d.	0.59a	92.8c	34.9a
		AC1	65.5ab	30.1a	17.9ab	1.19a	n.d.	0.90a	115.6ab	34.9a
		AC2	77.0a	28.2a	21.4a	1.14a	n.d.	0.92a	128.6a	33.7a
		AC3	54.4ab	22.9a	14.1b	0.91a	n.d.	0.79a	93.2c	34.2a
	Tachinagaha	CTR	39.8a	21.7a	18.9a	0.96a	n.d.	0.72a	82.2a	33.1a
		AC1	37.5a	19.1a	16.2a	0.87a	n.d.	0.56a	74.1a	32.5a
		AC2	44.9a	26.7a	18.0a	0.78a	n.d.	0.58a	90.9a	32.2a
		AC3	37.4a	22.7a	14.9a	0.94a	n.d.	0.75a	76.6a	32.9a
	TnVRNN4	CTR	63.9a	34.6a	23.6a	0.99a	n.d.	1.07a	124.2a	23.8a
		AC1	46.1a	34.3a	18.9a	0.98a	n.d.	1.06a	101.4a	24.5a
		AC2	50.0a	36.2a	21.5a	0.96a	n.d.	0.95a	109.6a	23.5a
		AC3	40.0a	38.2a	14.6a	0.92a	n.d.	0.96a	94.6a	23.1a
ANOVA *P* values		Y	NS	***	***	***	N/A	***	***	**
		G	***	***	NS	***	N/A	***	NS	***
		T	NS	NS	NS	NS	N/A	NS	NS	NS
		Y x G	**	**	**	**	N/A	***	**	NS
		Y x T	NS	NS	NS	NS	N/A	NS	NS	NS
		G x T	NS	NS	NS	NS	N/A	NS	NS	NS
		Y x G x T	NS	NS	NS	NS	N/A	NS	NS	NS

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n = 3). * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$; NS-non-significant; n.d.-not detected; N/A.-not applicable.

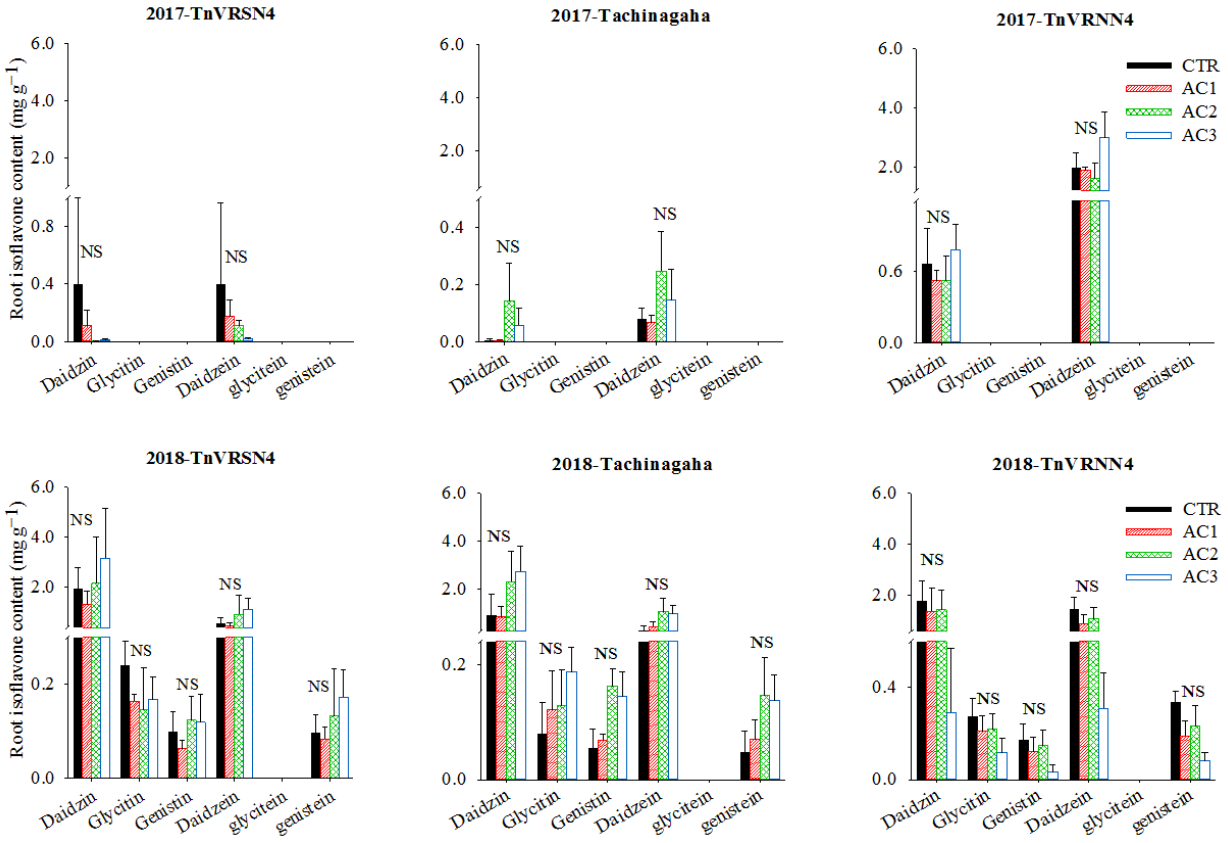

Figure 34. Effect of AC on the root isoflavone content of the soybean genotypes over the two years of cropping. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. NS-non-significant at $P < 0.05$. Values are mean ± standard error (n = 3).

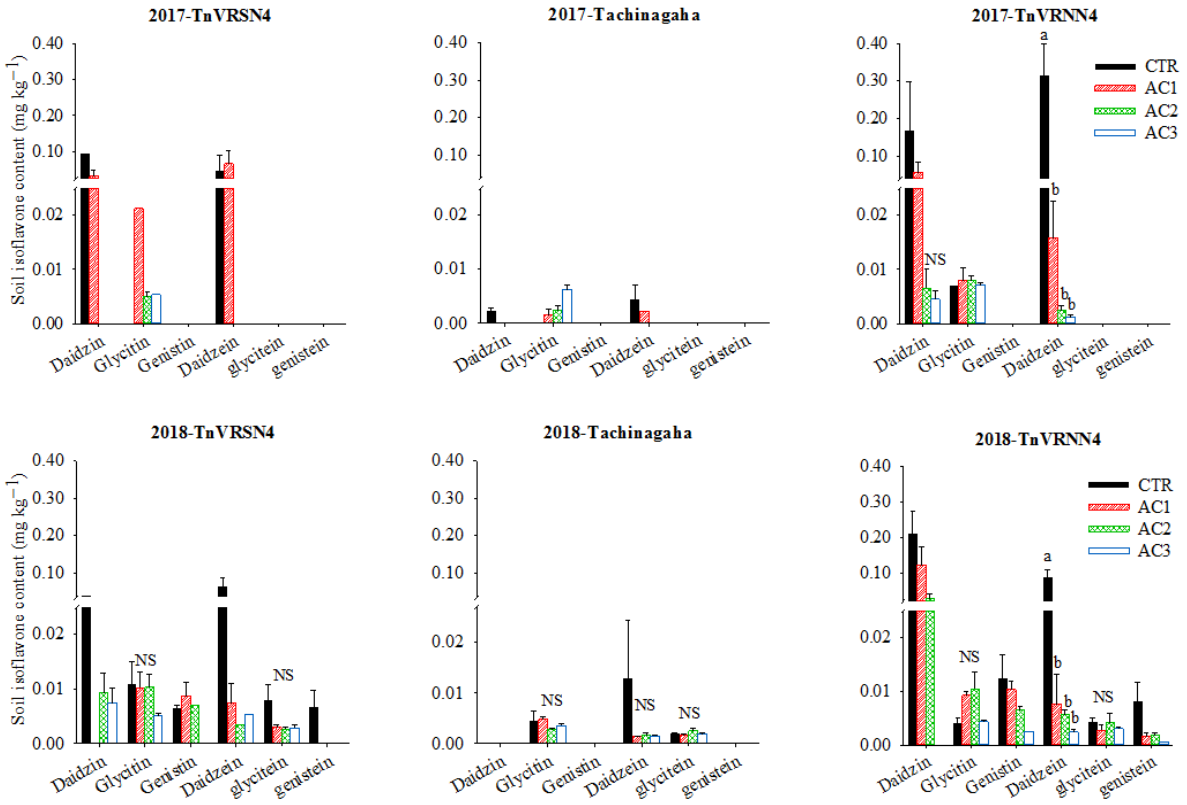

Figure 35. Effect of AC on the isoflavone content in soils of the soybean genotypes. TnVRSN4, Tachinagaha and TnVRNN4 have high, normal and low nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. For each isoflavone, different letters indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test. NS-non-significant. Values are mean ± standard error (n = 3). Bars without error bars represents data obtained from only one replication. Missing data points on the bar graphs show that the isoflavones were not detected in the treatments.

5.4. Discussion

5.4.1. Greenhouse gas emissions

Soil N_2O emissions from the high nodulating genotype were not significantly different from the other genotypes, indicating that the nodules were not a significant source of N_2O emissions during the growing stages of the soybeans. Although there are no studies evaluating GHG emissions from the soybean genotypes used in the present study, an experiment conducted on an upland field (Andisols), with three soybean genotypes of varying nodulation i.e. normal nodulating cv. "Enrei", super-nodulating cv. "Sakukei 4" and non-nodulating cv. "En1282" showed that N_2O emissions were significantly highest in the super-nodulating cultivar and lowest in the non-nodulating cultivar especially during the full bloom and full pod stages (Kim et al., 2005) which suggested that nodules contribute significantly to N_2O emissions. Another study conducted on gray lowland soils showed that the root nodules of the normal-nodulating cv. "Enrei" had significantly higher N_2O emissions compared to the non-nodulating line "En1282" during the late growing period (Inaba et al., 2009). In the present study, the non-significantly higher N_2O emissions in soils of the high nodulating genotype as compared to the other genotypes, especially in the absence of AC could be attributed to the fact that the sandy soils used are poorly drained and have a low soil organic matter content, hence providing unsuitable conditions for microbial activity. Although the main effects were not significant, there could be a possibility of AC to reduce N_2O emissions as shown by the reducing tendency of these emissions in treatments with AC especially where the nodulation of the high nodulating genotype significantly increased in 2018 (**Figure 32, Table 13**). Moreover, Flynn et al. (2014) reported that the effect of soybean nodule activity on soil N_2O emissions is not limited to the time when soybeans are grown, but also occurs for crops grown in subsequent years following the soybean crop.

Although it may be possible for rhizobia in root nodules to denitrify and produce N_2O (O'Hara and Daniel, 1985), nitrification could be the dominant process for N_2O emissions considering the soil conditions such as moisture content of 10–40 % WFPS (**Figure 28**) and the fact that the sandy soil used in this study has low water holding capacity and high aeration which favors nitrification. Bateman and Baggs (2005) also reported that nitrification is the main source of N_2O in soils at 35–60% WFPS. Nitrification process is further promoted by AC and this could be attributed to the following reasons: (1) the adsorption of phenolic compounds and other secondary metabolites that have inhibitory effects on autotrophic nitrifying bacteria (Paavolainen

et al., 1998; DeLuca et al., 2002); (2) the adsorption of various soluble C substances which act as food source supply for microorganisms thereby facilitating the aggregation of nitrifiers around AC particles which could then produce a biofilm structure essential for nitrification (Berglund et al., 2004).

The reasons for the negative fluxes and different N_2O flux patterns were not fully understood in this study. Although Cowan et al. (2014) attributed the negative N_2O fluxes (N_2O uptake) in soils to non-biological factors such as moisture and high humidity as well as to artefact of measurement methodologies which results from instrumental uncertainty, the negative N_2O fluxes in this study could be due to biological factors like microbial N_2O uptake. N_2O uptake by soybean nodules has been reported and attributed to respiratory N_2O reductase thereby lessening N_2O emissions to the atmosphere from soybean fields (Sameshima-Saito et al., 2006). The effect of soybean growth on N_2O emission from soil varies with plant growth stages as available N for N_2O production is mainly from fertilizer N and organic mineralization during the early growth of soybean plants and later controlled by the quantity and quality of root exudates in the late season of soybean growth (Yang and Cai, 2006). Therefore, in the present study, the changes in N_2O emission patterns could be attributed to N fertilizer but later on turned to exudates and/or from nodules from mid to late stages of crop growth. The significantly higher N_2O emissions in 2018 could mostly be accounted for by the residual N (N from chemical fertilizer and root residues) that remained from the previous cropping and to a less extent the increase in nodulation especially in the high nodulating genotype.

CO_2 production in soil is mainly from respiration of plant roots and microorganisms (Smith et al., 2018). The significantly higher CO_2 emissions in the high nodulating genotype can mainly be attributed to microbial respiration. Although microbial growth was not measured in the present study, it can be speculated that since the high nodulating genotype had a significantly more number of nodules, it is possible that it had more microorganisms in the rhizosphere which through respiration, would increase the CO_2 emissions. This could be evidenced by the seasonal variation in CO_2 fluxes for the two years (**Figure 30**); the low CO_2 fluxes at the early growth stage could be due to the low microbial respiration rates which increased during the season and then declined during the late stages of crop growth. The significantly higher CO_2 emissions in 2018 as compared to 2017 could be attributed to the higher microbial activity, evidenced by the higher number of nodules in 2018 (**Table 13**). Since CO_2 fluxes are controlled by biological factors, the negative

CO_2 fluxes obtained on some sampling dates could be explained by other factors. Cueva et al. (2019) reported that soil CO_2 fluxes are bidirectional depending on the predominance of various biological and non-biological components in soils; with soils acting as a net sink of CO_2 when non-biological components outcompete the biological ones.

CH_4 producing bacteria are extremely sensitive to oxygen, and CH_4 production in soil only occurs under anaerobic highly reducing conditions in the absence of other potential electron acceptors like nitrate and sulphate (Topp and Pattey, 1997). Therefore, aerated soils are a sink for microbial methane through microbial oxidation (Smith et al., 2018) and since this experiment was conducted in sandy soils which are known for their high aeration, this explains the negligible effect of year of cropping, genotype and treatment on CH_4 emissions.

5.4.2. Nodulation and Agronomic traits

The present study showed that the effects of AC on nodulation depends mostly on the soybean genotype and the year of cropping and this could be explained by the seasonal changes in soil chemical and biological properties which might affect nodulation. The decrease in nodulation following AC amendment could be attributed to the reduction in the isoflavone concentration (daidzein) by AC. This is in conformity with Wurst and Van Beersum (2009) who reported that AC reduced nodulation and growth of *Lotus corniculatus* L. and attributed it to the deterred communication between the plant and rhizobia through altering signaling compounds in soil. In addition, Vantsis and Bond (1950) reported that the nodulation, dry weight and N content of inoculated maple field peas reduced with increasing levels of AC and attributed it to the charcoal adsorbing root exudates (signaling compounds). Similarly, this study showed the potential of AC to adsorb these compounds especially daidzein which is the most important signaling compound aiding nodulation in soybeans (**Figure 35**).

The significant increase in the number of nodules in the normal and low nodulating genotypes following AC application in 2017 could not be clearly explained. However, the stimulation of nodule formation can be attributed to the ability of charcoal to adsorb nodulation inhibitory compounds exuded by the plant roots although the effects may vary with the concentration of these compounds (Turner, 1955). One of the limitations of using AC is that it does not only adsorb allelopathic compounds but also signaling compounds like isoflavones responsible for the interaction between plants and microorganisms (Weißhuhn and Prati, 2009; Wurst and Van Beersum, 2009). The significant increase in the nodules per plant in the low

nodulating genotype in 2017 despite the fact that AC decreased the concentration of daidzein could imply that since this soybean genotype is genetically non-nodulating, we may not be able to use changes in soil isoflavone concentration as a factor to explain the effects of AC on nodulation in this genotype. The increase in nodulation of the soybean genotypes in 2018 could be accounted for by the increase in soil available P (**Table 14**). This is further supported by Kakiuchi and Kamiji (2015) who reported an increase in number of root nodules at higher P fertilization. Furthermore, even in the absence of a legume in their cropping history, sandy soils could have some low populations of indigenous rhizobia despite their poor drainage and low organic matter content (Zengeni et al., 2006), and can multiply in soil upon introduction of a legume crop. Senescing root nodules release rhizobium bacteroids back in the soil resulting in an increase in rhizobial population (Gresshoff and Rolfe, 1978), which subsequently increases nodulation in the succeeding soybean crop as observed in the present study **(Figure 32 and Table 13)**.

AC may increase or decrease plant growth and development (Lau et al., 2008; Jakob et al., 2012; Yuan et al., 2014; Oleszczuk et al., 2017) but may also have no effects on plant growth (Chen et al., 2010). In the present study, the interaction between genotype and treatment for the seeds per pod, seeds per plant and seed weight per plant implies that the effect of AC on these parameters varies with the genetic traits of the soybean genotypes which may influence root exudation as well as plant nutrient uptake. The significant year x genotype effects especially on the seed weight per plant could be attributed to the changes in the soil nutrient and microbial status which can affect plant nutrient availability. Furthermore, the changes in the effects of AC on crop yield could be associated with its role on soil physical, chemical and biological properties. Although AC generally increased the soil chemical properties, this was not reflected in the yield of the plants after harvest implying that there could be other factors associated with the AC-related effects on plant growth. For instance, in tomatoes grown in hydroponic solutions under greenhouse conditions, Yu et al. (1993) observed an increase in plant dry weight and fruit yield in AC treatments and attributed it to the ability of the charcoal to adsorb phytotoxic organic substances in the residual nutrient solutions.

The high agronomic performance of the high nodulating genotype was attributed to the presence of a large number of root nodules which could sustain the crop in addition to the chemical fertilizer applied at the start of each crop season thereby increasing N availability for plant growth. Similarly, Takahashi et al. (2003) reported that the super-nodulating genotype “En-b0-1-2” had

higher yield compared to other genotypes and attributed it to its unique growth characteristics and high seed weight especially in fields with low available N.

Interestingly, AC significantly reduced the nodules of the high nodulating genotype and seed yield was expected to follow the same trend but this did not occur. This implied that the BNF process was still efficient even at lower levels of nodulation that resulted from AC application and this means that under the same crop growth conditions, there might be a minimum threshold for the number of root nodules needed to have a significant effect on crop yield but more studies are still required to clarify this phenomenon. Moreover, since nodulation requires high energy demands in form of ATP (Ferguson, 2013), excessive nodulation may not be beneficial to these plants and may not necessarily increase crop yield. Furthermore, it might be that the positive effects of AC on soil chemical properties and the adsorption as well as the removal of chemical compounds that could be toxic to plants facilitated higher crop growth to compensate for the low nodulation of soybeans in AC amended soils. These results could not thoroughly explain the negative effects of AC on crop growth and yield. However, the reduction in plant growth following AC addition might be caused by the reduction in the positive plant-soil interactions such as symbiosis and pathogen defense (Nolan et al., 2015). Furthermore, it could be explained by the ability of AC to directly restrict nutrient availability (Jeffery et al., 2012) as well as limiting available organic nutrients through adsorption or indirectly via the effects of microorganisms (Wallstedt et al., 2002). Data for nutrient uptake might have partly explained this but unfortunately, the pot experiment design in this study could not favor destructive sampling for plant nutrient analysis due to the limitations in the number of plants. Therefore, further studies should be carried out to evaluate the effects of AC on nutrient uptake in soybeans.

5.4.3. Soil chemical properties

Most of the functions of AC amendment have been largely related to adsorption of chemical compounds but its effects in soil may change the dynamics of soil chemical properties which can affect plant growth and development. Studies have shown no effect of AC on soil pH, total N and available P (Berglund et al., 2004; DeLuca et al., 2006; Yuan et al., 2014). AC can also increase soil pH and K, but decrease Ca and Mg (Lau et al., 2008). In the present study, significant interactions between the genotype and treatment for soil pH, available P, exchangeable K, Ca and Mg showed that the effects of AC on soil properties depend on the soybean variety planted at a particular time. In studies using biochar amendments in soil, the increase in soil pH following

biochar application has been attributed to the alkalinity and base cations in biochar (Steiner et al., 2007; Hailegnaw et al., 2019). Since the AC had high amounts of base cations and a high pH (**Table 11**), the increase in soil pH can also be attributed to the increase in soil exchangeable Ca content. These results show that AC has a potential to increase soil pH in sandy soils under soybean production but this might vary with the soybean variety grown.

The tendency of AC to reduce soil NO_3^--N content could be attributed to microbial immobilization and denitrification (Ippolito et al., 2016) or to the adsorption of NO_3^--N on the surfaces of AC. Similarly, Lawrinenko (2014) reported the adsorption of NO_3^--N on biochar and attributed it to the anion exchange capacity of biochar. Contrary, Lau et al. (2008) showed the potential of AC to increase NO_3^--N and decrease NH_4^+-N concentrations of leachates from soil. This implies that the effects of AC on soil inorganic N are not similar and may vary with other factors such as plants grown, soil types and their fertility management.

A portion of the N fixed by leguminous plants remains in soil as root residues and nodules or returned as litter fall (Cooper and Scherer, 2012). Therefore, since the roots and nodules were not removed from the pots, it is possible that as these residues decomposed, they released the N fixed in the previous season hence increasing the total N at the end of the 2018 crop season. This, in addition to the residual N from chemical fertilizer explains the higher total N in 2018 than 2017 crop season and it was further evidenced by the fact that the total N was higher in soils where the high nodulating genotype was planted as compared to the other genotypes. The significant increase in available P in 2018 can also be explained by the residual P that remained from the previous cropping in form of chemical fertilizers, and from decomposition of root nodules which are well known for their capacity to act as strong sinks for P (Cooper and Scherer, 2012). In addition, the authors reported the role of P in the energy metabolism of plants since it plays an important role in N_2 fixation due to the high ATP demand from the nitrogenase reaction. Ferguson (2013) highlighted that the super-nodulating soybean mutants invest too much energy into forming nodule structures. Therefore, the lower soil P in the high nodulating genotype compared to the other genotypes at the end of each crop season might be explained by the high P uptake by the plants for use in root nodule formation.

Varietal changes in soil properties may also be attributed to the differences in plant growth which could affect root exudation and plant nutrient uptake. For instance, the exchangeable K and Ca in soil after harvesting the soybeans at the end of each crop season was generally highest in the

low nodulating genotype due to the poor plant growth. K is one of the nutrients required by plants in the largest amount after N and when it is abundant in soil, 'luxury consumption' by the plant occurs (Hawkesford et al., 2012). Therefore, the poor growth of low nodulating plants due to N deficiency might have resulted in low K uptake hence leaving higher exchangeable K in soil. Contrary, the higher growth of the high nodulating plants might have resulted in increased K uptake hence reducing soil exchangeable K. The increase in soil nutrients after addition of AC can be attributed to its ability to release the inherent nutrients in the soil.

5.4.4. Seed Isoflavone and protein content

Soybean seeds are important sources of proteins, oil and isoflavones which are essential for human consumption. N is an important element required for the synthesis of proteins, nucleic acids, chlorophyll, coenzymes, phytohormones and secondary metabolites (Hawkesford et al., 2012). Therefore, any genetic or environmental manipulation that affects plant N uptake may affect isoflavone and protein content of seeds.

Isoflavones are important secondary metabolites that accumulate in soybean seeds during soybean development and have attracted much attention in prevention and treatment of cancer and cardiovascular diseases (Coward et al., 1993; Munro et al., 2003). The individual isoflavone content of soybeans vary with the soybean varieties, genotypes, growing conditions and year of cropping among others (Lee et al., 2003; Teekachunhatean et al., 2013). This was also observed in the present study where there were significant year x genotype interactions for isoflavone content of seeds (**Table 15**). The significant effect of genotype on isoflavones showed that genetic factor plays an important role in the accumulation of isoflavones in soybean seeds (Zhang et al., 2014). The variations in isoflavone content of seeds among the years could be attributed to the changes in environmental conditions such as soil moisture and high temperature during the crop growth stages (Tsukamoto et al., 1995; Lozovaya et al., 2005). Although mature seeds may constitute the greatest concentration of isoflavones in soybeans plants, they can vary significantly even under identical conditions (Dhaubhadel et al., 2003) and the variation could possibly be higher when soybean plants are grown in different soil amendments.

Amendment of AC did not generally alter the isoflavone and protein content of soybean seeds which suggested that there was no change in the quality of the seeds obtained. However, AC could have a potential to increase, decrease or have no effect on the individual and total isoflavone content in seeds but this could vary with the application rate. Therefore, the potential effect of AC

as a soil amendment on seed isoflavone content should not be ignored and further studies using AC as well as other pyrogenic carbonaceous soil amendments such as biochar should be further evaluated to clarify this. Since the information regarding the effect of AC on seed isoflavones is still scarce, it is difficult to compare the results of this study with those from other researchers. However, in a study that involved use of organic amendments, Taie et al. (2008) reported that organic fertilization with different levels of compost did not have any effect on daidzein and genistein in soybean seeds.

The seeds of all the genotypes were mostly dominated by isoflavone glycosides especially daidzin and a relatively lower concentration of aglycones. This corroborates other findings which reported that daidzin and genistin were predominant isoflavones in soybean seeds while their corresponding aglycones forms were undetectable or detected in negligible amounts (Tsukamoto et al., 1995; Teekachunhatean et al., 2013). Glycitein was not detected in any of the three genotypes during the second year of cropping and this observation is also similar to other studies that did not detect glycitein in soybean seeds due to its naturally low concentration in the seeds (Mujić et al., 2011; Šertovic et al., 2012; Teekachunhatean et al., 2013; Wang et al., 2016). The total isoflavone content of the soybean genotypes in this study was within the range obtained from soybean cultivars in different locations. For instance, seed isoflavone content of soybean cultivars ranged from 71.2 to 133.8 mg $100g^{-1}$ (Šertovic et al., 2012) and 80.7 to 213.6 mg $100g^{-1}$ (Mujić et al., 2011) in Croatia while soybean cultivars grown in Brazil had isoflavone contents ranging from 12 to 461 mg $100g^{-1}$ (Carrão-Panizzi et al., 2009). Furthermore, Sakthivelu et al. (2008) also reported isoflavone content ranging from 55.8 to 104.9 mg $100g^{-1}$ in Indian soybean cultivars and 62.7 to 171.7 mg $100g^{-1}$ in Bulgarian cultivars. Nodulation did not have any significant effects on total isoflavone content among the three genotypes since there was no clear trend like the one observed in protein content. This could imply that changes in nodulation either due to genotypic difference or due to changes in sandy soil status by AC amendment might not have any significant influence on soybean seed total isoflavone content under conditions of this study.

The differences in protein content among the soybean genotypes were attributed to the variations in N availability due to the different nodulation capacities of the soybeans. Furthermore, the changes in soybean protein content between the years could be attributed to the changes in environmental factors such as temperature, soil moisture status and photoperiod (Piper and Boote,

1999; Bellaloui et al., 2010) which can affect N uptake. Seed protein content was influenced more by genotypic factors rather than soil factors (AC amendments).

5.4.5. Isoflavone content in roots and soil

Roots exude picomolar concentrations of isoflavones including daidzein and genistein which are responsible for inducing signals to rhizobia, and glycitein which is an inactive form of isoflavones in the soil (Pueppke et al., 1998). However, the isoflavones in root exudates may vary according to the cultivation system, growth conditions, plant species, sampling and extraction procedures (Cesco et al., 2010) which makes it difficult to compare data from different studies. Isoflavones can be exuded from roots through root injury, senescence and decomposition, as well as exudation by active roots (Shaw et al., 2006). Root exudation involves two major isoflavone secretion pathways; (1) The ATP-dependent active transport of isoflavone aglycones especially daidzein and genistein (Sugiyama et al., 2007; Sugiyama and Yazaki, 2014), and (2) the secretion of isoflavone glucosides into the apoplast, followed by hydrolysis of glucosides with isoflavone conjugate-hydrolyzing β-glucosidase (ICHG) (Suzuki et al., 2006).

In the present study, daidzein and its glucoside conjugate (daidzin) were the most dominant isoflavones in the roots and soils of the soybean genotypes. This agrees with previous studies that showed that daidzein is the most dominant isoflavone in roots and root exudates in the absence or presence of rhizobia (Suzuki et al., 2006; Cesco et al., 2010; Sugiyama et al., 2017). The differences in the isoflavone content among the genotypes demonstrated that there are variations in root exudation abilities among the cultivars of the same species. In this study, the variation in isoflavone content among the genotypes is mostly attributed to the genotypic effects due to their nodulation capacities which might indirectly influence the nutritional status of the plants. The high concentration of isoflavones in soils of the low nodulating genotype could be attributed to N deficiency due to its low nodulation ability which implied that the inorganic fertilizer supplied was not enough to sustain proper plant growth. A number of stress factors such as P and N deficiency are associated with up-regulation of the phenylpropanoid metabolism which increases the biosynthesis and release of flavonoids into the rhizosphere (Cho and Harper, 1991; Cesco et al., 2010). This could also explain the high concentration of daidzein and daidzin in the roots of the low nodulating genotype in 2017 (**Figure 34**). However, since the diversity and concentration of root isoflavones generally increased in 2018 for all genotypes, this means that N deficiency might not always result in high concentration of isoflavones; other factors like climate variation and

changes in soil status could influence the isoflavones content in roots. Although the high isoflavone content in soil has been attributed to the N deficiency, further research should aim at quantifying the isoflavones in the rhizosphere of this genotype in conditions where N is not limiting.

The adsorption abilities of AC for daidzein and genistein and their glucoside conjugates was attributed to its high surface area and porous structures (Miao et al., 2013; Nanda et al., 2016; Zhang et al., 2019b). AC did not adsorb glycitin and glycitein and this could be attributed to the very low levels of these compounds in the soil which may not be clearly distinguished in the HPLC system during analysis. The non-significant effects of AC on root isoflavone content suggest that the effect of AC on isoflavone dynamics could be more prominent in soil than in plant roots.

5.5. Conclusion

The effects of AC in soil are beyond the adsorption of chemical compounds in the rhizosphere. This study highlights the potential of AC to somewhat reduce N_2O emissions but not CO_2 and CH_4 emissions. The N_2O reduction tendency by AC in the high nodulating genotype (TnVRSN4) especially in the second year of cropping could be indirect through the reduction in the number of root nodules. Seed yield and CO_2 emissions were significantly highest in the high nodulating genotype and lowest in the low nodulating genotype (TnVRNN4) which further clarified the role of genotype on GHG emissions. The effects of AC on seed yield depended on the genotype where it was observed that seed yield was not affected by AC in the high nodulating genotype but either reduced or did not affect seed yield in the other genotypes depending on the year of cropping. Furthermore, this study showed that AC generally increases soil total N, total C and C/N ratio but its effect on soil pH, available P and exchangeable cations may vary with the soybean genotype grown. Although AC did not significantly affect total isoflavone and protein content of seeds, it reduced the concentration of daidzein and daidzin in soil and this was more evident in the low nodulating genotype. This implies that the effects of AC in soil under a particular soybean genotype may not have significant effects on the quality of the seeds under conditions of this study. Although the low nodulating genotype had lower GHG emissions, its yield potential was significantly very low at the 45 kg N ha^{-1} applied in soil. Therefore, further studies should be done to compare the amounts of GHG emitted if more chemical N fertilizer is added to soils of the low nodulating genotype to give similar yield as that in the high nodulating genotype. The results from this study also suggest that the high nodulating genotype can perform better in marginalized sandy

soils with a low nutrient status but it is necessary to further evaluate and compare these three genotypes in terms of productivity and GHG emissions under field conditions.

CHAPTER SIX

Effect of activated carbon on N_2O and CO_2 emissions from decomposing root nodules of soybean genotypes with varying nodulation capacities under sandy soil conditions

6.1. Introduction

Agriculture is among the major sources of GHG emissions especially N_2O, CO_2 and CH_4 (IPCC, 2013). N_2O is mainly emitted from upland fields following addition of different N sources such as chemical N fertilizers, manures, legume Biological N fixation (BNF), and mineralization of crop residues especially those from N rich sources such as vegetables and legumes in soil (Mosier et al., 1998; Velthof et al., 2002). Soybean (*Glycine max* (L.) Merr.) is a major leguminous crop grown mainly for its health benefits, with a high N fixation potential. Although BNF is considered a source of N_2O emission, its contribution is relatively low and is considered an effective and environmental-friendly alternative to N fertilizer. However, BNF can be an indirect source of N_2O emission through the decomposition of plant parts especially the root nodules during the late stages of crop growth (Yang and Cai, 2006; Shah, 2014). In chapter five, it was observed that nodules were not a significant source of N_2O emission during the crop growing seasons of the three soybean genotypes with different nodulation capacities under sandy soil conditions.

Compared to the above ground soybean plant parts (leaves, stems and branches), root nodule decomposition is an important indirect source of post-harvest N_2O emissions from soybean cropping systems especially when the soil conditions are favorable for N_2O emission (Uchida and Akiyama, 2013; Sanchez and Minamisawa, 2019). The organic N inside the root nodules is mineralized to NH_4^+ and then followed by nitrification to produce NO_3^- which is denitrified to produce N_2O that is either emitted to the atmosphere or further reduced to N_2 gas by N_2O reductase (N_2OR), encoded by the *nosZ* gene (Inaba et al., 2009, 2012; Akiyama et al., 2016). Moreover, Marinho et al. (2006) reported that the maximum N_2O flux after harvesting soybeans was 12–16 times more than that of the growing season. This provides evidence that mitigation of N_2O emissions from soybean fields should mainly focus more on the post-harvest rather than the crop growing seasons.

The enhancement of microbial N_2OR activity either at the level of genes or proteins through plant breeding has been suggested as an N_2O mitigation option (Richardson et al., 2009). One of the recently proposed methods for mitigating post-harvest N_2O emissions is the inoculation

of N_2O reducing bacteria on soybean roots with *nosZ*+ and *nosZ*++ strains (mutants with increased N_2OR activity) of *Bradyrhizobia* (Inaba et al., 2012; Itakura et al., 2013; Akiyama et al., 2016). Although this approach of mitigation of post-harvest N_2O emissions from soybean plants could have a high potential as a future mitigation option, its use has shortcomings in terms of requiring time, cost and technical skills in generating mutants and this necessitates further research on more efficient and cost-effective methods.

Pyrogenic carbonaceous soil amendments such as biochar have been suggested to reduce N_2O emissions through a number of mechanisms including the increase in the abundance of *nosZ* genes in microbial communities (Cayuela et al., 2013, 2014) and reduction of available N through immobilization and N fixation on the surfaces as well as improving other soil chemical properties such as pH (Clough et al., 2013). In chapter five, it was observed that activated carbon (AC), which is another type of pyrogenic carbonaceous soil amendment tended to reduce N_2O emissions from the soils of the high nodulating genotype (TnVRSN4) during the crop growing season. However, there is no information on the effect of AC on N_2O and CO_2 emissions from decomposing root nodules of soybean genotypes with varying nodulation capacities under sandy soil conditions.

An incubation experiment was conducted to; (i) quantify N_2O and CO_2 emissions from decomposing root nodule residues of soybean genotypes with varying nodulation capacities; (ii) assess the effect of AC on N_2O and CO_2 emissions from decomposing root nodules; (iii) assess the effects of root nodule incorporation on soil chemical properties. The underlying hypotheses were: (i) N_2O and CO_2 emissions from nodules of the high nodulating genotype are higher than those of the normal nodulating genotype; (ii) AC reduces N_2O but not CO_2 emissions; (iii) Root nodule incorporation improves soil chemical properties.

6.2. Materials and methods

6.2.1. Establishment of the experiment

On 28th June 2019, seeds of all the three soybean genotypes used in the previous experiment (Chapter five) i.e. TnVRSN4, Tachinagaha and TnVRNN4 with high, normal and low nodulation capacities respectively were sown in seedling trays. One seedling (V1 stage) of each genotype was transplanted on 10th July 2019 in the same pots used in the 2017 and 2018 crop seasons (Chapter five). The soybean was transplanted in pots where the root residues had not been previously removed during the end of each of the previous years of cropping. Before transplanting, chemical fertilizers were applied at rates equivalent to 45 kg N ha^{-1}, 150 kg P ha^{-1}, 150 kg K ha^{-1} as NPK,

TSP, and K_2SO_4, with dolomite at 500 kg ha^{-1}. The fertilizer and AC were thoroughly mixed in soil at 10 cm depth (7.2 kg air dry basis). Similar to the 2018 season, activated carbon (AC) was also not applied in 2019 because it had been applied once at the start of the 2017 season at rates equivalent to 0, 2.4, 4.8, and 9.6 t ha^{-1} (0, 12, 24, and 48 g per pot) and each soybean genotype had been continuously grown in the respective pots as explained in section 5.2.1. Each of the soybean genotypes was grown at the four levels of AC in three replications.

On 3rd September 2019, the plants were uprooted and the roots were thoroughly washed to remove the soil particles adhering to the roots. At that time, the plants had reached the reproductive stage and were between the R5 (beginning seed) and R6 (full seed) stages. The root nodules were removed from the roots and later counted and weighed. Some of the nodules were oven dried at 72°C for 3 days and later ground by use of a mortar and pestle to obtain the powder which was taken for total C and total N analysis using the C/N corder (JM1000CN, J-SCIENCE LAB, Kyoto, Japan); the other nodules were then preserved in air sealed glass jars and stored and at 4°C before being used for the incubation experiment. The root nodules from each treatment were stored separately. Soil samples were also collected at 0–10 cm soil depth on the same day from each treatment and later air-dried and then passed through a 2 mm sieve to remove plant debris. There were very scarce or no nodules found on the roots of the low nodulating genotype (TnVRNN4). Therefore, only the high and normal nodulating genotypes (TnVRSN4 and Tachinagaha respectively) were selected for this incubation experiment.

After air-drying, the soil from the three replications of each treatment was thoroughly mixed and a sub-sample (about 500 g air-dried soil) was taken for use in the incubation experiment. The soil chemical properties are shown in **Table 16**; soil inorganic-N analysis was performed on fresh soil. Before the start of the incubation experiment, 150 g of air-dried soil from each of the corresponding treatments of the two genotypes (TnVRSN4 and Tachinagaha) was loaded in 200 ml glass jars. Therefore, each glass jar consisted of AC amended soils at rates equivalent to 0, 2.4, 4.8, and 9.6 t ha^{-1} herein referred to as CTR, AC1, AC2, and AC3 treatments respectively with three replications. For each genotype, 3 g of fresh root nodules (>2 mm) from the corresponding treatments in the pots were added to the respective glass jars with soils of similar origin (same treatment) and then thoroughly mixed. In this experiment, jars with root nodules are referred to as N2 (root nodules applied at 2% w/w). A similar set of jars were also established for each genotype but without addition of root nodules (N0).

Table 16. Initial soil chemical properties before the incubation experiment.

Soybean genotype (G)	Treatment (T)	pH (H_2O)	EC mS m^{-1}	Total C	Total N	C/N	NH_4^+-N	NO_3^--N
				g kg^{-1}			mg kg^{-1}	
TnVRSN4	CTR	6.54a	4.79a	0.99d	0.16c	6.1d	4.03a	2.60a
	AC1	6.39a	5.78a	1.62c	0.16bc	9.9c	4.41a	2.75a
	AC2	6.34a	4.78a	2.30b	0.18b	13.1b	4.24a	2.83a
	AC3	6.45a	3.46a	3.60a	0.19a	18.6a	4.26a	3.41a
Tachinagaha	CTR	6.57ab	4.11a	0.78d	0.16b	4.8d	3.33a	2.98a
	AC1	6.48b	4.65a	1.58c	0.18ab	9.0c	3.17a	2.69a
	AC2	6.62ab	4.65a	2.02b	0.17ab	11.8b	2.93a	3.38a
	AC3	6.77a	4.12a	3.35a	0.19a	17.9a	4.40a	2.73a

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4 and Tachinagaha have high and normal nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n = 3).

Figure 36 shows a pictorial summary of how this experiment was established and conducted. Furthermore, in order to compare the effect of nodules with those of inorganic fertilizer in soil, four urea fertilizer application rates were established by thoroughly mixing air-dried sandy soils (without any history of soybean cropping) collected from Tottori sand dunes, Tottori, Japan with urea at rates equivalent to 0, 61, 123 and 245 mg N kg^{-1} of soil (0, 20, 40 and 80 mg of urea in 150 g of soil) herein referred to as U0, U1, U2 and U3 respectively. The physicochemical properties of this sandy soil are shown in **Table 1**.

The jars were incubated in the dark for 3 months (from 13th September 2019 to 13th December 2019 inside the growth chamber (MLR-352H, Panasonic Healthcare Co., Ltd, Gunma, Japan) after adjusting the soil moisture content to 60% WFPS. They were covered with perforated lids to minimize water evaporation; soil moisture content was maintained at 60% WFPS by watering every 3 days and also opening for aeration as well. The temperature inside the growth chamber was maintained at 30°C, with a relative humidity of 65% during day while at night, the temperature was 25°C with a relative humidity of 70%.

6.2.2. Gas sampling and analysis

Headspace gas samples were collected on day 1, 2, 3, 4, 5, 6, 7, 10, 13, 18, 25, 33, 42, 53, 63, 74, 83 and 92 after the start of the experiment. Prior to gas sampling, the vials were opened for 30 min to renew the atmosphere inside and then resealed for 40 min with air tight covers containing rubber septa. After 40 min of sealing, the air inside the jars was mixed by flushing the syringe 3–4 times before collecting the gas samples. The headspace concentrations of gases were measured by sampling 35 ml of gas using a 60 ml plastic syringe. 5 ml of gas were used to flush the syringe needle and 30 ml were immediately injected into 15 ml pre-evacuated vials (Nichiden-Rika Glass Co. Ltd, Kobe, Japan) fitted with butyl rubber stoppers and then stored until analysis which was done at the Institute for Agro-Environmental Sciences (NIAES), Tsukuba, Japan. On each day of sampling, three air samples were taken to measure the atmospheric concentration of gases and their average was later used to calculate the gas fluxes. Using an autosampler (AOC-6000, Shimadzu, Kyoto, Japan), 1 ml of gas in each sample was injected into a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with the electron capture detector (ECD) and thermal conductivity detector (TCD) for determination of N_2O and CO_2 concentrations respectively. The N_2O and CO_2 fluxes were calculated based on the difference in gas concentration between the

atmosphere and the samples while the cumulative gas emissions were calculated from the gas fluxes by adding up the average daily emissions from every two gas sampling dates.

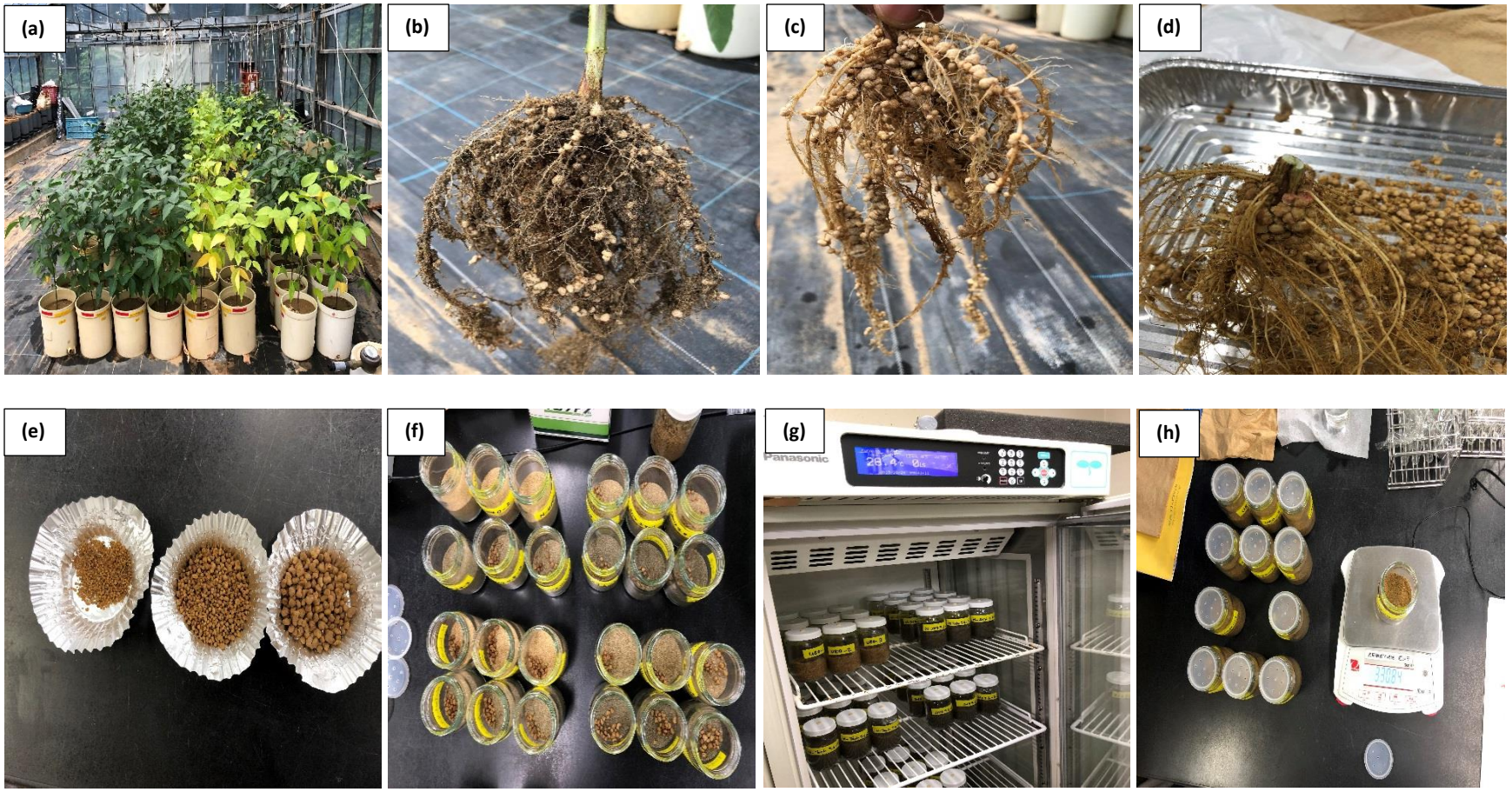

Figure 36. Establishment of the experiment. Soybean plants at the stage of root removal (**a**), plant root with nodules (**b**), after washing the roots (**c**), during nodule removal from roots (**d**), root nodules from a single TnVRSN4 plant (**e**), root nodules before mixing with soil in the glass jar (**f**), glass jars sealed during gas sampling (**g**), glass jar weighed before watering (**h**).

6.2.3. Soil sampling and analysis

At the end of the incubation, all the soils (fresh) were removed from the glass jars and separated in to two parts. The first part was stored at −80°C until further analysis for soil inorganic N (exchangeable NH_4^+-N and NO_3^--N) while the second part was air-dried and later analyzed for soil pH, EC, total N and total C. Before analysis, all the soil samples were passed through a 2 mm sieve to remove the root nodule debris. All the soil chemical properties were analyzed following procedures described in section 5.2.3. Soil inorganic N was done on the same day the samples were extracted.

6.2.4. Statistical analysis

All data was analyzed by analysis of variance (ANOVA) using IBM SPSS software (Version 20.0). A two way ANOVA was used to analyze the effects of genotype and treatment on the number and fresh weight of nodules per plant, nodule N and C/N ratio. A three way ANOVA examined the main effects and the interactions between the genotype, nodule application and treatment on N_2O and CO_2 emissions and soil chemical properties at the end of the incubation experiment. The statistical differences between treatment means were tested using Tukey's HSD test at 5% level of significance.

6.3. Results

6.3.1. Root nodules

There were no significant interactions between the genotype and treatment for the number and fresh weight of nodules as well as the root nodule N content and C/N ratio (**Figure 37**). The effect of genotype was significant for the number and weight of root nodules, root nodule N content and C/N ratio. The high nodulating genotype had the highest number and weight of root nodules. However, the N content (%) of the root nodules was significantly higher in the normal nodulating genotype than in the high nodulating genotype while the C/N ratio was significantly higher in the high nodulating genotype. Treatment was not significant for all the parameters except the fresh weight of nodules which were generally lowest in the AC3 treatment as compared to the other treatments.

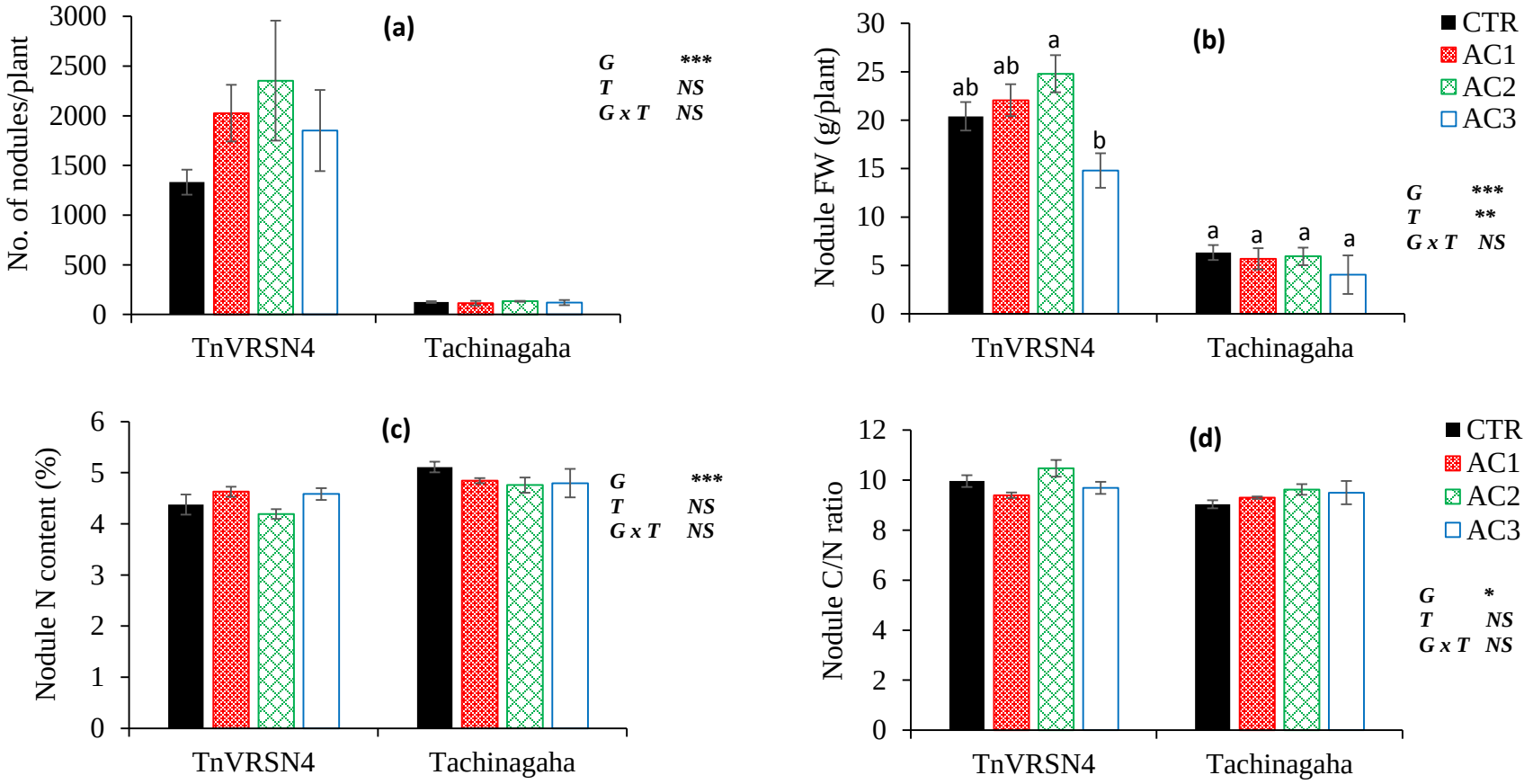

Figure 37. Number of nodules per plant **(a)**, Nodule fresh weight **(b)**, Nodule N content **(c)**, and C/N ratio **(d)** for the soybean genotypes. TnVRSN4 and Tachinagaha have high and normal nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. G and T represents genotype and treatment respectively while G x T represents interaction between genotype and treatment. Data points represent mean ± standard error (n = 3). * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P \leq 0.001$; NS-non-significant.

6.3.2. Greenhouse gas emissions

6.3.2.1. N_2O emissions

In the soils of both genotypes, root nodule application resulted in significantly higher N_2O fluxes compared to soils without root nodules (**Figure 38**). In the absence of root nodules, N_2O fluxes were below 0.16 µg N kg^{-1} h^{-1} and 0.30 µg N kg^{-1} h^{-1} in the soils of the high and normal nodulating genotypes respectively. However, in the presence of nodules, N_2O fluxes ranged from 0.02 to 13.5 µg N kg^{-1} h^{-1} and 0.02 to 8.3 µg N kg^{-1} h^{-1} in the soils of the high and normal nodulating genotypes respectively. The gas fluxes gradually increased and peaked within the first week after nodule addition to the soil. In soils of the high nodulating genotypes, the N_2O emissions from 19th to 30th September were higher in the control (CTR) than in the AC1, AC2 and AC3 treatments. In addition, from 19th September to 7th October, the N_2O emissions in the CTR and AC1 treatment from soils of the normal nodulating genotype were higher than those in AC2 and AC3 treatments.

The cumulative N_2O emissions ranged from 0.01 to 5.73 mg N kg^{-1} and 0.01 to 4.71 mg N kg^{-1} in the soils of the high and normal nodulating genotypes respectively (**Table 17**). Nodule application significantly increased N_2O emissions in the soils of the two genotypes. However, genotype and treatments did not significantly affect cumulative N_2O emissions. Also, all the interactions between genotype, nodule application and treatments were not significant.

Urea application also significantly increased N_2O fluxes (**Figure 39a**). The gas fluxes peaked on 25th September in the U1 and U2 treatments reaching 0.66 and 1.81 µg N kg^{-1} h^{-1} respectively while the U3 treatment peaked on 30th September at 1.60 µg N kg^{-1} h^{-1} and maintained higher N_2O emissions compared to the other treatments for the rest of the incubation period. The cumulative N_2O emissions from the urea treatments were 0.002, 0.14, 0.36 and 0.57 mg N kg^{-1} in the U0, U1, U2 and U3 treatments respectively (**Figure 39b**).

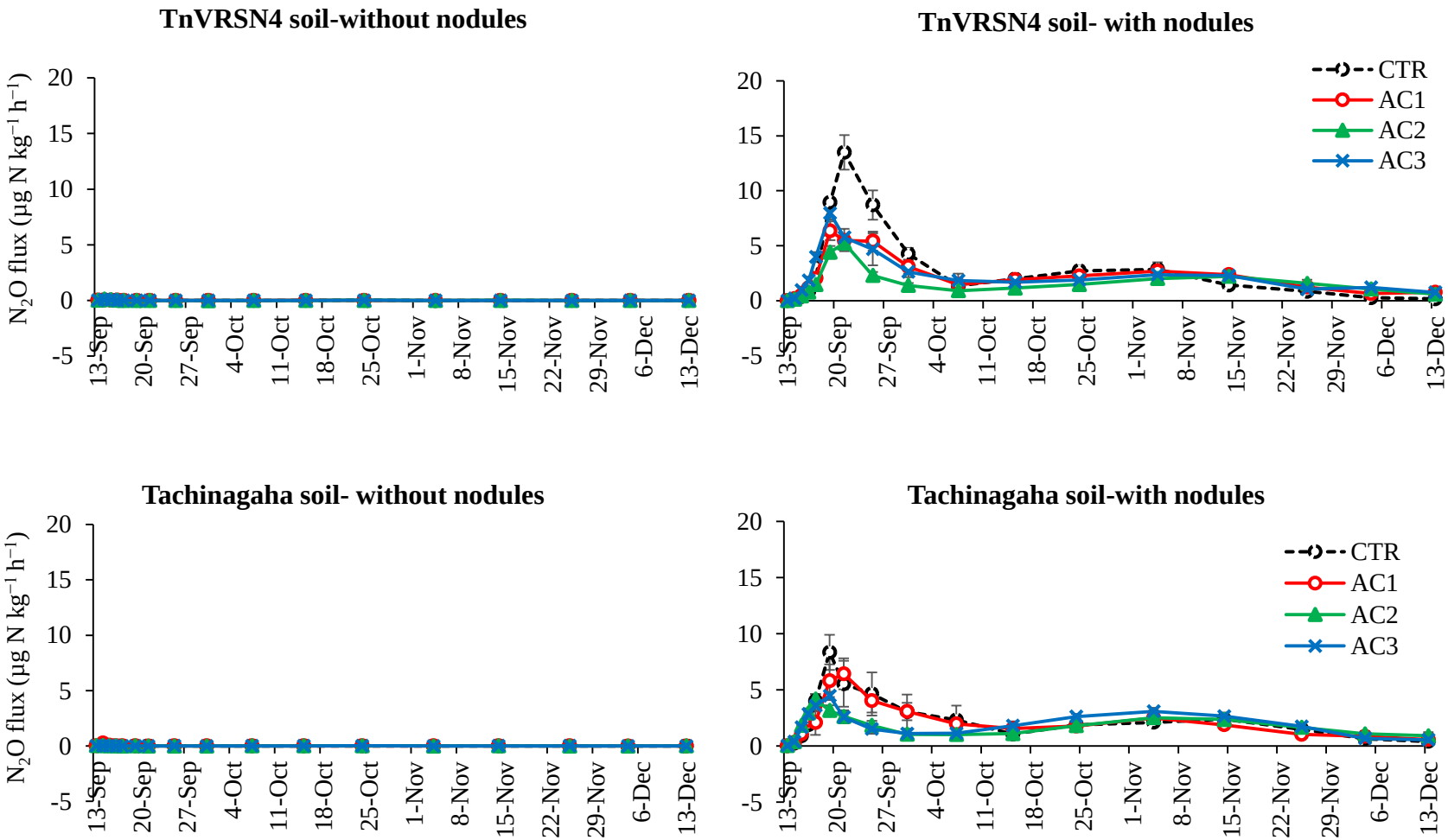

Figure 38. Temporal variation in N_2O fluxes in soils of the soybean genotypes over the incubation period. TnVRSN4 and Tachinagaha have high and normal nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Data points represent mean ± standard error (n=3).

Table 17. Cumulative N_2O and CO_2 emissions from the decomposing nodules of the two soybean genotypes.

Soybean genotype (G)	Nodule application (N)	Treatment (T)	N_2O emissions (mg N kg^{-1})	CO_2 emissions (mg C kg^{-1})
TnVRSN4	No nodules (N0)	CTR	0.01a	35.9a
		AC1	0.01a	35.9a
		AC2	0.02a	30.7a
		AC3	0.01a	27.6a
		Mean	**0.01**	**32.5**
	With nodules (N2)	CTR	5.73a	214.3a
		AC1	4.86a	207.6a
		AC2	3.64a	216.1a
		AC3	4.81a	222.6a
		Mean	**4.76**	**215.2**
Tachinagaha	No nodules (N0)	CTR	0.01ab	36.9a
		AC1	0.02a	34.6a
		AC2	0.01b	31.5a
		AC3	0.01b	43.7a
		Mean	**0.01**	**36.7**
	With nodules (N2)	CTR	4.71a	221.3a
		AC1	4.47a	218.3a
		AC2	3.76a	230.9a
		AC3	4.24a	214.7a
		Mean	**4.30**	**221.3**
ANOVA *P* values		G	NS	NS
		N	***	***
		T	NS	NS
		G x N	NS	NS
		G x T	NS	NS
		N x T	NS	NS
		G x N x T	NS	NS

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4 and Tachinagaha have high and normal nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n=3). *** indicates $P < 0.001$; NS indicates non-significant.

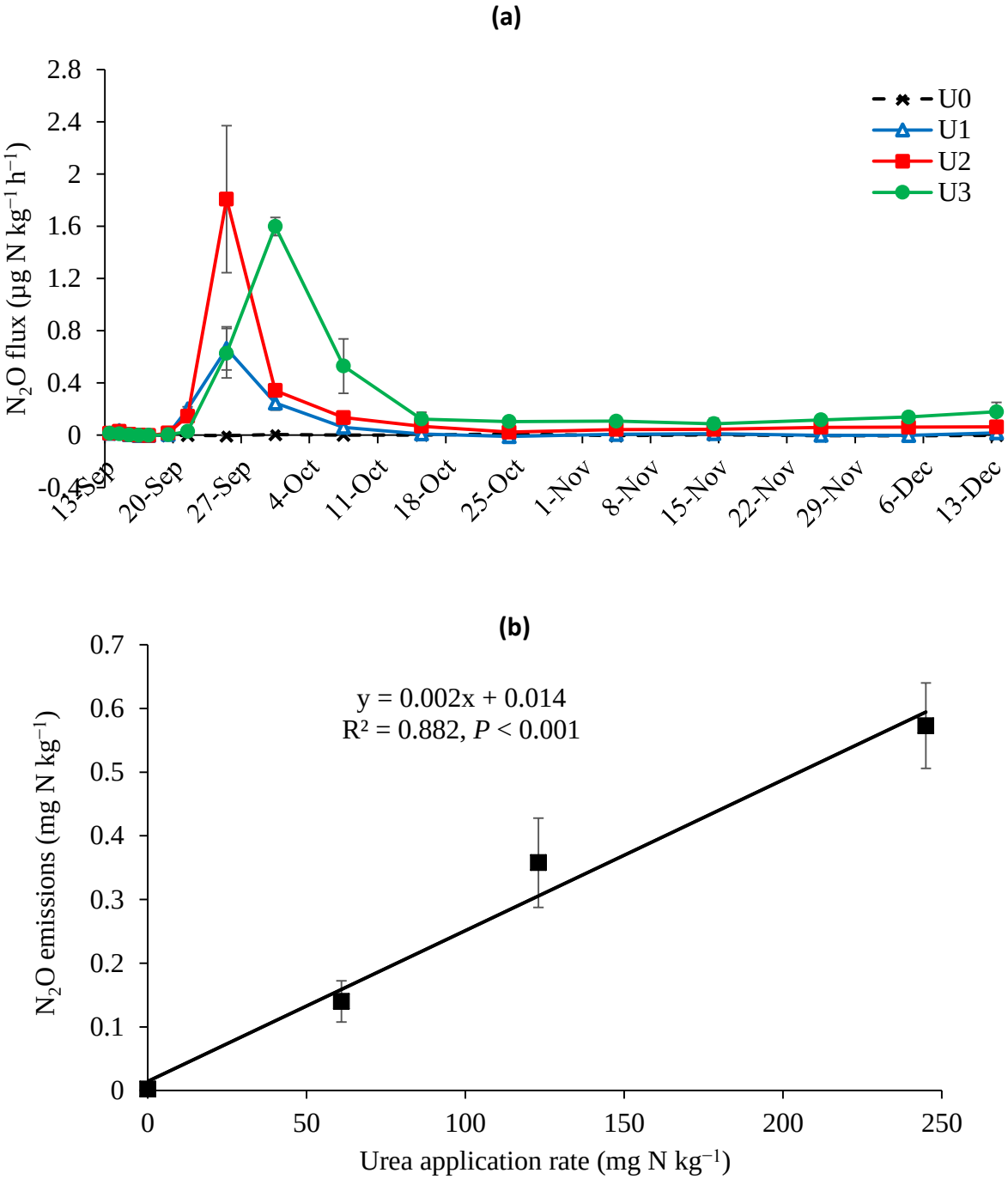

Figure 39. Variation in N_2O fluxes for the urea treatments throughout the incubation period **(a)**, correlation between urea application rate and cumulative N_2O emissions **(b)**. U0, U1, U2 and U3 represent urea application at 0, 61, 123 and 245 mg N kg^{-1} of soil respectively. Data points represent mean ± standard error (n=3).

6.3.2.2. CO_2 emissions

CO_2 fluxes were significantly higher in soils with nodules as compared to that without nodules of the two soybean genotypes (**Figure 40**). In soils without nodules, CO_2 fluxes were below 0.3 mg C kg^{-1} h^{-1} and were relatively high at the start of incubation but generally reduced over the following days. However, in soils with nodules, the CO_2 fluxes ranged from −0.0001 to 0.62 mg C kg^{-1} h^{-1} and from 0.02 to 0.72 mg C kg^{-1} h^{-1} in the soils of the high and normal nodulating genotypes respectively. The gas fluxes sharply increased after nodule application reaching the peak on 15th September and then significantly declined up to 30th September and remained relatively stable for the remaining period of incubation. There were no significant variations in CO_2 fluxes among the treatments on all gas sampling dates.

The cumulative CO_2 emissions ranged from 27.6 to 222.6 mg C kg^{-1} and 31.5 to 230.9 mg C kg^{-1} in the soils of the high and normal nodulating genotypes respectively (**Table 17**). In all the soils of the two genotypes, CO_2 emissions were significantly higher in the presence of nodules than in the absence of nodules. Genotype and treatment did not significantly influence CO_2 emissions. In addition, all the interactions between genotype, nodule application and treatment were not significant for CO_2 emissions. Urea application did not significantly affect CO_2 emissions (**Figure 41**).

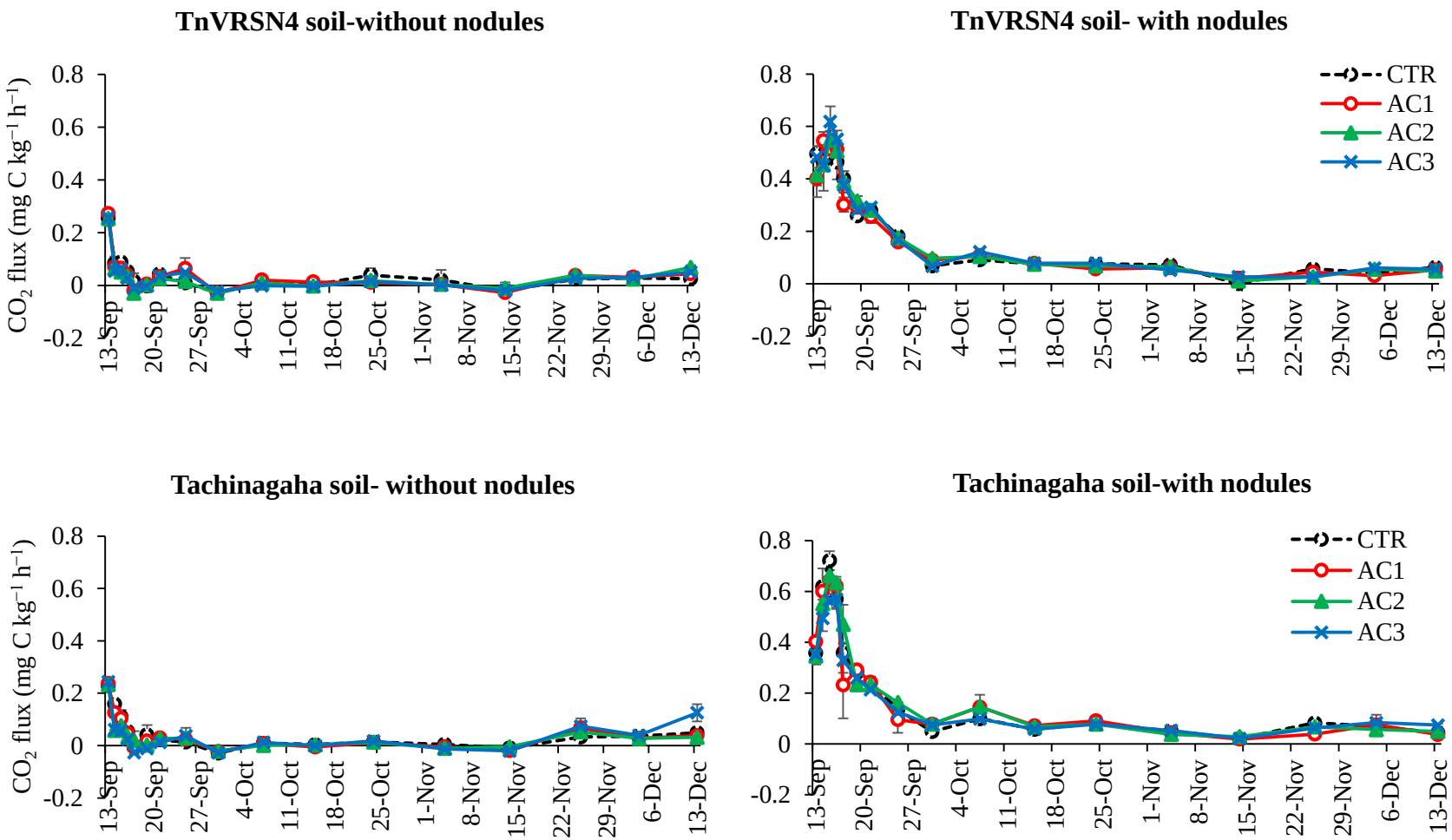

Figure 40. Temporal variation in CO_2 fluxes in soils of the soybean genotypes over the incubation period. TnVRSN4 and Tachinagaha have high and normal nodulating capacities respectively. CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. Data points represent mean ± standard error (n=3).

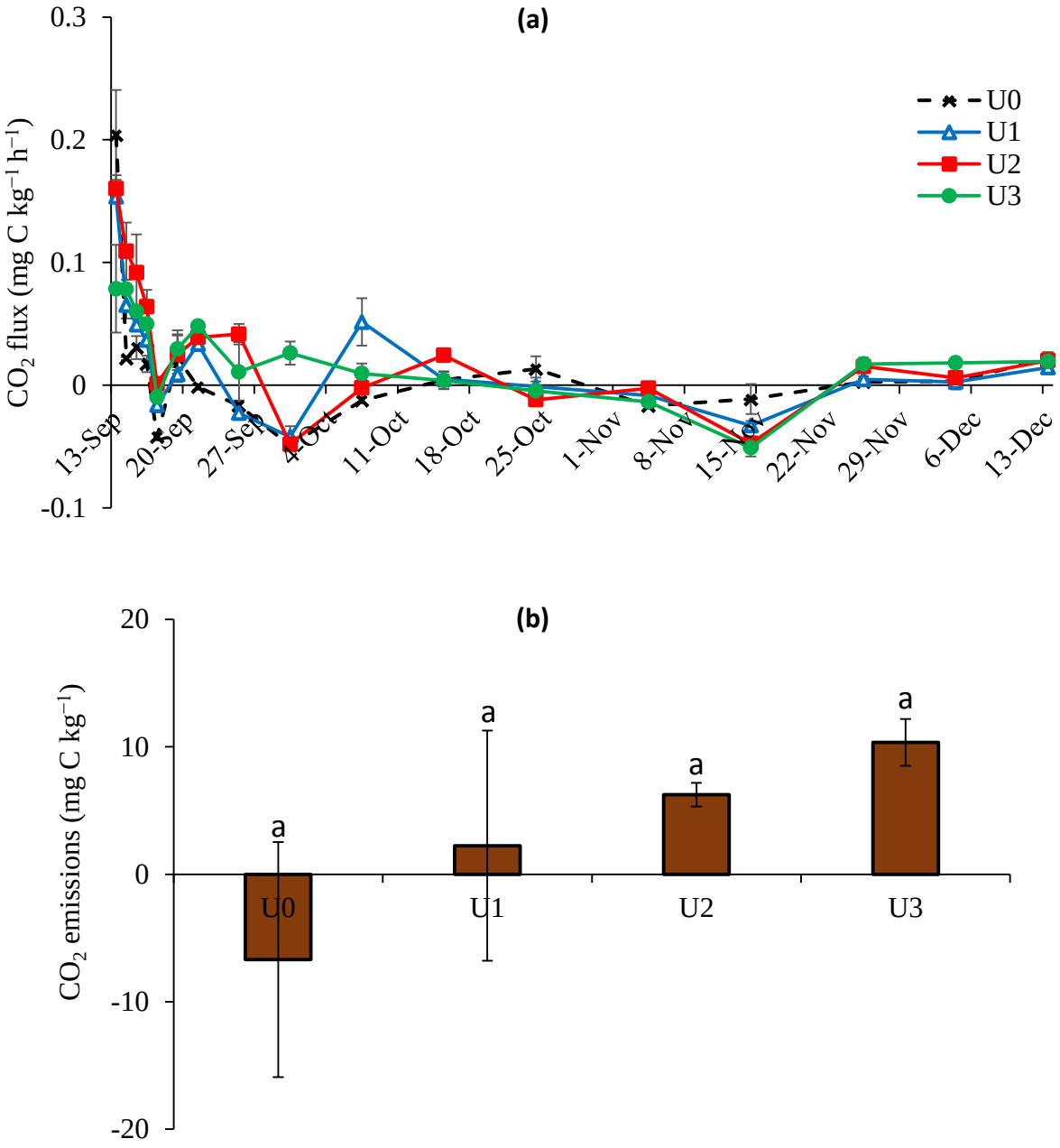

Figure 41. Variation in CO_2 fluxes **(a)** and cumulative CO_2 emissions **(b)** for the urea treatments throughout the incubation period. U0, U1, U2 and U3 represent urea application at 0, 61, 123 and 245 mg N kg^{-1} of soil respectively. Treatment means followed by the same letter are not significantly different at $P < 0.05$ using Tukey's HSD test. Data points represent mean ± standard error (n=3).

6.3.3. Soil chemical properties

After the incubation, there were significant interactions between the genotype, nodule applications and treatment for soil pH and soil EC but not for other soil properties (**Table 18**). Nodule application significantly incresed the soil pH, soil EC, total C, and total N in the soils of the two genotypes. The soil pH of the normal nodulating genotype was higher than that of the high nodulating genotype. The main effect of treatment on soil pH, soil EC, total C, total N and C/N ratio were significant. However, this was more evident and consistent for total C, total N and C/N ratio which significantly increased with AC application in both genotypes, with or without nodules. The main effects of genotype, nodule application, treatment and their interactions were not significant for soil exchangeable NH_4^+-N and NO_3^--N content except that there were significant interactions between nodule application and treatment for soil NO_3^--N. In the absence of nodules, AC decreased soil NO_3^--N in the AC3 treatment but in the presence of nodules, AC did not affect soil NO_3^--N in all the treatments.

The soil exchangeable NH_4^+-N content after incubation (**Table 18**) both in the presence or absence of root nodules was significantly lower than that of the initial soil before incubation (**Table 16**). On the contrary, soil NO_3^--N content and soil EC after incubation, both in the presence and absence of root nodules was significantly higher than that of the initial soil before incubation. However, soil pH, total N, total C and C/N ratio before and after incubation did not vary significantly.

Urea application significantly increased soil EC, total N, exchangeable NH_4^+-N and NO_3^--N content but significantly reduced soil pH, and C/N ratio, with no effect on Total C (**Table 19**).

Table 18. Soil chemical properties at the end of the incubation experiment.

Soybean genotype (G)	Nodule application (N)	Treatment (T)	pH (H_2O)	EC (mS m^{-1})	Total C	Total N	C/N	NH_4^+-N	NO_3^--N
					g kg^{-1}			mg kg^{-1}	
TnVRSN4	No nodules (N0)	CTR	6.87a	7.33b	0.80d	0.15c	5.4d	2.00a	13.6a
		AC1	6.48b	9.17a	1.56c	0.17b	9.4c	2.08a	13.1a
		AC2	6.44b	7.27b	2.17b	0.18b	12.3b	1.90a	10.6ab
		AC3	6.53b	5.60b	3.45a	0.19a	17.7a	2.91a	8.6b
		Mean	**6.58**	**7.34**	**1.99**	**0.17**	**11.2**	**2.22**	**11.5**
	With nodules (N2)	CTR	6.70a	9.42a	1.06d	0.18b	5.8d	1.76a	14.4a
		AC1	6.66a	8.94ab	1.58c	0.18b	8.7c	1.31a	13.7a
		AC2	6.65a	7.43ab	2.23b	0.19ab	11.7b	2.73a	10.9a
		AC3	6.65a	7.15b	3.55a	0.21a	16.9a	2.40a	15.7a
		Mean	**6.67**	**8.24**	**2.11**	**0.19**	**10.8**	**2.05**	**13.7**
Tachinagaha	No nodules (N0)	CTR	6.79b	6.73a	0.79d	0.15b	5.2d	1.88a	13.2ab
		AC1	6.73b	6.74a	1.49c	0.17ab	9.0c	2.34a	17.6a
		AC2	6.86ab	6.69a	2.02b	0.17ab	11.9b	2.61a	12.4ab
		AC3	7.00a	5.48b	3.17a	0.18a	17.4a	1.87a	7.4b
		Mean	**6.85**	**6.41**	**1.87**	**0.17**	**10.9**	**2.18**	**12.7**
	With nodules (N2)	CTR	6.88ab	7.61a	0.90d	0.17c	5.5d	1.54b	11.7a
		AC1	6.80b	8.97a	1.57c	0.17bc	9.0c	1.94ab	12.0a
		AC2	6.94ab	7.93a	2.21b	0.19ab	11.6b	3.04a	13.5a
		AC3	7.02a	8.21a	3.38a	0.20a	17.3a	1.88ab	13.4a
		Mean	**6.91**	**8.18**	**2.02**	**0.18**	**10.9**	**2.10**	**12.7**
ANOVA *P* values		G	***	*	***	**	NS	NS	NS
		N	***	***	***	***	NS	NS	NS
		T	***	***	***	***	***	NS	NS
		G x N	NS	*	NS	NS	NS	NS	NS
		G x T	***	**	*	NS	NS	NS	NS
		N x T	**	NS	NS	NS	NS	NS	*
		G x N x T	**	*	NS	NS	NS	NS	NS

CTR represents control (no AC) while AC1, AC2 and AC3 represents AC amendment at 2.4, 4.8 and 9.6 t ha^{-1} respectively. TnVRSN4 and Tachinagaha have high and normal nodulating capacities respectively. For each genotype, different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n = 3). * indicates $P < 0.05$; **indicates $P < 0.01$; *** indicates $P < 0.001$; NS indicates non-significant.

Table 19. Effect of urea application on soil chemical properties at the end of the incubation.

Treatment	pH (H_2O)	EC mS m^{-1}	Total C	Total N	C/N	NH_4^+-N	NO_3^--N
			g kg^{-1}			mg kg^{-1}	
U0	6.62a	1.4c	0.82a	0.16d	5.0a	1.9c	6.8d
U1	4.91b	10.9b	0.83a	0.21c	4.0b	1.9c	79.2c
U2	4.53c	21.4a	0.82a	0.26b	3.1c	24.0b	114.5b
U3	4.63bc	25.2a	0.82a	0.33a	2.5d	61.9a	153.2a

U0, U1, U2 and U3 represent urea application at 0, 61, 123 and 245 mg N kg^{-1} of soil respectively. Different letters within a column indicate significant differences among treatments at $P < 0.05$ using Tukey's HSD test (n=3).

6.4. Discussion

The results from this study revealed that root nodules are a significant source of N_2O and CO_2 emissions when they decompose in the soil, and this suggests that they should be taken into consideration when designing GHG mitigation strategies in soybean cropping systems. This corroborates results from Inaba et al. (2009) who reported significant increase in N_2O emissions from degraded root nodules as compared to fresh nodules and roots which had lower emissions. This implies that N_2O emissions are mainly from decomposed root nodules and not from fresh root nodules which explains why BNF is not a major source of N_2O emissions during the crop growing season of soybeans as observed in chapter five. In the present study, the addition of root nodules in soils created a significant increase in N_2O emissions which later gradually decreased over time implying that nodule decomposition creates a temporary abundance of available N and C for microorganisms. Therefore, the very low N_2O emissions in the absence of root nodules clarified that the nodules were a main source of N_2O emissions rather than nitrification and denitrification of soil N during nodule decomposition (Akiyama et al., 2016). Soil microbes are essential during root nodule decomposition as well as the organic N from the root nodules which is mineralized into NH_4^+ and the N_2O is produced from either nitrification or denitrification (Mosier et al., 1998; Inaba et al., 2012) depending on soil conditions.

The increase in N_2O emissions was attributed mainly to enhanced soil respiration and subsequent reduction in oxygen levels creating anaerobic microsites in soil (Azam et al., 2002; Scheer et al., 2017). The high N_2O emissions during the first week of incubation suggested that denitrification could have been the dominant process for N_2O production. This could be attributed to the high amounts of oxidizable C from root nodules which stimulated soil respiration hence lowering oxygen partial pressures in the soil thus creating anaerobic conditions from denitrifiers (Giles et al., 2012). The NH_4^+ did not only provide the NO_3^- through nitrification but also stimulated microbial activity which created favorable conditions for denitrification. The stimulation of microbial activity can be evidenced by the rapid increase in the CO_2 emissions during the first two days of the incubation. The levelling off of N_2O fluxes following an initial increase suggested that the easily oxidizable C was exhausted while nitrification still continued and contributed to N_2O emissions (Azam et al., 2002).

The concurrent decrease in soil exchangeable NH_4^+-N concentration coupled with an increase in NO_3^--N concentration at the end of the incubation (**Table 18**) compared to that before

the incubation (**Table 16**) provides evidence that nitrification existed and was the most dominant N_2O emission process for most of the duration in this study. A strong correlation between N_2O fluxes and increase in soil NO_3^- coupled with a decrease in NH_4^+ concentration suggests that nitrification significantly contributes to N_2O production (Baggs et al., 2000a). Soil moisture also influences the N_2O production processes and nitrification is reported to occur between 35 and 60% WFPS (Bateman and Baggs, 2005) which was in the range at which the soils in the present study were maintained (60% WFPS). The increased O_2 availability at lower WFPS hinders the activity of denitrifying microorganisms but increases the activity of nitrifying microorganisms (Cayuela et al., 2014).

Contrary to the hypothesis that N_2O and CO_2 emissions from nodules of the high nodulating genotype are higher than those from the normal nodulation genotype, the results showed that the N_2O and CO_2 emissions from root nodules of the two genotypes applied in soil at the same amount did not significantly differ. Although the N content of the root nodules of the normal nodulating genotype was significantly higher than that of the high nodulating genotype (**Figure 37c**), the N_2O emissions from these genotypes did not significantly vary which implies that the N content (% or mg g^{-1}) of individual nodules is not the major factor influencing N_2O emissions between these two genotypes but instead, it is the total N content (mg N per plant) of all the nodules per plant. The total N content of nodules per plant would be higher in the high nodulating genotype since it has a high nodule density compared to the normal nodulating genotype (**Figure 37a**) hence accounting for the high N_2O emissions reported in high nodulating soybean genotypes. Therefore, in studies that have reported significantly higher N_2O emissions in the high nodulating soybean genotypes as compared to the normal and non-nodulating soybeans (Kim et al., 2005), it could be that the gas emissions are due to the high numbers of nodules and not to the individual nodules. This could also account for the significantly higher CO_2 emissions reported in the high nodulating genotype compared to the normal and low nodulating genotypes explained in the previous study (chapter five). Furthermore, the results showed that it might not be feasible to use of root nodule total N in predicting the extent of N_2O emissions from the root nodules of the different genotypes. Instead, the measurement and usage of inorganic N (NH_4^+-N and NO_3^--N) content of root nodules could be a better approach to estimate emissions from the different root nodules.

The short-term (from the second to fourth week) reduction in N_2O emissions by AC in soils with root nodules especially those of the high nodulating genotype could be explained by the AC-related changes in denitrification. The decrease in total N denitrified, and the enhancement of further reduction of N_2O to N_2 are the two different pathways that can lead to lowering denitrification derived N_2O emissions in soil (Cayuela et al., 2013). Therefore, a reduction in soil NO_3^--N content would cause a decrease in the total N available for denitrification. Although AC did not significantly influence soil NO_3^--N content in the presence of root nodules at the end of the experiment (**Table 18**), there is a possibility that it could adsorb the NO_3^- in soil as evidenced by the significant reduction of NO_3^--N in the absence of nodules (**Table 18**). The adsorption of NO_3^--N could be related to anion exchange capacity (AEC) (Lawrinenko, 2014) of the AC. Although NO_3^--N was not measured during the second to fourth week, it can be speculated that there could have been a short-term adsorption of NO_3^--N hence accounting to the reduction in N_2O emissions during the early stages of nodule decomposition. The reduction in N_2O emissions by AC during the early stages could also be attributed to the enhanced activity of nitrous oxide reductase (N_2OR) which facilitates further reduction of N_2O to N_2 (Cayuela et al., 2013). In section 1.2.1, synthetic fertilizers were identified as significant sources of N_2O and this was also evidenced in this study where N_2O emissions increased with urea application rates due to the increased availability of available N to enhance microbial activities.

In this study, AC did not absorb any NH_4^+-N by the end of the experiment which provides evidence that more N was available for nitrification in all the treatments. Crop residues are significant sources of nutrients in the soil and this explains why nodule application significantly increased soil EC, total C and total N in this study. The increase in soil total C and N in the presence of AC is attributed to its inherent potential to directly release these nutrients which further clarifies the role of pyrogenic carbonaceous soil amendments in soil fertility management programs. Compared to urea application which significantly reduced soil pH (acidification effect of chemical fertilizer), nodule application in soil significantly increased soil pH. This is in agreement with Hue (2011) who reported a moderate increase in soil pH after crop residue (cowpea and pineapple) addition and attributed it to the liming effect of basic cations contained in crop residues and by ligand exchange between the terminal OH at the soil surface and organic anions derived from the decomposing residues. Therefore, nodule decomposition in soil could provide more benefits in improving the availability of nutrients in soil through increasing soil pH.

6.5. Conclusion

Soybean root nodules are significant sources of GHG emissions. This study showed that root nodules of the high and normal nodulating genotypes were important sources of N_2O and CO_2 emissions. The results clarified that the N_2O and CO_2 emissions from root nodules of the two genotypes applied in soil at the same amount did not significantly differ. This implied that the GHG emissions are due to the nodule density on each plant and not to the individual nodules. AC did not have significant effects on cumulative N_2O and CO_2 emissions from the root nodules which suggests that the effect of AC on GHG emissions from root nodules may vary with the time from the start of nodule decomposition in soil. Root nodule application significantly improved soil chemical properties and this suggests that nodule decomposition could improve the soil nutrient status. Therefore, to achieve sustainable soybean production, it could be possible to mitigate GHG emissions from the decomposing root nodules and increase soil nutrients by incorporating pyrogenic carbonaceous soil amendments after crop harvest. Further studies should also focus on quantifying the GHG emissions from decomposing root nodules of these genotypes in different soil types under field conditions using different pyrogenic carbonaceous soil amendments such as biochar.

CHAPTER SEVEN

General conclusions and recommendations

The adoption of agricultural practices that can increase agricultural production to feed the world's increasing population with minimum effects on environment are necessary for sustainable development. Synthetic fertilizer, crop residues and BNF in leguminous crops (through decomposition of plant parts especially root nodules) were identified as major sources of GHG emissions from the agricultural sector. The use of readily available and cheaper options like the incorporation of pyrogenic carbonaceous soil amendments such as biochar in soil has been proposed to increase crop productivity while mitigating GHG emissions from the agricultural sector. This study generally focused on assessing the effect of pyrogenic carbonaceous soil amendments on GHG emissions and crop productivity in vegetable and soybean production systems.

The investigation of the long term effects of biochar is necessary through studying the residual effects and aging effects of biochar on nutrient availability and crop growth in soils like sandy soils that have very poor nutrient retention. This is because it is necessary to consider the challenges farmers may face while using biochar, especially those in Sub Saharan Africa and South East Asia who might not be able to supply additional fertilizer following biochar application in the next crop growing season. Therefore, before advising them to apply biochar in their fields, a thorough study of the residual effects of biochar in soils is mandatory. Biochar application in soils with fertilizer did not significantly influence crop yield and N uptake during the first crop cycle. However, with increased cultivation in these soils without fertilizer application, biochar hindered N availability to the plants resulting in to negative residual effects on crop growth, yield and chlorophyll content during the second and third crop cycles. The negative residual effects of biochar necessitate the need for more seasonal N fertilizer application in sandy soils where it has been previously used as a soil amendment. Moreover, while considering the need for more additional N fertilizer application in biochar amended soils, it is also necessary to assess the role of biochar in mitigating the negative effects especially N_2O emissions and soil acidification that might result from fertilizer application.

To test the above hypothesis, the experiment in chapter three was conducted and the results showed that even after 1 year of application in the sandy soil, biochar still showed a potential to

reduce N_2O emissions following additional basal chemical fertilizer. This was because biochar still retained its functions of reducing N availability through adsorption of inorganic N, mainly NH_4^+-N. Soil pH is among the factors that affect nutrient availability to plants, and fertilizer application in the soil significantly results in changes in soil pH. Soil acidification is also one of the problems that occur after seasonal fertilizer application especially in vegetable crops where excessive fertilizer application occurs. The results of this study also showed that as with fresh biochar, the liming ability in aged biochar still existed and was able to offset soil acidification in biochar amended soils. Generally, both fresh and aged biochar application with fertilizer significantly increased plant tissue K and Ca content but decreased N, P and Mg content. When fresh biochar was applied at higher application rates, it had negative effects on crop yield but as it aged (after 1 year), the negative effects were offset and this was as a result of similar variation in N uptake. Therefore, since seasonal N fertilizer application seems to be inevitable in vegetable production, addition of biochar could be a possible way of counteracting the effects of excessive fertilizer use. Although the relatively high biochar rates (equivalent 85–250 t ha^{-1}) used in this study could somewhat mitigate N_2O emissions and reduce soil acidification, they might not be economically feasible under large-scale field conditions because of the enormous quantities of feedstock (palm shells) that could be needed to make the biochar. This work could be a springboard for further research exploring the aging effects of different biochars at varying application rates in different biochar types and crops before recommending it for use in the different soil types.

The strategies for N_2O mitigation from broccoli fields are most likely to vary with the season of crop residue incorporation in soil which depends on the time of transplanting. In this study, the incorporation of broccoli crop residues in soil significantly increased N_2O and CO_2 emissions but not CH_4 emissions during the fallow season. The increase in N_2O emissions following crop residue incorporation was mainly attributed to their high N content (248 kg N ha^{-1}), low C/N ratio (15.8) and the high moisture content (> 85%) while increase in CO_2 emission was due to the labile C provided to microorganisms for decomposition during the fallow season. Although the application of palm shell biochar did not significantly affect the seasonal GHG emissions, plant residue biomass and N uptake after harvest, the effects of biochar could be beneficial in longer-term (more than one year) as it ages in the soil. The study highlights the potential for palm shell biochar (at application rates ≥40 t ha^{-1}) to improve on the soil nutrient status in soils under vegetable production. Therefore, instead of removing broccoli crop residues

after harvest, they should rather be incorporated with biochar because it could be beneficial to soil through maintaining a high soil nutrient status which may improve productivity of the subsequent crops. Furthermore, farmers should consider thorough harvesting of the broccoli heads since they are most likely to emit more GHG than other plant parts (stems, leaves and roots) due to their low C/N ratio. This study did not look at the effect of biochar on crop growth and yield of broccoli. Therefore, future work will be needed to assess the effects of biochar on the productivity of broccoli as well as the further evaluation of the effects of combined incorporation of broccoli crop residues with biochar on GHG emissions and soil nutrient status in different soils under various tillage systems.

BNF was not a major source of N_2O emissions during the crop growing season of soybeans. The results further highlighted the potential of AC to somewhat reduce N_2O emissions but not CO_2 and CH_4 emissions. The reduction in nodulation by AC could be responsible for the N_2O reduction tendency in the high nodulating genotype (TnVRSN4). The role of genotype was evident especially on seed yield and CO_2 emissions which were significantly highest in the high nodulating genotype and lowest in the low nodulating genotype (TnVRNN4). Furthermore, the effects of AC on seed yield depended on the genotype; seed yield was not affected by AC in the high nodulating genotype but either reduced or did not affect seed yield in the other genotypes depending on the year of cropping. AC generally increased soil total N, total C and C/N ratio but its effect on soil pH, available P and exchangeable cations varied with the soybean genotype grown. Although AC did not significantly affect total isoflavone and protein content of seeds, it reduced the concentration of daidzein and daidzin in soil especially in the low nodulating genotype which implies that the effects of AC in soil under a particular soybean genotype may not have significant effects on the quality of the seeds. The fertilizer rate applied in this study could not sustain proper crop growth in the low nodulating genotype and this necessitates further studies to compare the amounts of GHG emitted if more chemical N fertilizer is added to soils of the low nodulating genotype to give similar yield as that in the high nodulating genotype. The high nodulating genotype can perform better in sandy soils with a low nutrient status but further studies should evaluate and compare these three genotypes in terms of productivity and GHG emissions under field conditions. In addition, since the soybean seeds used in this study were not inoculated with *Bradyrhizobia*, further studies could also focus on assessing the effect of pyrogenic carbonaceous

soil amendments on GHG emissions, crop productivity and seed quality as well as changes in soil properties while comparing inoculated and non-inoculated soybeans.

The root nodules of both the high and normal nodulating soybean genotypes were important sources of N_2O and CO_2 emissions; at the same nodule application rate in soil, the emissions from the two genotypes did not vary significantly. Although AC reduced the N_2O emissions during the early stages of the incubation experiment, it did not have significant effects on the cumulative N_2O and CO_2 emissions from the root nodules which implied that its effect on GHG emissions from root nodules may vary with the time from the start of nodule decomposition in soil. Therefore, to achieve sustainable soybean production, it is possible to mitigate GHG emissions from the decomposing root nodules as well as increasing soil nutrients by incorporating pyrogenic carbonaceous soil amendments after harvesting the soybeans.

Pyrogenic carbonaceous soil amendments have a potential to significantly contribute to GHG mitigation (especially N_2O) while maintaining crop productivity in vegetable and soybean cropping systems. Compared to AC which could be expensive to most of the farmers, biochar could be an easily available and cheaper option to use since it can be made at a local scale. There is need for long term studies on the different techniques of biochar production and usage (when to apply, how to apply and how much to apply) in soil while focusing on the changes in quality of the crop products obtained after biochar amendments to ensure safety for human consumption.

REFERENCES

Abdullah, N., Sulaiman, F., 2013. The Oil Palm Wastes in Malaysia. Chap. 3 in Biomass Now - Sustainable Growth and Use, edited by Miodrag Darko Matovic, 75–100. IntechOpen, doi: 10.5772/55302. Available from: https://www.intechopen.com/books/biomass-now-sustainable-growth-and-use/the-oil-palm-wastes-in-malaysia.

Abrishamkesh, S., Gorji, M., Asadi, H., Bagheri-Marandi, G. H., Pourbabaee, A. A., 2015. Effects of rice husk biochar application on the properties of alkaline soil and lentil growth. Plant, Soil Environ. 61 (11), 475–482. https://doi.org/10.17221/117/2015-PSE.

Agegnehu, G., Bass, A.M., Nelson, P.N., Bird, M.I., 2016. Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. Sci. Total Environ. 543, 295–306. https://doi.org/10.1016/j.scitotenv.2015.11.054.

Agneessens, L., De Waele, J., De Neve, S., 2014. Review of alternative management options of vegetable crop residues to reduce nitrate leaching in intensive vegetable rotations. Agronomy 4, 529–555. https://doi.org/10.3390/agronomy4040529.

Ahmed, M.B., Hasan Johir, M.A., Zhou, J.L., Ngo, H.H., Nghiem, L.D., Richardson, C., Moni, M.A., Bryant, M.R., 2019. Activated carbon preparation from biomass feedstock: Clean production and carbon dioxide adsorption. J. Clean. Prod. 225, 405–413. https://doi.org/10.1016/j.jclepro.2019.03.342.

Ajayi, A.E., Horn, R., 2016. Modification of chemical and hydrophysical properties of two texturally differentiated soils due to varying magnitudes of added biochar. Soil Tillage Res. 164, 34–44. https://doi.org/10.1016/j.still.2016.01.011.

Akhtar, S.S., Li, G., Andersen, M.N., Liu, F., 2014. Biochar enhances yield and quality of tomato under reduced irrigation. Agric. Water Manag. 138, 37–44. https://doi.org/10.1016/j.agwat.2014.02.016.

Akiyama, H., Hoshino, Y. T., Itakura, M., Shimomura, Y., Wang, Y., Yamamoto, A., Tago, K., Nakajima, Y., Minamisawa, K., Hayatsu, M., 2016. Mitigation of soil N_2O emission by

inoculation with a mixed culture of indigenous *Bradyrhizobium diazoefficiens.* Sci. Rep. 6, 32869. https://doi.org/10.1038/srep32869.

Ameloot, N., De Neve, S., Jegajeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y.N., Prins, W., Bouckaert, L., Sleutel, S., 2013. Short-term CO_2 and N_2O emissions and microbial properties of biochar amended sandy loam soils. Soil Biol. Biochem. 57, 401–410. https://doi.org/10.1016/j.soilbio.2012.10.025.

Amkha, S., Inubushi, K., 2009. Effects of Controlled-release Fertilizer Application with Cow Manure on Yield (*Brassica napa* L), Drainage Water Quality and N_2O Emission. The Proceedings of the International Plant Nutrition Colloquium XVI; University of California, Davis: Department of Plant Sciences. https://escholarship.org/uc/item/6tm8g19h.

Amkha, S., Sakamoto, A., Tachibana, M., Inubushi, K., 2009. Controlled mineralizing acetaldehyde condensation urea (CM-CDU) fertilizer can reduce nitrate leaching and N_2O emission from an Andisol with continuous cropped Komatsuna (*Brassica napa* L.). Soil Sci. Plant Nutr. 55 (6), 772–777. https://doi.org/10.1111/j.1747-0765.2009.00418.x.

Arami-Niya, A., Daud, W.M.A.W., Mjalli, F. S., 2010. Using granular activated carbon prepared from oil palm shell by $ZnCl_2$ and physical activation for methane adsorption. J. Analytical and Applied Pyrolysis 89 (2), 197–203. https://doi.org/10.1016/j.jaap.2010.08.006.

Asai, H., Samson, B. K., Stephan, H. M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T. and Horie, T., 2009. Biochar Amendment Techniques for Upland Rice Production in Northern Laos: 1. Soil Physical Properties, Leaf SPAD and Grain Yield. Field Crops Res. 111, 81–84. https://doi.org/10.1016/j.fcr.2008.10.008.

Azam, F., Müller, C., Weiske, A., Benckiser, G., Ottow, J.C.G., 2002. Nitrification and denitrification as sources of atmospheric nitrous oxide - role of oxidizable carbon and applied nitrogen. Biol. Fertil. Soils 35 (1), 54–61. https://doi.org/10.1007/s00374-001-0441-5.

Badagliacca, G., Ruisi, P., Rees, R.M., Saia, S., 2017. An assessment of factors controlling N_2O and CO_2 emissions from crop residues using different measurement approaches. Biol. Fertil. Soils 53, 547–561. https://doi.org/10.1007/s00374-017-1195-z.

Baggs, E.M., 2011. Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. Current Opinion in Environmental Sustainability 3, 321–327. https://doi.org/10.1016/j.cosust.2011.08.011.

Baggs, E.M., Rees, R.M., Smith, K.A., Vinten, A.J.A., 2000a. Nitrous oxide emission from soils after incorporating crop residues. Soil Use Manag. 16, 82–87.

Baggs, E.M., Watson, C.A., Rees, R.M., 2000b. The fate of nitrogen from incorporated cover crop and green manure residues. Nutr. Cycl. Agroecosystems 56, 153–163. https://doi.org/10.1023/A:1009825606341.

Bakken, L.R., Bergaust, L., Liu, B., Frostegard, A., 2012. Regulation of denitrification at the cellular level: a clue to the understanding of N_2O emissions from soils. Philosophical Transactions of The Royal Society. B 367, 1226–1234. https://doi.org/10.1098/rstb.2011.0321.

Bakker, C.J., Swanton, C.J., McKeown, A.W., 2009. Broccoli growth in response to increasing rates of pre-plant nitrogen. I. Yield and quality. Can. J. Plant Sci. 89, 527–537. https://doi.org/10.4141/CJPS08036.

Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biol. Fertil. Soils 41, 379–388. https://doi.org/10.1007/s00374-005-0858-3.

Batjes, N.H., 1999. Management options for reducing CO_2-concentrations in the atmosphere by increasing carbon sequestration in the soil. Report 410-200-031, Dutch National Research Programme on Global Air Pollution and Climate Change & Technical Paper 30, International Soil Reference and Information Centre, Wageningen.

Bellaloui, N., Bruns, H.A., Gillen, A.M., Abbas, H.K., Zablotowicz, R.M., Mengistu, A., Paris, R.L., 2010. Soybean seed protein, oil, fatty acids, and mineral composition as influenced by soybean-corn rotation. Agric. Sci. 1, 102–109. https://doi.org/10.4236/as.2010.13013.

Berglund, L.M., DeLuca, T.H., Zackrisson, O., 2004. Activated carbon amendments to soil alters nitrification rates in Scots pine forests. Soil Biol. Biochem. 36, 2067–2073. https://doi.org/10.1016/j.soilbio.2004.06.005.

Biederman, L. A., Harpole, W. S., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5 (2), 202–214. https://doi.org/10.1111/gcbb.12037.

Blossfeld, S., Gansert, D., Thiele, B., Kuhn, A. J., Lösch, R., 2011. The dynamics of oxygen concentration, pH value, and organic acids in the rhizosphere of Juncus spp. Soil Biol. Biochem. 43 (6), 1186–1197. https://doi.org/10.1016/j.soilbio.2011.02.007.

Blossfeld, S., Perriguey, J., Sterckeman, T., Morel, J. L., Lösch, R., 2010. Rhizosphere pH dynamics in trace-metal-contaminated soils, monitored with planar pH optodes. Plant and Soil 330, 173–184. https://doi.org/10.1007/s11104-009-0190-z.

Boersma, M., Wrobel-Tobiszewska, A., Murphy, L., Eyles, A., 2017. Impact of biochar application on the productivity of a temperate vegetable cropping system. New Zealand J. of Crop and Hortic. Sci. 45, 277–288. https://doi.org/10.1080/01140671.2017.1329745.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature. https://doi.org/10.1038/nature08930.

Borchard, N., Siemens, J., Ladd, B., Möller, A., Amelung, W., 2014. Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. Soil Tillage Res. 144, 184–194. https://doi.org/10.1016/j.still.2014.07.016.

Buss, W., Graham, M.C., Shepherd, J.G., Mašek, O., 2016. Risks and benefits of marginal biomass-derived biochars for plant growth. Sci. Total Environ. 569–570, 496-506. https://doi.org/10.1016/j.scitotenv.2016.06.129.

Butnan, S., Deenik, J. L., Toomsan, B., Antal, M. J., Vityakon, P., 2015. Biochar characteristics and application rates affecting corn growth and properties of soils contrasting in texture and mineralogy. Geoderma 237–238, 105–116. https://doi.org/10.1016/j.geoderma.2014.08.010.

Carrão-Panizzi, M.C., Berhow, M., Mandarino, J.M.G., Oliveira, M.C.N. de., 2009. Environmental and genetic variation of isoflavone content of soybean seeds grown in Brazil. Pesqui. Agropecuária Bras. 44, 1444–1451. https://doi.org/10.1590/s0100-204x2009001100011.

Case, S.D.C., McNamara, N.P., Reay, D.S., Stott, A.W., Grant, H.K., Whitaker, J., 2015. Biochar suppresses N_2O emissions while maintaining N availability in a sandy loam soil. Soil Biol. Biochem. 81, 178–185. https://doi.org/10.1016/j.soilbio.2014.11.012.

Castellini, M., Giglio, L., Niedda, M., Palumbo, A.D., Ventrella, D., 2015. Impact of biochar addition on the physical and hydraulic properties of a clay soil. Soil & Tillage Research 154, 1–13. https://doi.org/10.1016/j.still.2015.06.016.

Cayuela, M. L., Sanchez-Monedero, M. A., Roig, A., Hanley, K. Enders, A., Lehmann, J., 2013. Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions. Sci. Rep. 3, 1732. https://doi.org/10.1038/srep01732.

Cayuela, M. L., Van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A., Sánchez-Monedero, M. A., 2014. Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agric. Ecosyst. Environ. 191, 5–16. https://doi.org/10.1016/j.agee.2013.10.009.

Cesco, S., Neumann, G., Tomasi, N., Pinton, R., Weisskopf, L., 2010. Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. Plant Soil 329, 1–25. https://doi.org/10.1007/s11104-009-0266-9.

Cha, J.S., Park, S.H., Jung, S.C., Ryu, C., Jeon, J.K., Shin, M.C., Park, Y.K., 2016. Production and utilization of biochar: A review. J. Ind. Eng. Chem. 40, 1–15. https://doi.org/10.1016/j.jiec.2016.06.002.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of greenwaste biochar as a soil amendment. Aust. J. Soil Res. 45 (8), 629–634. https://doi.org/10.1071/SR07109.

Chapman, H.D., 1965. Cation Exchange Capacity. In: Black, C.A., Ed., Methods of Soil Analysis, American Society of Agronomy, Madison, 891–901.

Chen, H.L., Lu, C.J., Lai, H.Y., 2010. Amendments of activated carbon and biosolid on the growth and cadmium uptake of soybean grown in potted Cd-contaminated soils. Water. Air. Soil Pollut. 209, 307–314. https://doi.org/10.1007/s11270-009-0199-1.

Chintala, R., Mollinedo, J., Schumacher, T.E., Malo, D.D., Julson, J.L., 2013. Effect of biochar on

chemical properties of acidic soil. Arch. Agron. Soil Sci. 60, 393–404. https://doi.org/10.1080/03650340.2013.789870.

Cho, M.J., Harper, J.E., 1991. Effect of inoculation and nitrogen on isoflavonoid concentration in wild-type and nodulation-mutant soybean roots. Plant Physiol. 95, 435–442. https://doi.org/10.1104/pp.95.2.435.

Clough, T., Condron, L., Kammann, C., Müller, C., 2013. A Review of Biochar and Soil Nitrogen Dynamics. Agronomy 3, 275–293. https://doi.org/10.3390/agronomy3020275.

Cooper, J.E., Scherer, H.W., 2012. Nitrogen Fixation. In: Marschner. P. (Ed.), Marschner`s Mineral Nutrition of Higher Plants. Academic press, London, pp.389–408.

Cowan, N.J., Famulari, D., Levy, P.E., Anderson, M., Reay, D.S., Skiba, U.M., 2014. Investigating uptake of N_2O in agricultural soils using a high-precision dynamic chamber method. Atmos. Meas. Tech. 7, 4455–4462. https://doi.org/10.5194/amt-7-4455-2014.

Coward, L., Barnes, N.C., Setchell, K.D.R., Barnes, S., 1993. Genistein, Daidzein, and Their β-Glycoside Conjugates: Antitumor Isoflavones in Soybean Foods from American and Asian Diets. J. Agric. Food Chem. 41, 1961–1967. https://doi.org/10.1021/jf00035a027.

Cueva, A., Volkmann, T.H.M., van Haren, J., Troch, P.A., Meredith, L.K., 2019. Reconciling Negative Soil CO_2 Fluxes: Insights from a Large-Scale Experimental Hillslope. Soil Syst. 3, 10. https://doi.org/10.3390/soilsystems3010010.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Sci. Soc. Am. J. 70, 448–453. https://doi.org/10.2136/sssaj2005.0096.

DeLuca, T.H., Nilsson, M.C., Zackrisson, O., 2002. Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. Oecologia 133, 206–214. https://doi.org/10.1007/s00442-002-1025-2.

De Neve, S., Sáez, S.G., Daguilar, B.C., Sleutel, S., Hofman, G., 2004. Manipulating N mineralization from high N crop residues using on- and off-farm organic materials. Soil Biol. Biochem. 36, 127–134. https://doi.org/10.1016/j.soilbio.2003.08.023.

Dharmakeerthi, R., Chandrasiri, J.A.S., Edirimanne, V.U., 2012. Effect of rubber wood biochar on nutrition and growth of nursery plants of *Hevea brasiliensis* established in an Ultisol. SpringerPlus. 1, 84. http://www.springerplus.com/content/1/1/84.

Dhaubhadel, S., McGarvey, B.D., Williams, R., Gijzen, M., 2003. Isoflavonoid biosynthesis and accumulation in developing soybean seeds. Plant Mol. Biol. 53, 733–743. https://doi.org/10.1023/B:PLAN.0000023666.30358.ae.

Ding, W.X., Chen, Z.M., Yu, H.Y., Luo, J.F., Yoo, G.Y., Xiang, J., Zhang, H.J., Yuan, J.J., 2015. Nitrous oxide emission and nitrogen use efficiency in response to nitrophosphate, N-(n-butyl) thiophosphoric triamide and dicyandiamide of a wheat cultivated soil under sub-humid monsoon conditions. Biogeosciences 12, 803–815. https://doi.org/10.5194/bg-12-803-2015.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar to improve soil fertility. A review. Agron. Sustain. Dev. 36: 36. https://doi.org/10.1007/s13593-016-0372-z.

Duan, P., Zhang, X., Zhang, Q., Wu, Z., Xiong, Z., 2018. Field-aged biochar stimulated N_2O production from greenhouse vegetable production soils by nitrification and denitrification. Sci. Total Environ. 642, 1303–1310. https://doi.org/10.1016/j.scitotenv.2018.06.166.

Duressa, D., Soliman, K., Cebert, E., 2010. Protein and polyphenol profile changes in soybean roots under aluminum stress. Int. J. Plant Physiol. Biochem. 2, 38–45.

Dutaur, L., Verchot, L.V., 2007. A global inventory of the soil CH_4 sink. Global Biogeochemical Cycles, 21, GB4013. https://doi.org/10.1029/2006GB002734.

Fang, J., Liu, S., Zhao, K., 1998. Factors affecting soil respiration in reference with temperature's role in the global scale. Chinese Geographical Science 8, 246–255.

FAO, 2006. Fertilizer Use by Crop. FAO Fertilizer and Plant Nutrition Bulletin. Rome, Italy. Available at http://www.fao.org/tempref/docrep/fao/009/a0787e/A0787E00.pdf.

FAO, 2016a. Soils and Pulses; Symbiosis for Life. Rome, Italy. Available at http://www.fao.org/documents/card/en/c/56244a4c-d35a-48f8-b465-89f46f343312/.

FAO, 2016b. The state of food and agriculture; Climate change, agriculture and food security. Rome, Italy. Available at http://www.fao.org/publications/sofa/2016/en/.

FAO, 2019. FAOSTAT database. Available online: http://www.fao.org/faostat/en/#data (accessed on 31 March 2020).

FAO/IIASA/ISRIC/ISSCAS/JRC, 2009. Harmonized World Soil Database (version 1.2). FAO, Rome, Italy and IIASA, Laxenburg, Austria.

Ferguson, B.J., 2013. The Development and Regulation of Soybean Nodules. In: Board, J. (Ed). A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships. IntechOpen, London. DOI: 10.5772/52573. https://www.intechopen.com/books/a-comprehensive-survey-of-international-soybean-research-genetics-physiology-agronomy-and-nitrogen-relationships/the-development-and-regulation-of-soybean-nodules.

Filiberto, D.M., Gaunt, J.L., 2013. Practicality of biochar additions to enhance soil and crop productivity. Agriculture 3, 715–725. https://doi.org/10.3390/agriculture3040715.

Flynn, B., Graham, A., Scott, N., Layzell, D.B., Dong, Z., 2014. Nitrogen fixation, hydrogen production and N_2O emissions. Can. J. Plant Sci. 94, 1037–1041. https://doi.org/10.4141/CJPS2013-210.

Fujihara, S., Sasaki, H., Sugahara, T., 2010. Nitrogen-to-Protein Conversion Factors for Some Pulses and Soybean Products. J. Integr. Study of Dietery Habits 21, 60–66. https://doi.org/10.2740/jisdh.21.60.

Fujita, K., Chan, J.M., Bollinger, J.A., Alvarez, M.L., Dooley, D.M., 2007. Anaerobic purification, characterization and preliminary mechanistic study of recombinant nitrous oxide reductase from *Achromobacter cycloclastes.* J. Inorg. Biochem. 101, 1836–1844. https://doi.org/10.1016/j.jinorgbio.2007.06.029.

Gai, X., Wang, H., Liu, J., Zhai, L., Liu, S., Ren, T., Liu, H., 2014. Effects of Feedstock and Pyrolysis Temperature on Biochar Adsorption of Ammonium and Nitrate. PLoS ONE 9 (12): 1–19. https://doi.org/10.1371/journal.pone.0113888.

Gao, J., Xie, Y., Jin, H., Liu, Y., Bai, X., Ma, D., Zhu, Y., Wang, C., Guo, T., 2016. Nitrous oxide emission and denitrifier abundance in two agricultural soils amended with crop residues and urea in the North China Plain. PLoS ONE 11, 1–15. https://doi.org/10.1371/journal.pone.0154773.

Gelfand, I., Shcherbak, I., Millar, N., Kravchenko, A.N., Robertson, G.P., 2016. Long-term nitrous oxide fluxes in annual and perennial agricultural and unmanaged ecosystems in the upper Midwest USA. Glob. Chang. Biol. 22, 3594–3607. https://doi.org/10.1111/gcb.13426.

Giles, M., Morley, N., Baggs, E.M., Daniell, T.J., 2012. Soil nitrate reducing processes - Drivers, mechanisms for spatial variation, and significance for nitrous oxide production. Front. Microbiol. 3, 1–16. https://doi.org/10.3389/fmicb.2012.00407.

Glaser, B., Guggenberger, L.H.G., Zech, W., 2001. The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. Naturwissenschaften 88, 37–41. http://dx.doi.org/10.1007/s001140000193.

Gremaud, M.F., Harper, J.E., 1989. Selection and initial characterization of partially nitrate tolerant nodulation mutants of Soybean. Plant Physiol. 89, 169–173. https://doi.org/10.1104/pp.89.1.169.

Gresshoff, P.M., Rolfe, B.G., 1978. Viability of Rhizobium Bacteroids Isolated from Soybean Nodule Protoplasts. Planta 142, 329–333. https://doi.org/10.1007/BF00385085.

Griffin, D.E., Wang, D., Parikh, S.J., Scow, K.M., 2017. Short-lived effects of walnut shell biochar on soils and crop yields in a long-term field experiment. Agric. Ecosyst. Environ. 236, 21–29. https://doi.org/10.1016/j.agee.2016.11.002.

Gunarathne, V., Mayakaduwa, S., Vithanage, M., 2017. Biochar's Influence as a Soil Amendment for Essential Plant Nutrient Uptake. In: Naeem, M., Ansari, A.A., Gill, S.S (Eds.). Essential Plant Nutrients. Springer International Publishing. pp47–67. doi: 10.1007/978-3-319-58841-4.

Guo, Z.Y., Kong, C.H., Wang, J.G., Wang, Y.F., 2011. Rhizosphere isoflavones (daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped

soybean soil in field and controlled conditions. Soil Biol. Biochem. 43, 2257–2264. https://doi.org/10.1016/j.soilbio.2011.07.022.

Hagemann, N., Harter, J., Kaldamukova, R., Guzman-Bustamante, I., Ruser, R., Graeff, S., Kappler, A., Behrens, S., 2017. Does soil aging affect the N_2O mitigation potential of biochar? A combined microcosm and field study. GCB Bioenergy 9, 953–964. https://doi.org/10.1111/gcbb.12390.

Hagner, M., Kemppainen, R., Jauhiainen, L., Tiilikkala, K., Setälä, H., 2016. The effects of birch (*Betula spp.*) biochar and pyrolysis temperature on soil properties and plant growth. Soil Tillage Res. 163, 224–234. https://doi.org/10.1016/j.still.2016.06.006.

Hailegnaw, N.S., Mercl, F., Pračke, K., Száková, J., Tlustoš, P., 2019. Mutual relationships of biochar and soil pH, CEC, and exchangeable base cations in a model laboratory experiment. J. Soils and Sediments. 19, 2405–2416. https://doi.org/10.1007/s11368-019-02264-z.

Harter, J., Krause, H., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., Kappler, A., Behrens, S., 2014. Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. Int. Soc. Microb. Ecol. 8, 660–674.

Hassan, S., Mathesius, U., 2012. The role of flavonoids in root-rhizosphere signalling: Opportunities and challenges for improving plant-microbe interactions. J. Exp. Bot. 63, 3429–3444. https://doi.org/10.1093/jxb/err430.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Moller, I.S., White, P., 2012. Functions of Macronutrients. In: Marschner. P. (Ed.), Marschner`s Mineral Nutrition of Higher Plants. Academic press, London, pp.135–189.

Hawthorne, I., Johnson, M.S., Jassal, R.S., Black, T.A., Grant, N.J., Smukler, S.M., 2017. Application of biochar and nitrogen influences fluxes of CO_2, CH_4 and N_2O in a forest soil. J. Environ. Manag. 192, 203–214. https://doi.org/10.1016/j.jenvman.2016.12.066.

He, Y., Zhou, X., Jiang, L., Li, M., Du, Z., Zhou, G., Shao, J., Wang, X., Xu, Z., Bai, S.H., Wallace, H., Xu, C., 2017. Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis. GCB Bioenergy 9, 743–755. https://doi.org/10.1111/gcbb.12376.

Heffer, P., Gruère, A., Roberts, T., 2017. Assessment of Fertilizer Use by Crop at the Global Level. International Fertilizer Association (IFA) and International Plant Nutrition Institute (IPNI). Available at https://www.fertilizer.org/images/Library_Downloads/2017_IFA_AgCom_17_134%20rev_FUBC%20assessment%202014.pdf.

Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2007. The transformation and mobility of charcoal in a fire-impacted watershed. Geochim. Cosmochim. Acta 71, 3432–3445. https://doi.org/10.1016/j.gca.2007.02.023.

Hooper, A. B., Terry, K. R., 1979. Hydroxylamine Oxidoreductase of *Nitrosomonas* production of Nitric oxide from hydroxylamine. Biochimica et Biophysica Acta, 571, 12–20. https://doi.org/10.1016/0005-2744(79)90220-1.

Huang, T., Gao, B., Hu, X., Lu, X., Well, R., Christie, P., Bakken, L.R., Ju, X., 2014. Ammonia-oxidation as an engine to generate nitrous oxide in an intensively managed calcareous Fluvo-aquic soil. Scientific Reports. 4, 3950. https://doi.org/10.1038/srep03950.

Huang, Y., Zou, J., Zheng, X., Wang, Y., Xu, X., 2004. Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. Soil Biol. Biochem. 36, 973–981. https://doi.org/10.1016/j.soilbio.2004.02.009.

Hue, N.V., 2011. Alleviating soil acidity with crop residues. Soil Sci. 176, 543–549. https://doi.org/10.1097/SS.0b013e31822b30f1.

Hungria, M., Mendes, I.C., 2015. Nitrogen Fixation with Soybean: The Perfect Symbiosis? In: De Bruijn, F.J. (Ed.), Biological Nitrogen Fixation. John Wiley & Sons., New Jersey, pp.1005–1019. https://doi.org/10.1002/9781119053095.ch99.

Hussain, M., Farooq, M., Nawaz, A., Al-Sadi, A.M., Solaiman, Z.M., Alghamdi, S.S., Ammara, U., Ok, Y.S., Siddique, K.H.M., 2017. Biochar for crop production: potential benefits and risks. Journal of Soils and Sediments 17, 685–716. https://doi.org/10.1007/s11368-016-1360-2.

Ikeda, H., Osawa, T., 1981. Nitrate- and Ammonium-N Absorption by Vegetables from Nutrient Solution Containing Ammonium Nitrate and the Resultant Change of Solution pH. J.

Japanese Soc. Hortic. Sci. 50, 225–230. https://doi.org/10.2503/jjshs.50.225.

Inaba, S., Ikenishi, F., Itakura, M., Kikuchi, M., Eda, S., Chiba, N., Katsuyama, C., Suwa, Y., Mitsui, H., Minamisawa, K., 2012. N_2O emission from degraded soybean nodules depends on denitrification by *Bradyrhizobium japonicum* and other microbes in the rhizosphere. Microbes Environ. 27, 470–476. https://doi.org/10.1264/jsme2.me12100.

Inaba, S., Tanabe, K., Eda, S., Ikeda, S., Higashitani, A., Mitsui, H., Minamisawa, K., 2009. Nitrous oxide emission and microbial community in the rhizosphere of nodulated soybeans during the late growth period. Microbes Environ. 24, 64–67. https://doi.org/10.1264/jsme2.ME08544.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

IPCC, 2014. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Stechow, C., Zwickel, T., Minx, J.C. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Ippolito, J.A., Ducey, T.F., Cantrell, K.B., Novak, J.M., Lentz, R.D., 2016. Designer, acidic biochar influences calcareous soil characteristics. Chemosphere 142, 184–191. https://doi.org/10.1016/j.chemosphere.2015.05.092.

Islam, M. A., Adjesiwor, A. T., 2018. Nitrogen Fixation and Transfer in Agricultural Production Systems. http://dx.doi.org/10.5772/intechopen.71766.

Itakura, M., Uchida, Y., Akiyama, H., Hoshino, Y. K., Shimomura, Y., Morimoto, S., Tago, K., Wang, Y., Hayakawa, C., Uetake, Y., Sanchez, C., Eda, S., Hayatsu, M., Minamisawa, K., 2013. Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium*

japonicum inoculation. Nature Clim. Change 3, 208–212. https://doi.org/10.1038/nclimate1734.

Jakob, L., Hartnik, T., Henriksen, T., Elmquist, M., Brändli, R.C., Hale, S.E., Cornelissen, G., 2012. PAH-sequestration capacity of granular and powder activated carbon amendments in soil, and their effects on earthworms and plants. Chemosphere 88, 699–705. https://doi.org/10.1016/j.chemosphere.2012.03.080.

Jeffery, S., Abalos, D., Spokas K.A., Verheijen F.G.A., 2012. Biochar effects on crop yield. In: Lehmann, J., Joseph, S (Eds.). Biochar for environmental management: Science, Technology and implementation. Routledge, London and New York, pp. 301–325.

Jeffery, S., Verheijen, F. G. A., Kammann, C., Abalos, D., 2016. Biochar effects on methane emissions from soils: A meta-analysis. Soil Biol. Biochem. 101, 251–258. https://doi.org/10.1016/j.soilbio.2016.07.021.

Jia, J., Li, B., Chen, Z., Xie, Z., Xiong, Z., 2012a. Effects of biochar application on vegetable production and emissions of N_2O and CH_4. Soil Sci. Plant Nutr. 58, 503–509. https://doi.org/10.1080/00380768.2012.686436.

Jia, J.X., Ma, Y.C., Xiong, Z.Q., 2012b. Net ecosystem carbon budget, net global warming potential and greenhouse gas intensity in intensive vegetable ecosystems in China. Agric. Ecosyst. Environ. 150, 27–37. https://doi.org/10.1016/j.agee.2012.01.011.

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy D.V., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol. Biochem. 45, 113–124. https://doi.org/10.1016/j.soilbio.2011.10.012.

Joo, S.J., Park, S., Park, M., Lee, C.S., 2012. Estimation of soil respiration using automated chamber systems in an oak (*Quercus mongolica*) forest at the Nam-San site in Seoul, Korea. Sci. Total Environ. 416, 400-409. https://doi.org/10.1016/j.scitotenv.2011.11.025.

Kakiuchi, J., Kamiji, Y., 2015. Relationship between phosphorus accumulation and dry matter production in soybeans. Plant Prod. Sci. 18, 344–355. https://doi.org/10.1626/pps.18.344.

Kamkar, B., Akbari, F., Teixeira da Silva, J.A., Movahedi Naeini, S.A., 2014. The Effect of Crop Residues on Soil Nitrogen Dynamics and Wheat Yield. Adv. Plants Agric. Res. 1(1): 00004. http://dx.doi.org/10.15406/apar.2014.01.00004.

Kanthle, A.K., Lenka, N.K., Lenka, S., Tedia, K., 2016. Biochar impact on nitrate leaching as influenced by native soil organic carbon in an Inceptisol of central India. Soil Tillage Res. 157, 65–72. https://doi.org/10.1016/j.still.2015.11.009.

Karhu, K., Mattila, T., Bergström, I., Regina, K., 2011. Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity - Results from a short-term pilot field study. Agric. Ecosyst. Environ. 140, 309–313. https://doi.org/10.1016/j.agee.2010.12.005.

Khalil, K., Mary, B., Renault, P., 2004. Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O_2 concentration. Soil Biol. Biochem. 36, 687–699. https://doi.org/10.1016/j.soilbio.2004.01.004.

Kim, E.H., Lee, O.K., Kim, J.K., Kim, S.L., Lee, J., Kim, S.H., Chung, I.M., 2014. Isoflavones and anthocyanins analysis in soybean (*Glycine max* (L.) Merill) from three different planting locations in Korea. F. Crop. Res. 156, 76–83. https://doi.org/10.1016/j.fcr.2013.10.020.

Kim, Y., Nakayama, N., Nakamura, T., Takahashi, M., Shimada, S., Arihara, J., 2005. NO and N_2O emissions from fields in the different nodulated genotypes of soybean. Jpn. J. Crop Sci. 74, 427–430.

Konsolakis, M., 2015. Recent Advances on Nitrous Oxide (N_2O) Decomposition over Non-Noble-Metal Oxide Catalysts: Catalytic Performance, Mechanistic Considerations, and Surface Chemistry Aspects. ACS Catal. 5, 6397–6421. https://doi.org/10.1021/acscatal.5b01605.

Koulibaly, B., Dakuo, D., Traoré, O., Ouattara, K., Lompo, F., 2017. Long-term Effects of Crops Residues Management on Soil Chemical Properties and Yields in Cotton - Maize - Sorghum Rotation System in Burkina Faso. J. Agric. Ecol. Res. Int. 10, 1–11. https://doi.org/10.9734/JAERI/2017/31178.

Kuppusamy, S., Thavamani, P., Megharaj, M., Venkateswarlu, K., Naidu, R., 2016. Agronomic and remedial benefits and risks of applying biochar to soil: Current knowledge and future research directions. Environ. Int. 87, 1–12. https://doi.org/10.1016/j.envint.2015.10.018.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. 38, 425–448. https://doi.org/10.1016/j.soilbio.2005.08.020.

Lau, J.A., Puliafico, K.P., Kopshever, J.A., Steltzer, H., Jarvis, E.P., Schwarzländer, M., Strauss, S.Y., Hufbauer, R.A., 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. New Phytol. 178, 412–423. https://doi.org/10.1111/j.1469-8137.2007.02360.x.

Lawrinenko, M., 2014. Anion exchange capacity of biochar. Green Chem. 17, 4628–4636. https://doi.org/10.1039/C5GC00828J.

Lee, S.J., Yan, W., Ahn, J.K., Chung, I.M., 2003. Effects of year, site, genotype and their interactions on various soybean isoflavones. F. Crop. Res. 81, 181–192. https://doi.org/10.1016/S0378-4290(02)00220-4.

Lehmann, J., Da Silva, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. Plant Soil 249, 343–357. https://doi.org/10.1023/A:1022833116184.

Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems- a review. Mitigation and Adaptation Strategies for Global Change 11, 403–427. http://dx.doi.org/10.1007/s11027-005-9006-5.

Lehmann, J., Joseph, S., 2009. Biochar for Environmental Management: Science and technology. 416. London, UK: Earthscan.

Lehmann, J., Joseph, S., 2015. Biochar for Environmental Management: Science, Technology and Implementation. Routledge. https://doi.org/10.4324/9781849770552.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota - A review. Soil Biol. Biochem. 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.

Lehtinen, T., Schlatter, N., Baumgarten, A., Bechini, L., Krüger, J., Grignani, C., Zavattaro, L., Costamagna, C., Spiegel, H., 2014. Effect of crop residue incorporation on soil organic

carbon and greenhouse gas emissions in European agricultural soils. Soil Use Manag. 30, 524–538. https://doi.org/10.1111/sum.12151.

Li, B., Fan, C.H., Xiong, Z.Q., Li, Q.L., Zhang, M., 2015a. The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N_2O emissions from an intensively managed vegetable field in southeastern China. Biogeosciences 12, 2003–2017. https://doi.org/10.5194/bg-12-2003-2015.

Li, B., Fan, C.H., Zhang, H., Chen, Z.Z., Sun, L.Y., Xiong, Z.Q., 2015b. Combined effects of nitrogen fertilization and biochar on the net global warming potential, greenhouse gas intensity and net ecosystem economic budget in intensive vegetable agriculture in southeastern China. Atmos. Environ. 100, 10–19. https://doi.org/10.1016/j.atmosenv.2014.10.034.

Li, X., Sørensen, P., Olesen, J.E., Petersen, S.O., 2016. Evidence for denitrification as main source of N_2O emission from residue-amended soil. Soil Biol. Biochem. 92, 153–160. https://doi.org/10.1016/j.soilbio.2015.10.008.

Licker, R., Johnston, M., Foley, J.A., Barford, C., Kucharik, C.J., Monfreda, C., Ramankutty, N., 2010. Mind the gap: how do climate and agricultural management explain the 'yield gap' of croplands around the world? Global Ecol. Biogeogr. 19, 769–782. https://doi.org/10.1111/j.1466-8238.2010.00563.x.

Lin, X.W., Xie, Z.B., Zheng, J.Y., Liu, Q., Bei, Q.C., Zhu, J.G., 2015. Effects of biochar application on greenhouse gas emissions, carbon sequestration and crop growth in coastal saline soil. Eur. J. Soil Sci. 66, 329–338. https://doi.org/10.1111/ejss.12225.

Linn, D.M., Doran, J.W., 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48, 1267–1272.

Liu, C.W., Murray, J.D., 2016. The role of flavonoids in nodulation host-range specificity: An update. Plants 5, 33. https://doi.org/10.3390/plants5030033.

Liu, Q., Liu, B., Ambus, P., Zhang, Y., Hansen, V., Lin, Z., Shen, D., Liu, G., Bei, Q., Zhu, J., Wang, X., Ma, J., Lin, X., Yu, Y., Zhu, C., Xie, Z., 2015. Carbon footprint of rice production under biochar amendment - a case study in a Chinese rice cropping system. GCB Bioenergy

8, 148–159. https://doi.org/10.1111/gcbb.12248.

Lone, A.H., Najar, G.R., Ganie, M.A., Sofi, J.A., Ali, T., 2015. Biochar for Sustainable Soil Health: A Review of Prospects and Concerns. Pedosphere 25(5), 639–653. https://doi.org/10.1016/S1002-0160(15)30045-X.

Longdoz, B., Yernaux, M., Aubinet, M., 2000. Soil CO_2 efflux measurements in a mixed forest: impact of chamber disturbances, spatial variability and seasonal evolution. Global Change Biology 6, 907–917.

Lozovaya, V.V., Lygin, A.V., Ulanov, A.V., Nelson, R. L., Dayde, J., Widholm, J. M., 2005. Effect of Temperature and Soil Moisture Status during Seed Development on Soybean Seed Isoflavone Concentration and Composition. Crop Sci. 45, 1934–1940. https://doi.org/10.2135/cropsci2004.0567.

Luo, Y., Zhou, X., 2006. Soil respiration and the Environment. Academic Press, USA https://doi.org/10.1016/B978-012088782-8/50002-4.

Major, J., Rondon, M., Molina, D., Riha, S.J., Lehmann, J., 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil 333, 117–128. https://doi.org/10.1007/s11104-010-0327-0.

Marchand, L., Pelosi, C., Gonzalez-Centeno, M. R., Maillard, A., Ourry, A., Galland, W., Teissedre, P., Bessoule, J., Mongrand, S., Morvan-Bertrand, A., Zhang, Q., Grosbellet, C., Bert, V., Oustriere, N., Mench, M., Brunel-Muguet, S., 2016. Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol. Chemosphere 156, 150–162. https://doi.org/10.1016/j.chemosphere.2016.04.129.

Marinho, E.V.A., DeLaune, R.D., Lindau, C.W., 2006. Nitrous Oxide Flux from Soybeans Grown on Mississippi Alluvial Soil. Communications in Soil Sci. Plant analysis 35, 1–8. https://doi.org/10.1081/CSS-120027630.

Marks, E.A.N., Mattana, S., Alcañiz, J.M., Pérez-Herrero, E., Domene, X., 2016. Gasifier biochar effects on nutrient availability, organic matter mineralization, and soil fauna activity in a

multi-year Mediterranean trial. Agric. Ecosyst. Environ. 215, 30–39. https://doi.org/10.1016/j.agee.2015.09.004.

Marschner, H., Römheld V., 1983. In vivo measurement of root induced pH changes at the soil-root interface: effect of plant species and nitrogen source. Zeitschrift für Pflanzenphysiologie 111 (3), 241–251. https://doi.org/10.1016/S0044-328X(83)80083-X.

Marschner, P., 2012. Marschner's Mineral Nutrition of Higher Plants. London: Academic Press.

Martinsen, V., Alling, V., Nurida, N. L., Mulder, J., Hale, S. E., Ritz, C., Rutherford, D. W., Heikens, A., Breedveld, G. D., Cornelissen, G., 2015. pH effects of the addition of three biochars to acidic Indonesian mineral soils. Soil Sci. Plant Nutr. 61, 821–834. https://doi.org/10.1080/00380768.2015.1052985.

Mete, F.Z., Mia, S., Dijkstra, F.A., Abuyusuf, M., Hossain, A.S.M.I., 2015. Synergistic Effects of Biochar and NPK Fertilizer on Soybean Yield in an Alkaline Soil. Pedosphere 25, 713–719. https://doi.org/10.1016/S1002-0160(15)30052-7.

Miao, Q., Tang, Y., Xu, J., Liu, X., Xiao, L., Chen, Q., 2013. Activated carbon prepared from soybean straw for phenol adsorption. J. Taiwan Inst. Chem. Eng. 44, 458–465. https://doi.org/10.1016/j.jtice.2012.12.006.

Minamikawa, K., Tokida, T., Sudo, S., Padre, A., Yagi, K., 2015. Guidelines for measuring CH_4 and N_2O emissions from rice paddies by a manually operated closed chamber method. National Institute for Agro-Environmental Science, Tsukuba, Japan.

Moinet, G.Y.K., Cieraad, E., Hunt, J.E., Fraser, A., Turnbull, M.H., Whitehead, D., 2016. Soil heterotrophic respiration is insensitive to changes in soil water content but related to microbial access to organic matter. Geoderma 274, 68–78. http://dx.doi.org/10.1016/j.geoderma.2016.03.027.

Mørkved, P.T., Dörsch, P., Bakken, L.R., 2007. The N_2O product ratio of nitrification and its dependence on long-term changes in soil pH. Soil Biol. Biochem. 39, 2048–2057. https://doi.org/10.1016/j.soilbio.2007.03.006.

Morley, N., Baggs, E.M., 2010. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biol. Biochem. 42, 1864–1871. https://doi.org/10.1016/j.soilbio.2010.07.008.

Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., 1998. Closing the global N_2O budget : nitrous oxide emissions through the agricultural nitrogen cycle inventory methodology. Nutr. Cycl. Agroecosystems 52, 225–248. https://doi.org/10.1023/A:1009740530221.

Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol. Biochem. 59, 72–85. http://dx.doi.org/10.1016/j.soilbio.2013.01.002.

Mujić, I., Šertović, E., Jokić, S., Sarić, Z., Alibabić, V., Vidović, S., Živković, J., 2011. Isoflavone content and antioxidant properties of soybean seeds. Croat. J. Food Sci. Technol. 3, 16–20.

Mukome, F.N.D., Parikh, S.J., 2016. Chemical, Physical, and Surface Characterization of Biochar. In: Ok, Y.S., Uchimiya, S.M., Chang, S.X., Bolan, N. (Eds.), Biochar: Production, Characterization, and Applications. CRC Press, Boca Raton, Florida, USA, pp. 68–96.

Munro, I.C., Harwood, M., Hlywka, J.J., Stephen, A.M., Doull, J., Flamm, W.G., Adlercreutz, H., 2003. Soy isoflavones: A safety review. Nutr. Rev. 61, 1–33. https://doi.org/10.1301/nr.2003.janr.1-33.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31–36.

Nanda, S., Dalai, A.K., Berruti, F., Kozinski, J.A., 2016. Biochar as an Exceptional Bioresource for Energy, Agronomy, Carbon Sequestration, Activated Carbon and Specialty Materials. Waste and Biomass Valorization 7, 201–235. https://doi.org/10.1007/s12649-015-9459-z.

Neeteson, J.J., Carton, O.T., 2001. The environmental impact of nitrogen in field vegetable production. Acta Hortic. 563, 21–28. https://doi.org/10.17660/ActaHortic.2001.563.1.

Nelson, N. O., Agudelo, S. C., Yuan, W., Gan, J., 2011. Nitrogen and phosphorus availability in biochar-amended soils. Soil Sci. 176 (5), 218–226. Doi:10.1097/SS.0b013e3182171eac.

Nett, L., Fuß, R., Flessa, H., Fink, M., 2015. Emissions of nitrous oxide and ammonia from a sandy soil following surface application and incorporation of cauliflower leaf residues. J. Agric. Sci. 153, 1341–1352. https://doi.org/10.1017/S0021859615000027.

Nett, L., Sradnick, A., Fuß, R., Flessa, H., Fink, M., 2016. Emissions of nitrous oxide and ammonia after cauliflower harvest are influenced by soil type and crop residue management. Nutr. Cycl. Agroecosystems 106, 217–231. https://doi.org/10.1007/s10705-016-9801-2.

Nguyen, D.H., Biala, J., Grace, P.R., Scheer, C., Rowlings, D.W., 2014. Greenhouse gas emissions from sub-tropical agricultural soils after addition of organic by-products. Springerplus 3, 1–14. https://doi.org/10.1186/2193-1801-3-491.

Nguyen, D.H., Scheer, C., Rowlings, D.W., Grace, P.R., 2016. Rice husk biochar and crop residue amendment in subtropical cropping soils: effect on biomass production, nitrogen use efficiency and greenhouse gas emissions. Biol. Fertil. Soils 52, 261–270. https://doi.org/10.1007/s00374-015-1074-4.

Nigussie, A., Kissi, E., Misganaw, M., Ambaw, G., 2012. Effect of biochar application on soil properties and nutrient uptake of lettuces (*Lactuca sativa*) grown in chromium polluted soils. Am. J. Agric. Environ. Sci. 12, 369–376.

Niu, Y., Luo, J., Liu, D., Müller, C., Zaman, M., Lindsey, S., Ding, W., 2018. Effect of biochar and nitrapyrin on nitrous oxide and nitric oxide emissions from a sandy loam soil cropped to maize. Biol. Fertil. Soils 54, 645–658. https://doi.org/10.1007/s00374-018-1289-2.

Nolan, N.E., Kulmatiski, A., Beard, K.H., Norton, J.M., 2015. Activated carbon decreases invasive plant growth by mediating plant-Microbe interactions. AoB Plants 7. https://doi.org/10.1093/aobpla/plu072.

Novoa, R.S.A., Tejeda, H.R., 2006. Evaluation of the N_2O emissions from N in plant residues as affected by environmental and management factors. Nutr. Cycl. Agroecosystems 75, 29–46. https://doi.org/10.1007/s10705-006-9009-y.

Obia, A., Mulder, J., Martinsen, V., Cornelissen, G., Børresen, T., 2016. In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils. Soil Tillage Res. 155, 35–44. https://doi.org/10.1016/j.still.2015.08.002.

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., Erasmi, S., 2016. Greenhouse gas emissions from soils—A review. Chemie der Erde 76, 327–352. http://dx.doi.org/10.1016/j.chemer.2016.04.002.

O'Hara, G.W., Daniel, R.M., 1985. Rhizobial denitrification: A review. Soil Biol. Biochem. 17 (1), 1–9. https://doi.org/10.1016/0038-0717(85)90082-3.

Oleszczuk, P., Godlewska, P., Reible, D.D., Kraska, P., 2017. Bioaccessibility of polycyclic aromatic hydrocarbons in activated carbon or biochar amended vegetated (*Salix viminalis*) soil. Environ. Pollut. 227, 406–413. https://doi.org/10.1016/j.envpol.2017.04.064.

Oo, A.Z., Nguyen, L., Win, K.T., Cadisch, G., Bellingrath-Kimura, S.D., 2013. Toposequential variation in methane emissions from double-cropping paddy rice in Northwest Vietnam. Geoderma 209–210, 41–49. https://doi.org/10.1016/j.geoderma.2013.05.025.

Oo, A.Z., Sudo, S., Win, K.T., Shibata, A., Gonai, T., 2018. Influence of pruning waste biochar and oyster shell on N_2O and CO_2 emissions from Japanese pear orchard soil. Heliyon 4, e00568. https://doi.org/10.1016/j.heliyon.2018.e00568.

Paavolainen, L., Kitunen, V., Smolander, A., 1998. Inhibition of nitrification in forest soil by monoterpenes. Plant Soil 205, 147–154. https://doi.org/10.1023/A:1004335419358.

Pal, P., 2016. Biochar Effects on Greenhouse Gas Emissions. In: Ok, Y.S., Uchimiya, S.M., Chang, S.X., Bolan, N. (Eds.), Biochar: Production, Characterization, and Applications. CRC Press, Boca Raton, Florida, USA, pp. 359–386.

Paraskevopoulos, K., Antonyuk, S.V., Sawers, R.G., Eady, R.R., Hasnain, S.S., 2006. Insight into Catalysis of Nitrous Oxide Reductase from High-resolution Structures of Resting and Inhibitor-bound Enzyme from *Achromobacter cycloclastes*. Journal of Molecular Biology 362, 55–65. https://doi.org/10.1016/j.jmb.2006.06.064.

Pavlíková, D., Zemanová, V., Břendová, K., Kubátová, P., Tlustoš, P., 2017. Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard. Plant Soil Environ. 63, 322–327. https://doi.org/10.17221/318/2017-PSE.

Petter, F.A., Borges de Lima, L., Marimon Júnior, B.H., Alves de Morais, L., Marimon, B.S., 2016. Impact of biochar on nitrous oxide emissions from upland rice. J. Environ. Manag. 169, 27–33. https://doi.org/10.1016/j.jenvman.2015.12.020.

Pietikäinen, J., Kiikkilä, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231–242. https://doi.org/10.1034/j.1600-0706.2000.890203.x.

Piper, E.L., Boote, K.I., 1999. Temperature and cultivar effects on soybean seed oil and protein concentrations. J. Am. Oil Chem. Soc. 76, 1233–1241. https://doi.org/10.1007/s11746-999-0099-y.

Pueppke, S.G., Bolaños-Vásquez, M.C., Werner, D., Bec-Ferté, M.P., Promé, J.C., Krishnan, H.B., 1998. Release of flavonoids by the soybean cultivars McCall and Peking and their perception as signals by the nitrogen-fixing symbiont *Sinorhizobium fredii*. Plant Physiol. 117, 599–608. https://doi.org/10.1104/pp.117.2.599.

Pugesgaard, S., Petersen, S.O., Chirinda, N., Olesen, J.E., 2017. Crop residues as driver for N_2O emissions from a sandy loam soil. Agric. For. Meteorol. 233, 45–54. https://doi.org/10.1016/j.agrformet.2016.11.007.

Quilliam, R.S., Marsden, K.A., Gertler, C., Rousk, J., DeLuca, T.H., Jones, D.L., 2012. Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric. Ecosyst. Environ. 158, 192–199. https://doi.org/10.1016/j.agee.2012.06.011.

Rahn, C.R., Bending, G.D., Lillywhite, R.D., Turner, M.K., 2003. Novel techniques to reduce environmental N pollution from high nitrogen content crop residues. Acta Hortic. 627, 105–111. https://doi.org/10.17660/ActaHortic.2003.627.12.

Rajkovich, S., Enders, A., Hanley, K., Hyland, C., Zimmerman, A.R., Lehmann, J., 2012. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol. Fertil. Soils 48, 271–284. https://doi.org/10.1007/s00374-011-0624-7.

Rascio, N., Rocca N. L., 2008. Biological Nitrogen Fixation. Encyclopedia of Ecology, pp. 412–419. https://doi.org/10.1016/B978-008045405-4.00273-1.

Rastogi, M., Singh, S., Pathak, H., 2002. Emission of carbon dioxide from soil. Current Science 82, 510–517.

Rezaei Rashti, M., Wang, W.J., Chen, C.R., Reeves, S.H., Scheer, C., 2017. Assessment of N_2O emissions from a fertilised vegetable cropping soil under different plant residue management strategies using ^{15}N tracing techniques. Sci. Total Environ. 598, 479–487. https://doi.org/10.1016/j.scitotenv.2017.04.030.

Rezaei Rashti, M., Wang, W.J., Reeves, S.H., Harper, S.M., Moody, P.W., Chen, C.R., 2016. Linking chemical and biochemical composition of plant materials to their effects on N_2O emissions from a vegetable soil. Soil Biol. Biochem. 103, 502–511. https://doi.org/10.1016/j.soilbio.2016.09.019.

Richardson, D., Felgate, H., Watmough, N., Thomson, A., Baggs, E., 2009. Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle – could enzymic regulation hold the key? Trends in Biotech. 27 (7), 388–397. https://doi.org/10.1016/j.tibtech.2009.03.009.

Rochette, P., Janzen, H.H., 2005. Towards a revised coefficient for estimating N_2O emissions from legumes. Nutr. Cycl. Agroecosystems 73, 171–179. https://doi.org/10.1007/s10705-005-0357-9.

Rogovska, N., Laird, D., Cruse, R.M., Trabue, S., Heaton, E., 2012. Germination tests for assessing biochar quality. J. Environ. Qual. 41, 1014–1022. https://doi.org/10.2134/jeq2011.0103.

Saarnio, S., Heimonen, K., Kettunen, R., 2013. Biochar addition indirectly affects N_2O emissions via soil moisture and plant N uptake. Soil Biol. Biochem. 58, 99–106. https://doi.org/10.1016/j.soilbio.2012.10.035.

Saarnio, S., Räty, M., Hyrkäs, M., Virkajärvi, P., 2018. Biochar addition changed the nutrient content and runoff water quality from the top layer of a grass field during simulated snowmelt. Agric. Ecosyst. Environ. 265, 156–165. https://doi.org/10.1016/j.agee.2018.06.007.

Sabry, K.H., 2015. Synthetic Fertilizers; Role and Hazards. Fertilizer Technology Vol. 1: Synthesis. https://doi.org/10.13140/RG.2.1.2395.3366.

Sakthivelu, G., Akitha Devi, M.K., Giridhar, P., Rajasekaran, T., Ravishankar, G.A., Nikolova, M.T., Angelov, G.B., Todorova, R.M., Kosturkova, G.P., 2008. Isoflavone composition, phenol content, and antioxidant activity of soybean seeds from India and Bulgaria. J. Agric. Food Chem. 56, 2090–2095. https://doi.org/10.1021/jf072939a.

Sameshima-Saito, R., Chiba, K., Hirayama, J., Itakura, M., Mitsui, H., Eda, S., Minamisawa, K., 2006. Symbiotic *Bradyrhizobium japonicum* reduces N_2O surrounding the soybean root system via nitrous oxide reductase. Appl. Environ. Microbiol. 72, 2526–2532. https://doi.org/10.1128/AEM.72.4.2526-2532.2006.

Sanchez, C., Minamisawa, K., 2019. Nitrogen Cycling in Soybean Rhizosphere: Sources and Sinks of Nitrous Oxide (N_2O). Front. Microbiol. 10, 1943. https://doi.org/10.3389/fmicb.2019.01943.

Sanchez-Garcia, M., Roig, A., Sanchez-Monedero, M.A., Cayuela, M.L., 2014. Biochar increases soil N_2O emissions produced by nitrification-mediated pathways. Frontiers in Environmental Science 2, 25. https://doi.org/10.3389/fenvs.2014.00025.

Sant'Anna, S.A.C., Martins, M.R., Goulart, J.M., Araújo, S.N., Araújo, E.S., Zaman, M., Jantalia, C.P., Alves, B.J.R., Boddey, R.M., Urquiaga, S., 2018. Biological nitrogen fixation and soil N_2O emissions from legume residues in an Acrisol in SE Brazil. Geoderma Reg. 15, e00196. https://doi.org/10.1016/j.geodrs.2018.e00196.

Saunois, M., A.R. Stavert, B. Poulter et al., 2019: The Global Methane Budget 2000–2017, *Earth System Science Data*. https://doi.org/10.5194/essd-2019-128.

Scheer, C., Rowlings, D., Firrell, M., Deuter, P., Morris, S., Riches, D., Porter, I., Grace, P., 2017. Nitrification inhibitors can increase post-harvest nitrous oxide emissions in an intensive vegetable production system. Sci. Rep. 7, 1–9. https://doi.org/10.1038/srep43677.

Scheer, C., Rowlings, D.W., Firrel, M., Deuter, P., Morris, S., Grace, P.R., 2014. Impact of nitrification inhibitor (DMPP) on soil nitrous oxide emissions from an intensive broccoli production system in sub-tropical Australia. Soil Biol. Biochem. 77, 243–251. https://doi.org/10.1016/j.soilbio.2014.07.006.

Schimmelpfennig, S., Müller, C., Grünhage, L., Koch, C., Kammann, C., 2014. Biochar, hydrochar

and uncarbonized feedstock application to permanent grassland-Effects on greenhouse gas emissions and plant growth. Agric. Ecosyst. Environ. 191, 39–52. https://doi.org/10.1016/j.agee.2014.03.027.

Schmidt, C.S., Richardson, D.J., Baggs, E.M., 2011. Constraining the conditions conducive to dissimilatory nitrate reduction to ammonium in temperate arable soils. Soil Biol. Biochem. 43, 1607–1611. https://doi.org/10.1016/j.soilbio.2011.02.015.

Seghers, D., Siciliano, S.D., Top, E.M., Verstraete, W., 2005. Combined effect of fertilizer and herbicide applications on the abundance, community structure and performance of the soil methanotrophic community. Soil Biol. Biochem. 37, 187–193. https://doi.org/10.1016/j.soilbio.2004.05.025.

Šertovic, E., Mujić, I., Jokic, S., Alibabic, V., Saric, Z., 2012. Effect of soybean cultivars on the content of isoflavones in soymilk. Rom. Biotechnol. Lett. 17, 7151–7159.

Setyanto, P., Rosenani, A.B., Makarim, A.K., Ishak, C.F., Bidin, A., Suharsih, A., 2002. Soil controlling factors of methane gas production from flooded rice fields in Pati District, Central Java. Indonesian Journal of Agricultural Science 3 (1), 1–11.

Shah, A., 2014. Determination of biological nitrogen fixation induced N_2O emission from arable soil by using a closed chamber technique. Appl. Environ. Soil Sci. 2014. https://doi.org/10.1155/2014/685168.

Shan, J., Yan, X., 2013. Effects of crop residue returning on nitrous oxide emissions in agricultural soils. Atmos. Environ. 71, 170–175. https://doi.org/10.1016/j.atmosenv.2013.02.009.

Shaw, L.J., Morris, P., Hooker, J.E., 2006. Perception and modification of plant flavonoid signals by rhizosphere microorganisms. Environ. Microbiol. 8, 1867–1880. https://doi.org/10.1111/j.1462-2920.2006.01141.x.

Shi, P., Qin, Y., Liu, Q., Zhu, T., Li, Z., Li, P., Ren, Z., Liu, Y., Wang, F., 2020. Soil respiration and response of carbon source changes to vegetation restoration in the Loess Plateau, China. Sci. Total Environ. 707, 135507. https://doi.org/10.1016/j.scitotenv.2019.135507.

Shiro, Y., 2012. Structure and function of bacterial nitric oxide reductases Nitric oxide reductase, anaerobic enzymes. Biochimica et Biophysica Acta 1817, 1907–1913. https://doi.org/10.1016/j.bbabio.2012.03.001.

Shrestha, G., Traina, S.J., Swanston, C.W., 2010. Black carbon's properties and role in the environment: A comprehensive review. Sustainability 2, 294–320. https://doi.org/10.3390/su2010294.

Signor, D., Cerri, C. E. P., 2013. Nitrous oxide emissions in agricultural soils: a review. *Pesquisa Agropecuária Tropical* Goiânia 43 (3), 322–338. http://dx.doi.org/10.1590/S1983-40632013000300014.

Singh, Y., Singh, B., Timsina, J., 2005. Crop residue management for nutrient cycling and improving soil productivity in rice-based cropping systems in the tropic. Advances in Agronomy 85, 269–407. https://doi.org/10.1016/S0065-2113(04)85006-5.

Singla, A., Dubey, S.K., Iwasa, H., Inubushi, K., 2013. Nitrous oxide flux from komatsuna (*Brassica rapa*) vegetated soil: A comparison between biogas digested liquid and chemical fertilizer. Biol. Fertil. Soils 49, 971–976. https://doi.org/10.1007/s00374-013-0796-4.

Singla, A., Iwasa, H., Inubushi, K., 2014. Effect of biogas digested slurry based-biochar and digested liquid on N_2O, CO_2 flux and crop yield for three continuous cropping cycles of Komatsuna (*Brassica rapa* var. perviridis). Biol. Fertil. Soils 50, 1201–1209. https://doi.org/10.1007/s00374-014-0950-7.

Skiba, U.M., Rees, R.M., 2014. Nitrous oxide, climate change and agriculture. CAB Rev. 9, 1–7. https://doi.org/10.1079/PAVSNNR20149010.

Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A., 2018. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur. J. Soil Sci. 69, 10–20. https://doi.org/10.1111/ejss.12539.

Smith, K.A., Cresser, M.S., 2004. Soil and Environmental Analysis. *Modern Instrumentation Techniques,* 3rd Edition, Marcel Dekker Inc, New York.

Snyder, C.S., Bruulsema, T.W., Jensen, T.L., Fixen, P.E., 2009. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agric. Ecosyst. Environ. 133, 247–266. https://doi.org/10.1016/j.agee.2009.04.021.

Sohi, S., Lopez-Capel, E., Krull, E., Bol, R., 2009. Biochar's roles in soil and climate change: A review of research needs. CSIRO Land. Water Sci. Rep. 64 pp.

Song, X., Pan, G., Zhang, C., Zhang, L., Wang, H., 2016. Effects of biochar application on fluxes of three biogenic greenhouse gases: a meta-analysis. Ecosystem Health and Sustainability 2(2):e01202. https://doi.org/10.1002/ehs2.1202.

Spiro, S., 2012. Nitrous oxide production and consumption: regulation of gene expression by gas sensitive transcription factors. Philosophical Transactions of the Royal Society B 367, 1213–1225. doi:10.1098/rstb.2011.0309.

Spokas, K.A., 2013. Impact of biochar field aging on laboratory greenhouse gas production potentials. GCB Bioenergy 5, 165–176. https://doi.org/10.1111/gcbb.12005.

Spott, O., Russow, R., Stange, C.F., 2011. Formation of hybrid N_2O and hybrid N_2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. Soil Biol. Biochem. 43, 1995–2011. https://doi.org/10.1016/j.soilbio.2011.06.014.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., De Macêdo, J.L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil**.** Plant Soil 291, 275–290. https://doi.org/10.1007/s11104-007-9193-9.

Streubel, J.D., Collins, H.P., Garcia-Perez, M., Tarara, J., Granatstein, D., Kruger, C.E., 2011. Influence of contrasting biochar types on five soils at increasing rates of application. Soil Sci. Soc. Am. J., 75(4), 1402–1413. https://doi.org/10.2136/sssaj2010.0325.

Subedi, R., Taupe, N., Pelissetti, S., Petruzzelli, L., Bertora, C., Leahy, J.J., Grignani, C., 2016. Greenhouse gas emissions and soil properties following amendment with manure-derived biochars: Influence of pyrolysis temperature and feedstock type. J. Environ. Manage. 166, 73–83. https://doi.org/10.1016/j.jenvman.2015.10.007.

Suddick, E.C., Six, J., 2013. An estimation of annual nitrous oxide emissions and soil quality following the amendment of high temperature walnut shell biochar and compost to a small scale vegetable crop rotation. Sci. Total Environ. 465, 298–307. https://doi.org/10.1016/j.scitotenv.2013.01.094.

Sugiyama, A., 2019. The soybean rhizosphere: Metabolites, microbes, and beyond—A review. J. Adv. Res. 19, 67–73. https://doi.org/10.1016/j.jare.2019.03.005.

Sugiyama, A., Shitan, N., Yazaki, K., 2007. Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-Rhizobium symbiosis. Plant Physiol. 144, 2000–2008. https://doi.org/10.1104/pp.107.096727.

Sugiyama, A., Yamazaki, Y., Hamamoto, S., Takase, H., Yazaki, K., 2017. Synthesis and secretion of isoflavones by field-grown soybean. Plant Cell Physiol. 58, 1594–1600. https://doi.org/10.1093/pcp/pcx084.

Sugiyama, A., Yazaki, K., 2014. Flavonoids in plant rhizospheres: Secretion, fate and their effects on biological communication. Plant Biotechnol. 31, 431–443. https://doi.org/10.5511/plantbiotechnology.14.0917a.

Suzuki, H., Takahashi, S., Watanabe, R., Fukushima, Y., Fujita, N., Noguchi, A., Yokoyama, R., Nishitani, K., Nishino, T., Nakayama, T., 2006. An isoflavone conjugate-hydrolyzing β-glucosidase from the roots of soybean (*Glycine max*) seedlings: Purification, gene cloning, phylogenetics, and cellular localization. J. Biol. Chem. 281, 30251–30259. https://doi.org/10.1074/jbc.M605726200.

Syakila, A., Kroeze, C., 2011. The global nitrous oxide budget revisited. Greenh. Gas Meas. Manage. 1, 17–26. https://doi.org/10.3763/ghgmm.2010.0007.

Syuhada, A.B., Shamshuddin, J., Fauziah, C.I., Rosenani, A.B., Arifin, A., 2016. Biochar as soil amendment: Impact on chemical properties and corn nutrient uptake in a Podzol. Can. J. Soil Sci. 96, 400–412. https://doi.org/10.1139/cjss-2015-0044.

Taie, H. A. A., El-Mergawi, R., Radwan, S., 2008. Isoflavonoids, Flavonoids, Phenolic Acids Profiles and Antioxidant Activity of Soybean Seeds as Affected by Organic and Bioorganic Fertilization. Am. J. Agric. Environ. Sci. 4, 207–213.

Takahashi, M., Arihara, J., Nakayama, N., Kokubun, M., 2003. Characteristics of growth and yield formation in the improved genotype of supernodulating soybean (*Glycine max* L. Merr.). Plant Prod. Sci. 6, 112–118. https://doi.org/10.1626/pps.6.112.

Teekachunhatean, S., Hanprasertpong, N., Teekachunhatean, T., 2013. Factors Affecting Isoflavone Content in Soybean Seeds Grown in Thailand. Int. J. Agron. 2013, 1–11. https://doi.org/10.1155/2013/163573.

Tepavčević, V., Cvejić, J., Poša, M., Popović, J., 2011. Isoflavone Content and Composition in Soybean. Soybean - Biochem. Chem. Physiol. https://doi.org/10.5772/14736.

Thangarajan, R., Bolan, N.S., Tian, G., Naidu, R., Kunhikrishnan, A., 2013. Role of organic amendment application on greenhouse gas emission from soil. Sci. Total Environ. 465, 72–96. https://doi.org/10.1016/j.scitotenv.2013.01.031.

Thomas, R. L., Sheard, R. W., Moyer, J. R., 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agronomy Journal. 59, 240–243. https://doi.org/10.2134/agronj1967.00021962005900030010x.

Thomazini, A., Spokas, K., Hall, K., Ippolito, J., Lentz, R., Novak, J., 2015. GHG impacts of biochar: Predictability for the same biochar. Agric. Ecosyst. Environ. 207, 183–191. https://doi.org/10.1016/j.agee.2015.04.012.

Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. PNAS 108, 20260–20264. www.pnas.org/cgi/doi/10.1073/pnas.1116437108.

Toma, Y., Hatano, R., 2007. Effect of crop residue C:N ratio on N_2O emissions from Gray Lowland soil in Mikasa, Hokkaido, Japan. Soil Sci. Plant Nutr. 53, 198–205. https://doi.org/10.1111/j.1747-0765.2007.00125.x.

Topp, E., Pattey, E., 1997. Soils as sources and sinks for atmospheric methane. Can. J. Soil Sci. 77, 167–177. https://doi.org/10.4141/S96-107.

Trigo, C., Spokas, K.A., Cox, L., Koskinen, W.C., 2014. Influence of soil biochar aging on

sorption of the herbicides MCPA, nicosulfuron, terbuthylazine, indaziflam, and fluoroethyldiaminotriazine. J. Agric. Food Chem. 62, 10855–10860. https://doi.org/10.1021/jf5034398.

Troy, S.M., Lawlor, P.G., O' Flynn, C.J., Healy, M.G., 2013. Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. Soil Biol. Biochem. 60, 173–181. https://doi.org/10.1016/j.soilbio.2013.01.019.

Truog, E., 1930. The determination of readily available phosphorus in soils. Am Soc Agron. 22, 874–882.

Tsukamoto, C., Shimada, S., Igita, K., Kudou, S., Kokubun, M., Okubo, K., Kitamura, K., 1995. Factors Affecting Isoflavone Content in Soybean Seeds: Changes in Isoflavones, Saponins, and Composition of Fatty Acids at Different Temperatures during Seed Development. J. Agric. Food Chem. 43, 1184–1192. https://doi.org/10.1021/jf00053a012.

Tuckett, R.P., 2016. The Role of Atmospheric Gases, in: Letcher, T.M. (Ed.), Climate Change: Observed Impacts on Planet Earth, Elsevier, Amsterdam, Netherlands. pp. 375–397. http://dx.doi.org/10.1016/B978-0-444-63524-2.00024-5.

Turner, E.R., 1955. The effect of certain adsorbents on the nodulation of clover plants. Annals of Botany 19, 149–160. https://doi.org/10.1093/oxfordjournals.aob.a083415.

Uchida, Y., Akiyama, H., 2013. Mitigation of postharvest nitrous oxide emissions from soybean ecosystems: a review. Soil Sci. Plant Nutr. 59, 477–487. http://dx.doi.org/10.1080/00380768.2013.805433.

UN DESA, 2019. United Nations, Department of Economic and Social Affairs, Population Division. *World Population Prospects 2019: Highlights* (ST/ESA/SER.A/423).

UNEP, 2013. Drawing Down N_2O to Protect Climate and the Ozone Layer. A UNEP Synthesis Report. United Nations Environment Programme (UNEP), Nairobi, Kenya.

Uzoma, K. C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., Nishihara, E., 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use Manag. 27, 205–212. https://doi.org/10.1111/j.1475-2743.2011.00340.x.

Vantsis, J.T., Bond, G., 1950. The Effect of Charcoal on the Growth of Leguminous Plants in Sand Culture. Ann. Appl. Biol. 37, 159–168. https://doi.org/10.1111/j.1744-7348.1950.tb01036.x.

Velthof, G.L., Kuikman, P.J., Oenema, O., 2002. Nitrous oxide emission from soils ammended with crop residues. Nutr. Cycl. Agroecosystems 62, 249–261.

Verhoeven, E., Six, J., 2014. Biochar does not mitigate field-scale N_2O emissions in a Northern California vineyard: An assessment across two years. Agric. Ecosyst. Environ. 191, 27–38. https://doi.org/10.1016/j.agee.2014.03.008.

Wacal, C., Ogata, N., Basalirwa, D., Handa, T., Sasagawa, D., Acidri, R., Ishigaki, T., Kato, M., Masunaga, T., Yamamoto, S., Nishihara, E., 2019. Growth, Seed Yield, Mineral Nutrients and Soil Properties of Sesame (*Sesamum indicum* L.) as Influenced by Biochar Addition on Upland Field Converted from Paddy. Agronomy 9, 55. https://doi.org/10.3390/agronomy9020055.

Wallstedt, A., Coughlan, A., Munson, A.D., Nilsson, M.C., Margolis, H.A., 2002. Mechanisms of interaction between *Kalmia angustifolia* cover and *Picea mariana* seedlings. Can. J. For. Res. 32, 2022–2031. https://doi.org/10.1139/x02-124.

Walworth, J.L., Sumner, M.E., 1987. The Diagnosis and Recommendation Integrated System (DRIS). Vol. 6 of Advances in Soil Science of B. A. Stewart (Ed). New York: Springer-Verlag, 149–188.

Wang, F., Kuzuya, T., Hirai, S., Li, J., Li, T., 2014. Carbon dioxide absorption and release properties of pyrolysis products of dolomite calcined in vacuum atmosphere. Sci. World J. 2014, 862762. https://doi.org/10.1155/2014/862762.

Wang, H., Zheng, H., Jiang, Z., Dai, Y., Liu, G., Chen, L., Luo, X., Liu, M., Wang, Z., 2017. Efficacies of biochar and biochar-based amendment on vegetable yield and nitrogen utilization in four consecutive planting seasons. Sci. Total Environ. 593–594, 124–133. https://doi.org/10.1016/j.scitotenv.2017.03.096.

Wang, J., Chen, Z., Xiong, Z., Chen, C., Xu, X., Zhou, Q., Kuzyakov, Y., 2015a. Effects of biochar amendment on greenhouse gas emissions, net ecosystem carbon budget and properties of an

acidic soil under intensive vegetable production. Soil Use Manag. 31, 375–383. https://doi.org/10.1111/sum.12202.

Wang, M., Fu, Y., Liu, H., 2016. Nutritional quality and ions uptake to PTNDS in soybeans. Food Chem. 192, 750–759. https://doi.org/10.1016/j.foodchem.2015.07.002.

Wang, W., Lai, D.Y.F., Sardans, J., Wang, C., Datta, A., Pan, T., Zeng, C., Bartrons, M., Penuelas, J., 2015b. Rice straw incorporation affects global warming potential differently in early vs. late cropping seasons in Southeastern China. Field Crops Res. 181, 42–51. https://doi.org/10.1016/j.fcr.2015.07.007.

Weißhuhn, K., Prati, D., 2009. Activated carbon may have undesired side effects for testing allelopathy in invasive plants. Basic Appl. Ecol. 10, 500–507. https://doi.org/10.1016/j.baae.2008.10.009.

Weil, R.R., Brady, N.C., 2016. The Nature and Properties of Soils. Harlow, England: Pearson.

WMO, 2019. World Meteorological Organization: WMO Reactive Gases Bulletin: The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2018.

Woldetsadik, D., Drechsel, P., Marschner, B., Itanna, F., Gebrekidan, H., 2017. Effect of biochar derived from faecal matter on yield and nutrient content of lettuce (*Lactuca sativa*) in two contrasting soils. Environ. Syst. Res. 6, 2. https://doi.org/10.1186/s40068-017-0082-9.

Wrage, N., Velthof, G.L., Van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33, 1723–1732. https://doi.org/10.1016/S0038-0717(01)00096-7.

Wu, H., Yip, K., Kong, Z., Li, C., Liu, D., Yu, Y., Gao, X., 2011. Removal and Recycling of Inherent Inorganic Nutrient Species in Mallee Biomass and Derived Biochars by Water Leaching. Ind. Eng. Chem. Res. 50 (21), 12143–12151. https://doi.org/10.1021/ie200679n.

Wu, X., Wang, F., Li, T., Fu, B., Lv, Y., Liu, G., 2020. Nitrogen additions increase N_2O emissions but reduce soil respiration and CH_4 uptake during freeze–thaw cycles in an alpine meadow. Geoderma 363, 114157. https://doi.org/10.1016/j.geoderma.2019.114157.

Wu, Z., Dong, Y. B., Xiong, Z. Q., 2018a. Effects of biochar application three-years ago on global warming potentials of CH_4 and N_2O in a rice wheat rotation system. Chinese J. Appl. Ecol. 29, 141–148. https://doi.org/10.13287/j.1001-9332.201801.028.

Wu, Z., Zhang, X., Dong, Y., Xu, X., Xiong, Z., 2018b. Microbial explanations for field-aged biochar mitigating greenhouse gas emissions during a rice-growing season. Environ. Sci. Pollut. Res. 25, 31307–31317. https://doi.org/10.1007/s11356-018-3112-x.

Wurst, S., Van Beersum, S., 2009. The impact of soil organism composition and activated carbon on grass-legume competition. Plant Soil 314, 1–9. https://doi.org/10.1007/s11104-008-9618-0.

Xiao, L., Sun, Q., Yuan, H., Li, X., Chu, Y., Ruan, Y., Lu, C., Lian, B., 2016. A feasible way to increase carbon sequestration by adding dolomite and K-feldspar to soil. Cogent Geosci. 2, 1–11. https://doi.org/10.1080/23312041.2016.1205324.

Xu, L. J., Xu, X. L., Tang, X. J., Yang, G. X. Zhang, Z., Xin, X. P., 2017. Effect of different forage species on the nitrogen uptake in Hulunbeir. International symposium on Earth Observation for One Belt and One Road (EOBAR), Beijing, China. IOP Conference Series: Earth and Environmental Science 57, 012046. https://doi.org/10.1088/1755-1315/57/1/012046.

Xu, N., Tan, G., Wang, H., Gai, X., 2016. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur. J. Soil Biol. 74, 1–8. https://doi.org/10.1016/j.ejsobi.2016.02.004.

Yadav, V., Jain, S., Mishra, P., Khare, P., Shukla, A.K., Karak, T., Singh, A.K., 2019. Amelioration in nutrient mineralization and microbial activities of sandy loam soil by short term field aged biochar. Appl. Soil Ecol. 138, 144–155. https://doi.org/10.1016/j.apsoil.2019.01.012.

Yamaki, A., 2003. A rapid UV absorption method for determination of nitrate in soil extracts. Jpn. J. Soil Sci. Plant Nutr. 74, 195–197.

Yan, Z., Guan, H., Han, W., Han, T., Guo, Y., Fang, J., 2016. Reproductive organ and young tissues show constrained elemental composition in *Arabidopsis thaliana*. Ann. Bot. 117, 431–439. https://doi.org/10.1093/aob/mcv190.

Yang, L., Cai, Z., 2006. Effects of shading soybean plants on N_2O emission from soil. Plant Soil 283, 265–274. https://doi.org/10.1007/s11104-006-0017-0.

Yang, L., Cai, Z., 2005. The effect of growing soybean (*Glycine max*. L.) on N_2O emission from soil. Soil Biol. Biochem. 37, 1205–1209. https://doi.org/10.1016/j.soilbio.2004.08.027.

Yin, S.X., Chen, D., Chen, L.M., Edris, R., 2002. Dissimilatory nitrate reduction to ammonium and responsible microorganisms in two Chinese and Australian paddy soils. Soil Biol. Biochem. 34, 1131–1137. https://doi.org/10.1016/S0038-0717(02)00049-4.

Yu, J.Q., Lee, K.S., Matsui, Y., 1993. Effect of the addition of activated charcoal to the nutrient solution on the growth of tomato in hydroponic culture. Soil Sci. Plant Nutr. 39, 13–22. https://doi.org/10.1080/00380768.1993.10416970.

Yuan, Y., Tang, J., Leng, D., Hu, S., Yong, J.W.H., Chen, X., 2014. An invasive plant promotes its arbuscular mycorrhizal symbioses and competitiveness through its secondary metabolites: Indirect evidence from activated carbon. PLoS One 9, e97163 https://doi.org/10.1371/journal.pone.0097163.

Yue, Y., Cui, L., Lin, Q., Li, G., Zhao, X., 2017. Efficiency of sewage sludge biochar in improving urban soil properties and promoting grass growth. Chemosphere 173, 551–556. https://doi.org/10.1016/j.chemosphere.2017.01.096.

Zengeni, R., Mpepereki, S., Giller, K.E., 2006. Manure and soil properties affect survival and persistence of soyabean nodulating rhizobia in smallholder soils of Zimbabwe. Appl. Soil Ecol. 32, 232–242. https://doi.org/10.1016/j.apsoil.2005.06.001.

Zhang, A., Bian, R., Hussain, Q., Li, L., Pan, G., Zheng, J., Zhang, X., Zheng, J., 2013. Change in net global warming potential of a rice-wheat cropping system with biochar soil amendment in a rice paddy from China. Agric. Ecosyst. Environ. 173, 37–45. https://doi.org/10.1016/j.agee.2013.04.001.

Zhang, D., Pan, G., Wu, G., Kibue, G.W., Li, L., Zhang, X., Zheng, J., Zheng, J., Cheng, K., Joseph, S., Liu, X., 2016. Biochar helps enhance maize productivity and reduce greenhouse gas emissions under balanced fertilization in a rainfed low fertility inceptisol. Chemosphere 142, 106–113. https://doi.org/10.1016/j.chemosphere.2015.04.088.

Zhang, J., Ge, Y., Han, F., Li, B., Yan, S., Sun, J., Wang, L., 2014. Isoflavone content of soybean cultivars from maturity group 0 to VI grown in northern and southern China. JAOCS, J. Am. Oil Chem. Soc. 91, 1019–1028. https://doi.org/10.1007/s11746-014-2440-3.

Zhang, X., Duan, P., Wu, Z., Xiong, Z., 2019a. Aged biochar stimulated ammonia-oxidizing archaea and bacteria-derived N_2O and NO production in an acidic vegetable soil. Sci. Total Environ. 687, 433–440. https://doi.org/10.1016/j.scitotenv.2019.06.128.

Zhang, X., Mao, X., Pi, L., Wu, T., Hu, Y., 2019b. Adsorptive and capacitive properties of the activated carbons derived from pig manure residues. J. Environ. Chem. Eng. 7, 103066. https://doi.org/10.1016/j.jece.2019.103066.

Zhao, R., Coles, N., Kong, Z., Wu, J., 2014. Effects of aged and fresh biochars on soil acidity under different incubation conditions. Soil Tillage Res. 146, 133–138. https://doi.org/10.1016/j.still.2014.10.014.

Zhao, R., Coles, N., Wu, J., 2015. Carbon mineralization following additions of fresh and aged biochar to an infertile soil. Catena 125, 183–189. https://doi.org/10.1016/j.catena.2014.10.026.

Zhong, Z., Lemke, R.L., Nelson, L.M., 2009. Nitrous oxide emissions associated with nitrogen fixation by grain legumes. Soil Biol. Biochem. 41, 2283–2291. https://doi.org/10.1016/j.soilbio.2009.08.009.

Zhou, Y., Berruti, F., Greenhalf, C., Tian, X., Henry, H.A.L., 2017. Increased retention of soil nitrogen over winter by biochar application: Implications of biochar pyrolysis temperature for plant nitrogen availability. Agric. Ecosyst. Environ. 236, 61–68. https://doi.org/10.1016/j.agee.2016.11.011.

SUMMARY

Agricultural activities such as the use of chemical fertilizers, crop residue incorporation in soil, cultivation of leguminous crops which increase biological N fixation (BNF) have partly contributed to the significant increase in greenhouse gas (GHG) emissions especially nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) that have resulted in global warming. One of the relatively cheaper technologies to achieve GHG mitigation in agriculture is the use of pyrogenic carbonaceous soil amendments such as biochar and activated carbon (AC) because they are not only available on farms after crop harvest, but also sequester carbon in soil, as well as improving crop productivity. The main objective of this study was to assess the effect of pyrogenic carbonaceous soil amendments on greenhouse gas emissions in relation to crop productivity. The specific objectives were to assess (i) the residual effects of palm shell biochar (PSB) on growth and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under three continuous crop cycles without additional N fertilizer application after the first crop cycle; (ii) the impact of fresh and aged PSB on N_2O emissions, soil properties, nutrient content and yield of Komatsuna; (iii) the effect of crop residue and PSB incorporation on GHG emissions during the fallow and crop growing seasons of broccoli (*Brassica oleracea* var. *italica*); (iv) the effect of AC on GHG emissions, seed yield, soil chemical properties and isoflavone content of soybean genotypes with varying nodulation capacities; (v) the effect of AC on N_2O and CO_2 emissions from decomposing root nodules of the soybean genotypes.

In chapter two, a pot experiment was conducted to assess the residual effects of palm shell biochar (PSB) at 0, 6, 12, and 18% w/w of dry soil on growth and yield of Komatsuna. Biochar application in soils with fertilizer did not significantly influence crop yield and N uptake during the first crop cycle. However, with increased cultivation in these soils, biochar hindered N availability to plants and significantly reduced crop growth and yield during the third crop cycle. These results imply that the negative residual effects of PSB on crop growth and yield necessitate the need for more seasonal N fertilizer application in sandy soils which have been previously amended with biochar. It is necessary to assess the role of biochar in mitigating the negative effects (N_2O emissions and soil acidification) that might result from the seasonal fertilizer application.

Chapter three focused on assessing whether the PSB applied in the previous experiment could still mitigate N_2O emissions even when additional basal N fertilizers are applied one year after the initial biochar application while comparing the effects with fresh PSB. The aged PSB

non-significantly reduced N_2O emissions but significantly offset soil acidification, and maintained a high soil nutrient status. Biochar application with fertilizer significantly increased plant tissue K and Ca content but decreased N, P and Mg content compared to the treatments without biochar. At higher application rates, biochar had negative effects on crop yield but as it aged, the negative effects were offset as a result of the similar variation in plant N uptake. Since seasonal N fertilizer application seems to be inevitable in Komatsuna cultivation, addition of biochar could be a possible way of counteracting the effects of excessive fertilizer use.

In chapter four, a field experiment was conducted to evaluate the combined effect of broccoli crop residues and PSB incorporation on GHG emissions during the fallow (post-harvest) and crop growing seasons of broccoli. The treatments included; No-residues (NR), Residues (R), Residues + 10 t ha^{-1} PSB (R10), Residues + 20 t ha^{-1} PSB (R20) and Residues + 40 t ha^{-1} PSB (R40), arranged in a completely randomized block design. The results showed that the fallow season had significantly higher GHG emissions than the crop growing season. Incorporation of crop residues in soil significantly increased N_2O and CO_2 emissions but did not significantly affect CH_4 emissions when compared to those of the NR treatment. PSB amendment did not significantly affect N_2O, CO_2 and CH_4 emissions from crop residues and also the biomass and N uptake of the crop residues remaining after broccoli harvest. The application of PSB at 40 t ha^{-1} significantly increased the total N, total C, C/N ratio and exchangeable K but did not significantly affect soil pH, EC, available P, exchangeable Ca and Mg, and CEC. The large amounts of N_2O and CO_2 emissions emitted from broccoli crop residues during the fallow season may necessitate higher biochar application rates (>40 t ha^{-1}) to achieve the GHG mitigation potential of biochar while maintaining a high soil nutrient status.

Chapter five explains the effect of AC on GHG emissions, seed yield, soil chemical properties and isoflavone content of soybean genotypes with varying nodulation capacities under sandy soil conditions in a 2-year pot experiment. The soybean genotypes were TnVRSN4, Tachinagaha and TnVRNN4 with high, normal and low nodulation capacities respectively. AC was applied at rates equivalent to 0, 2.4, 4.8, and 9.6 t ha^{-1} in combination with inorganic fertilizers. AC tended to reduce soil N_2O emissions in the high nodulating genotype due to the significant reduction in nodulation but did not significantly affect CO_2 and CH_4 emissions. Highest CO_2 emissions and seed yield were observed in the high nodulating genotype and lowest in the low nodulating genotype. AC did not significantly affect seed yield of the high nodulating genotype

but significantly reduced seed yield of the low and normal nodulation genotypes in 2017 and 2018 respectively. Although AC generally increased soil total N, total C and C/N ratio, its effect on soil pH, available P and exchangeable cations significantly varied with the soybean genotype. AC did not significantly affect root isoflavone, seed protein and total isoflavone content but significantly reduced the concentration of daidzein and daidzin which were exuded from soybean roots in soil. This implies that the effects of AC in soil under a particular soybean genotype may not have significant effects on the quality of the seeds. These findings suggest that the high nodulating genotype can perform better than other genotypes in marginalized sandy soils with a low nutrient status.

In chapter six, an incubation experiment was conducted to assess the effect of AC on N_2O and CO_2 emissions from decomposing root nodules of the soybean genotypes described above. The results showed that root nodules of the high and normal nodulating genotypes were important sources of N_2O and CO_2 emissions. These results clarified that the N_2O and CO_2 emissions from root nodules of the two genotypes applied in soil at the same amount did not significantly differ. AC did not have significant effects on cumulative N_2O and CO_2 emissions from the root nodules. To achieve sustainable soybean production, it could be possible to mitigate GHG emissions from the decomposing root nodules and increase soil nutrients by incorporating pyrogenic carbonaceous soil amendments after crop harvest.

The results from the above studies showed that pyrogenic carbonaceous soil amendments have a potential to significantly contribute to GHG mitigation while maintaining crop productivity in vegetable and soybean cropping systems. Compared to AC which could be expensive to most of the farmers, biochar could be an easily available and cheaper option to use by the farmers since it can be made at a local scale. There is need for long term studies on the different techniques of biochar production and usage (when to apply, how to apply and how much to apply) in soil while focusing on the changes in quality of the crop products obtained after biochar application to ensure safety for human consumption. Further research should also focus on exploring the aging effects of different biochars at varying application rates in different biochar types and crops before recommending it for use in the different soil types.

SUMMARY IN JAPANESE（要約）

化学肥料の施用，収穫後の作物残さのすき込み，生物学的 N 固定（BNF）を含む土壌微生物などの一連の農業活動は，地球温暖化を引き起こす亜酸化窒素（N_2O），二酸化炭素（CO_2）とメタン（CH_4）のような温室効果ガス（GHG）排出増加に寄与している．この農業分野での GHG 排出緩和を達成するための比較的安価な技術の 1 つは，土壌改良材としてのバイオ炭や活性炭（AC）などの熱分解炭素質の利用であり，土壌中への炭素隔離をしながら同時に作物生産性を向上させ，この機能はある程度期間持続すると考えられている．しかし未だ不明な点が多い．そこで本研究の主な目的は，土壌改良材としてのバイオ炭や活性炭のような熱分解炭素質による作物生産に関与する GHG 量と作物生産性の両方に対する熱分解炭素質の土壌改良材としての評価を行い，新たな知見を得た．

第 2 章では，供試土壌を砂丘未熟土とし，栽培前にヤシガラ炭の施用量を 0, 6, 12 および 18% （w/w）と，元肥を施用した．2 作目からは新たなヤシガラ炭および肥料を施用せず 3 作連続で栽培した．この結果，1 作目はヤシガラ炭と窒素肥料を同時に施用すると化学肥料のみ区と同等の生育を示したが，3 作目のヤシガラ炭施用区では，化学肥料のみ区に比べ土壌中アンモニア態窒素含量の低下が認められたが，逆に硝酸態窒素同等か約 3 倍程度多く存在していたにもかかわらず生育が低下した．この原因は不明ではあるが，バイオ炭施用によって土壌中の窒素，特に硝酸態窒素に何らかの影響を及ぼしていることが現象として明らかとなった．

第 3 章では，1 年前に砂丘未熟土に混和したヤシガラ炭（旧）が，化学肥料の窒素由来の N_2O 排出を依然低減可能かどうか，栽培試験ごとに化学肥料を施用して評価を，新しいバイオ炭（新）を施用した区と比較しながら調査した．旧バイオ炭は依然 N_2O 排出量を大幅に削減したが，土壌に施肥した窒素の状態は１の結果同様の傾向を示した．この結果，バイオ炭施用をする時は，窒素肥料の施用は必要であると考えられた．

第 4 章では，アブラナ科ブロッコリーが，施肥量が他の園芸作物に比べ多く，収穫後のブロッコリー残渣量も多く，ほとんどは作付け前に同じ畑地にすき込まれることから，この残渣から GHGs 排出量が多い可能性があった．そこで本研究では，ヤシガラ炭施用（0，10，20 および 40 t ha^{-1}）の排出抑制効果を年 2 回作付けの現地ほ場で 1 年間モニタリングを行った．この結果，休閑期の GHG 排出量は栽培期間よりも有意に多いことが明らかとなり，土壌への作物残渣のすき込みは，特に N_2O と CO_2 の排出を有意に増加した．一方，バイオ炭施用効果は，作物残渣からの GHGs 排出に大きな影響を与えず、花蕾収穫後に残った作物残渣のバイオマスおよび N 吸収にも影響を与えなかった．バイオ炭の GHG 削減ポテンシャルを達成するために，40 t ha^{-1} 以上のバイオ炭施肥量を必要とする可能性があることが明らかとなった．

第 5 章では，ダイズの着生する根粒菌をモデルとした土壌微生物から排出される GHGs 量と作物生産性の両方に対する活性炭（AC）の影響を 2 年間評価した．供試ダイズは根粒着生能力が異なる 3 系統；TnVRSN4（高），Tachinagaha（中）および TnVRNN4（低）とした．AC 施肥量は 0，2.4，4.8 および 9.6 t ha^{-1} とした． 活性炭は，根粒着生を大幅に減少させ，特に TnVRSN4（根粒着生の高い系統）では土壌からの N_2O 排出量を減らす傾向にあった． TnVRSN4 は CO_2 排出量と子実収量が最も多く，TnVRNN4 で最も少なかった．AC 施用は，TnVRSN4 の子実収量に有意な影響を与えなかった． AC はダイズの根から滲出する根粒菌誘導物質の一種であるダイゼインおよびダイジン吸着した．このように，AC は N_2O のような GHGs の排出を低減させる一方で，本来ダイズ根から滲出する根粒菌の誘導物質を吸着させ，根粒菌の着生を減少させる効果があることを明らかにした．

第 6 章では供試した 2 系統に着生した根粒菌分解過程における N_2O および CO_2 排出に対する AC の効果を評価するために，インキュベーション実験を行った．この結果，TnVRSN4（高）および Tachinagaha（中）の根粒は，N_2O および CO_2 排出の重要な発生源の１つであることを明らかにした．また，系統が異なる根粒菌分解から放出される N_2O と CO_2 排出量には，有意差がないことが明らかとなった． 一方，活性炭（AC）は根粒分解過程から発生する積算 N_2O と CO_2 排出量に大きな影響を与えなかった．

以上から，土壌改良材としてのバイオ炭や活性炭のような熱分解炭素質による園芸作物の生育および収量と収穫後の作物残渣および根粒菌の腐敗から発生する GHGs 排出の関係を明らかにした．今後は，材料の違いやさまざまな施用手法の確立（いつ，どのように，どのくらい）に関する長期的な研究（バイオ炭の老化過程を含む）が必要であると考える．

LIST OF CONFERENCE PRESENTATIONS

Basalirwa, D., Sudo, S., Akae, F., Wacal, C., Oo, A.Z., Sasagawa, D., Koyama, S., Yamamoto, S., Masunaga, T., Nishihara, E., 2019. Post-harvest soil N_2O and CO_2 emissions from broccoli (*Brassica oleracea* var. *italica*) production are influenced by crop residue management and biochar application. The 3rd Agriculture and Climate Change Conference (Oral). Novotel Budapest City & Budapest Congress Centre, Budapest, Hungary (24th –26th March 2019).

Basalirwa, D., Sasagawa, D., Wacal, C., Acidri, R., Yamada, N., Nishihara, E., 2018. Seed isoflavone, protein content and yield of soybean (*Glycine max* (L.) Merr.) lines grown in sandy soils amended with activated carbon. The 246th Meeting of the Crop Science Society of Japan (Poster). Hokkaido University, Japan (5th – 6th September 2018). https://doi.org/10.14829/jcsproc.246.0_101.

Basalirwa, D., Sudo, S., Akae, F., Wacal, C., Oo, A.Z., Sasagawa, D., Koyama, S., Acidri, R., Ishigaki T., Handa, T., Nishihara, E., 2018. Effect of activated carbon on yield and greenhouse gas emissions in relation to isoflavones from different soybean (*Glycine max* (L.) Merr.) lines in sandy soil. The annual meetings, Japanese Society of Soil Science and Plant Nutrition, Volume 64 (Oral). Nihon University, Fujisawa, Kanagawa Japan (29th –31st August, 2018). https://doi.org/10.20710/dohikouen.64.0_153_1.

Basalirwa, D., Sudo, S., Wacal, C., Sasagawa, D., Acidri, R., Nishihara, E., 2017. Impact of fresh and one-year aged in-situ palm shell biochar on N_2O emissions, soil properties and yield of Komatsuna (*Brassica rapa* var. *perviridis*). The Annual meetings, Japanese Society of Soil Science and Plant Nutrition, Volume 63 (Oral). Tohoku University, Japan (5th September, 2017). https://doi.org/10.20710/dohikouen.63.0_169_2.

Basalirwa, D., Wacal, C., Sasagawa, D., Acidri, R., Nishihara, E., 2016. Residual effects of palm shell biochar on soil chemical properties, growth and nutrient uptake of Komatsuna (*Brassica rapa* var. *perviridis)* in sandy soil. The 13th International Joint Symposium between Korea and Japan. Agricultural, Food, Environmental and Life Sciences in Asia (AFELiSA), 2016 (Oral). Hotel Interciti, Daejeon, Korea. (8th –11th November 2016).

Basalirwa, D., Sudo, S., Acidri, R., Sasagawa, D., Wacal, C., Nishihara, E., 2016. Palm shell biochar application on N_2O emissions, soil chemical properties, growth and nutrient uptake of Komatsuna (*Brassica rapa* var. *perviridis*) in sandy soil. The Annual meetings, Japanese Society of Soil Science and Plant Nutrition, Volume 62 (Oral). Saga University, Japan (20th –22nd September, 2016) https://doi.org/10.20710/dohikouen.62.0_167_2.

LIST OF PEER REVIEWED JOURNAL PUBLICATIONS

Basalirwa, D., Sudo, S., Wacal, C., Namirembe, C., Sasagawa, D., Yamamoto, S., Masunaga, T., Nishihara, E., 2020. Effect of activated carbon on greenhouse gas emissions, seed yield, soil chemical properties and isoflavone content of soybean genotypes with varying nodulation capacities under sandy soil conditions. Rhizosphere 14, 100202. https://doi.org/10.1016/j.rhisph.2020.100202. (**Chapter five**).

Basalirwa, D., Sudo, S., Wacal, C., Akae, F., Oo, A.Z., Koyama, S., Sasagawa, D., Yamamoto, S., Masunaga, T., Nishihara, E., 2020. Assessment of crop residue and palm shell biochar incorporation on greenhouse gas emissions during the fallow and crop growing seasons of broccoli (*Brassica oleracea* var. *italica*). Soil & Tillage Research 196, 104435. https://doi.org/10.1016/j.still.2019.104435. (**Chapter four**).

Basalirwa, D., Sudo, S., Wacal, C., Oo, A.Z., Sasagawa, D., Yamamoto, S., Masunaga, T., Nishihara, E., 2020. Impact of fresh and aged palm shell biochar on N_2O emissions, soil properties, nutrient content and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under sandy soil conditions. Soil Science and Plant Nutrition 66 (2), 328–343. https://doi.org/10.1080/00380768.2019.1705737. (**Chapter three**).