Application of recycled slaughterhouse wastes as plant nutrients for vegetable crops cultivated in eastern India

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Dedicated to

My Family

DETAILS OF THE THESIS

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Bhunia, S., Bhowmik, A., Mallick, R., Debsarcar, A., Mukherjee, J., 2021. Application of recycled slaughterhouse wastes as an organic fertilizer for successive cultivations of bell pepper and amaranth. **Scientia Horticulturae (Elsevier)**. 280, 109927.

Bhunia, S., Bhowmik, A., Mallick, R., Mukherjee, J., 2021. Agronomic efficiency of animal-derived organic fertilizers and their effects on biology and fertility of soil: a review. **Agronomy (MDPI)**. 11(5), 823.

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Balu, S., **Bhunia, S.,** Gachhui, R. and Mukherjee, J., 2022. Polycyclic aromatic hydrocarbon sequestration by intertidal phototrophic biofilms cultivated in hydrophobic and hydrophilic biofilm-promoting culture vessels. **Journal of Hazardous Materials (Elsevier)**. 437, 129318.

Balu, S., **Bhunia, S.,** Gachhui, R. and Mukherjee, J., 2020. Assessment of polycyclic aromatic hydrocarbon contamination in the Sundarbans, the world's largest tidal mangrove forest and indigenous microbial mixed biofilm-based removal of the contaminants. **Environmental Pollution (Elsevier)**. 266, 115270.

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STATEMENT OF ORIGINALITY

I, **Shantanu Bhunia,** registered for the degree of **Doctor of Philosophy (Science)** in the School of Environmental Studies, Jadavpur University on **December 30, 2021** do hereby declare that the thesis entitled **"Application of recycled slaughterhouse wastes as plant nutrients for vegetable crops cultivated in eastern India"** contains original research.

This thesis has been prepared following the existing academic rules and ethical conduct of Jadavpur University, and I declare that all sources used have been cited properly in the text.

As per the "Policy on Anti Plagiarism, Jadavpur University, 2019", I have maintained the similarity level at **10%** for the thesis, and the similarity index is checked by the *i*- Thenticate software.

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Revised thesis submitted on 12/10/2023

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CERTIFICATE FROM THE SUPERVISOR

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DECLARATION

I do hereby declare that the thesis entitled **"Application of recycled slaughterhouse wastes as plant nutrients for vegetable crops cultivated in eastern India"** submitted by me for the degree of **Doctor of Philosophy (Science)** to Jadavpur University, is completely based on my own research work which was carried out under the supervision of **Dr. Joydeep Mukherjee** at the School of Environmental Studies of Jadavpur University. Neither this thesis nor any part of it has been submitted for either any degree/ diploma or any other academic award anywhere before. Furthermore, I have acknowledged all sources used and have cited these in the reference section accordingly.

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Signature of the Candidate with date Shantanu Bhunia
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INDEX

Abbreviations

%:	Percentage
<:	Less than
>:	Greater than
°C:	Degree Celsius
$\mu g\ g^{-1}\ hr^{-1}$:	Microgram per gram per hour
ASTM:	American Society for Testing and Materials
B:	Boron
BBRDM:	Bovine blood rumen digesta mixture
BH:	High dose of BBRDM
BL:	Low dose of BBRDM
BR:	Recommended dose of BBRDM
BSE:	Bovine spongiform encephalopathy
C:	Carbon
Ca:	Calcium
CF:	Chemical fertilizer
CH_4:	Methane
cm:	Centimetre
CO_2:	Carbon dioxide
Cu:	Copper
DAP:	Diammonium phosphate
DNA:	Deoxyribonucleic acid
EC:	European Commission

EDS:	Energy dispersive spectroscopy
ETH:	Electron high tension
EU:	European Union
FAO:	Food and Agriculture Organization
Fe:	Iron
g cm^{-3}:	Gram per cubic centimetre
g kg^{-1}:	Gram per kilogram
GC FID:	Gas chromatograph flame ionization detector
GDP:	Gross domestic product
GHG:	Greenhouse gas
H_2:	Hydrogen
HGT:	Horizontal gene transfer
IMD:	India Meteorological Department
INR kg^{-1}:	Indian rupee per kilogram
IPCC:	Intergovernmental Panel on Climate Change
ISO:	International Organization for Standardization
K:	Potassium
K_2PO_4:	Dipotassium phosphate
kg ha^{-1}:	Kilogram per hectare
kV:	Kilovolt
m^2 min^{-1}:	Meter square per minute
MCQ:	Multiple choice question
mg g^{-1}:	Milligram per gram
mg kg^{-1}:	Milligram per kilogram
ml L^{-1}:	Milliliter per litter

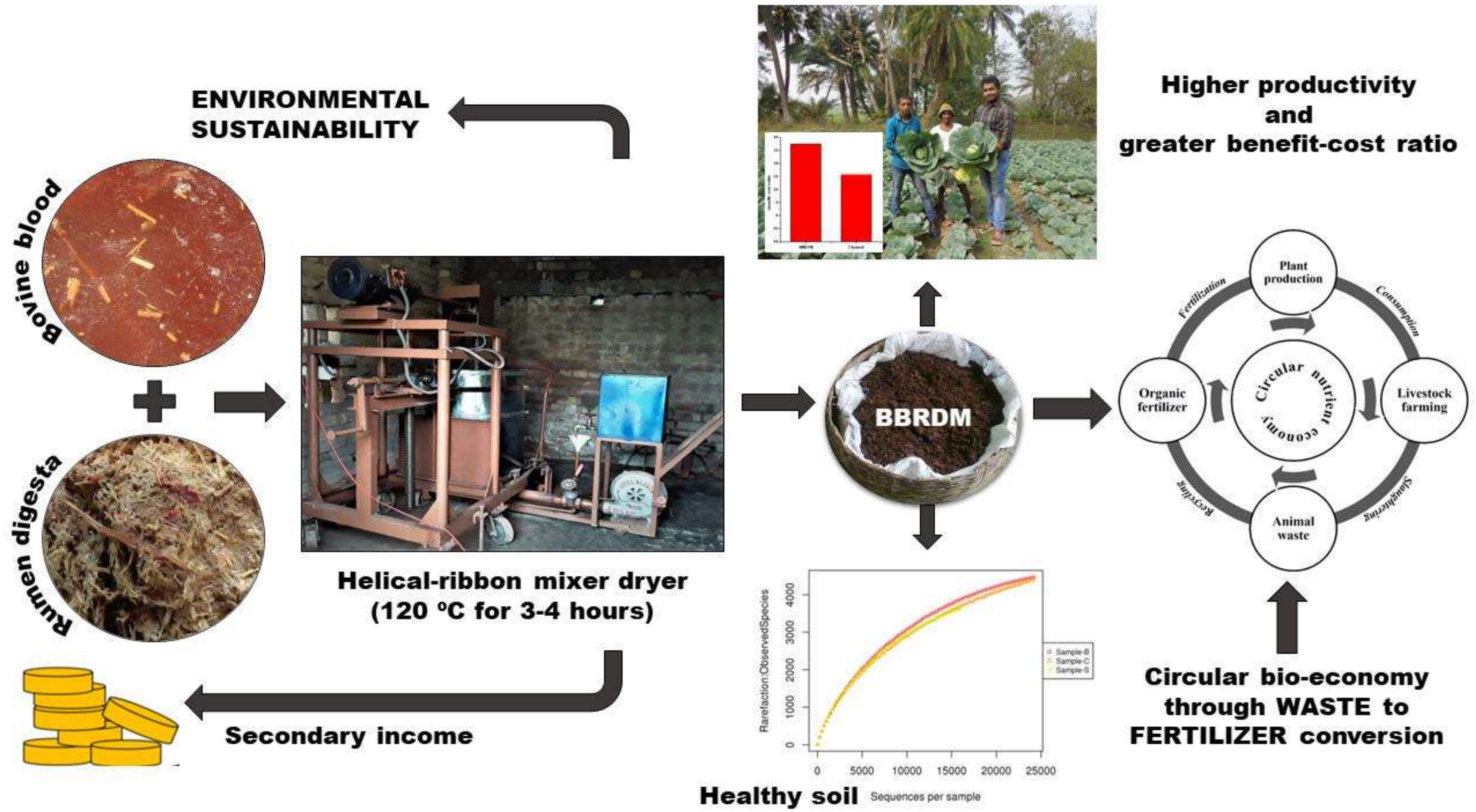
mm:	Millimetre
Mn:	Manganese
MSL:	Mean sea level
MSW:	Municipal solid waste
Mt yr^{-1}:	Metric tonne per year
N:	Nitrogen
N_2O:	Nitrous oxide
NCBI:	National Center for Biotechnology Information
NH_4^+ -N:	Ammonical nitrogen
NO_2^-:	Nitrite
NO_3^- -N:	Nitrate nitrogen
NO_3^-:	Nitrate
OC:	Organic carbon
OUT:	Operational taxonomic unit
P:	Phosphorus
p:	Probability
PCA:	Principal component analysis
PCR:	Polymerase chain reaction
QIIME:	Quantitative Insights into Microbial Ecology
RBD:	Randomized block design
RNA:	Ribonucleic acid
S:	Unfertilized soil
SEM:	Scanning electron microscopy
SOC:	soil organic carbon
SOM:	soil organic matter

SPSS:	Statistical Package for Social Science
SRA:	Sequence read archive
SWOT:	Strength, Weakness, Opportunity, and Threat
TKN:	Total Kjeldahl nitrogen
TN:	Total nitrogen
TP:	Total phosphorous
UNEP:	United Nations Environmental Programme
USDA FAS:	United States Department of Agriculture Foreign Agricultural Service
VC:	Vermicompost
WDS:	Waste disposal site
XRD:	X-ray diffraction
Zn:	Zinc
θ:	Theta

Abstract

The effectiveness of 'bovine-blood-rumen-digesta-mixture' (BBRDM), an abattoir-derived organic amendment was studied on growth and productivity of vegetable crops and soil health. Due to poor infrastructure and low waste generation in rural abattoirs, implementation of sophisticated waste management system is difficult resulting in social, environmental, and economical depletion. Using a newly designed helical-ribbon mixer dryer, mixture of waste blood and undigested rumen (in a 3:1 ratio) was converted at 90-110 °C for 2-3 hours to a non-hazardous nitrogen-rich organic product BBRDM having a C/N ratio of 4.68. The N/P/K content of BBRDM was approximately 8:1:2. The presence of boron, zinc, calcium, iron, and selenium in BBRDM that are essential for crop improvement was revealed by scanning electron microscopy combined with energy dispersive spectroscopy (SEM-EDS). The yield was significantly higher when BBRDM was amended at rates of 6 g kg^{-1} and 9 g kg^{-1} of soil than it was when inorganic fertilizer (N/P/K=10:26:26+urea) and vermicompost were applied. In contrast, the presence of labile C percentage in animal waste caused the highest BBRDM rate (13 g kg^{-1} of soil) to have detrimental impacts on the plant growth. Furthermore, compared to chemical treatment, residual production was almost double when soils were fertilized with the recommended BBRDM dose (9 g BBRDM kg^{-1} in field). Judicious supply of the BBRDM and decorous N application ameliorated soil organic carbon (SOC), enhanced macroaggregates percentage in soil, and encouraged copiotrophic richness in soil which in turn impacted soil enzymes to convert organic substance into plant accessible form. During the study, delayed nitrogen mineralization and sluggish nutrient release from BBRDM was evidenced that led to decreased fruit nitrate/ nitrite build-up while vegetables sold in markets typically had higher nitrate/ nitrite levels. The structure, abundance, and diversity of soil bacterial communities

were revealed by V3-V4 16S rRNA gene sequencing, and the metagenomic study also confirmed the absence of slaughterhouse pathogens in BBRDM fertilized soils. Application of BBRDM as an organic fertilizer seems to be a better option than open dumping in terms of emitted methane, as evidenced by the fact that air-soil methane flux (0.008 μg g^{-1} hr^{-1}) in fields treated with BBRDM was around 1787 times lesser than that produced from the abattoir dumping sites (14.30 μg g^{-1} hr^{-1}). The average cost-benefit ratio for BBRDM treatment was about 3.75 as opposed to 2.58 for NPK fertilization, showing that BBRDM use in commercial agriculture offers greater socio-economic advantage. Circular bio-nutrient economy, where nutrients recirculated along with economy, was also fostered over waste to fertilizer conversion, and organic fertilizers derived from animal waste may be deemed for sustainable green agriculture in the future. Although fertilizer availability and lack of consumer awareness for bio-based products could be the possible threat to circular economy of organic waste management.



Graphical overview

1.

Introduction

Advantages of organic farming

Healthy soils are vital for progressive agronomic activities. Misuse of synthetic fertilizers and nitrogen (N) addition at indiscriminate rates may result in deterioration of soil makeup, habitat and biodiversity loss, soil acidification, and even disturbance of various ecosystem services, thus impeding sustainable agricultural development (Guo et al., 2018; Urra et al., 2020). Careful application of well-prepared organic amendments rich in humus, organic substances, and growth-promoting microorganisms have a positive impact on agro-environment health by promoting soil organic matter (SOM) turnover, cohesion of soil aggregates, copiotrophic dominance, and soil enzymatic activity as well as keeps plants protected from *Phytopthora*, *Pythium*, *Verticillium*, *Fusarium*, and *Rhizoctonia* like soil-borne phytopathogens by developing synergistic consortia at the root-microbiome zone (De Corato, 2020). Few studies evidenced that plant disease suppression may also be attributed to volatile and non-volatile toxic chemicals produced during the breakdown of organic amendment supplied to soils (Chen et al., 1987; De Brito Alvarez et al., 1995). The major plant nutrients supplied by the organic amendments are nitrogen (N), phosphorus (P), and potassium (K). However, majority of the vegetable crops demand a constant supply of nitrogen instead of phosphorus and potassium for their growth and development. Chemical fertilizers mostly offer ammonium, nitrate, phosphate, and potassium in salt forms, whereas the organic amendments provide zinc (Zn), copper (Cu), boron (B), iron (Fe), and manganese (Mn), all of which are necessary for crop enhancement (Zhang et al., 2020). Most of these agricultural chemicals are persistent in nature, which not only results in nutrient runoff and non-point water pollution but also places an unbearable economic burden on the growers (Tal, 2018). Moreover, long-term inorganic fertilization may increase the risk of crop damage by pest and disease infestation (Yatoo et al., 2021).

In the next few decades, it is expected that the world's need for food would be doubled as a result of population growth and dietary shifts (Urra et al., 2019). Adoption of a more westernized diet, especially in India and China which combined account for 37% of the global population, can contribute around 50-70% of the overall rising need (Gandhi and Zhou, 2014). The United Nations- Food and Agriculture Organization (FAO) estimated that, by 2050, the world population may increase up to 9 billion (FAO, 2009) which might create tremendous pressure on the farmers to increase agricultural productivity. This can be achieved either by enhancing farming practises or increasing the usage of agricultural land (Bommarco et al., 2013). Sjauw-Koen-Fa (2010) estimated 9% expansion of cultivable land, 14% increase in cropping intensity, and 77% more yields to feed the constantly growing population, while Pretty and Bharucha (2014) suggested sustainable intensification of agro-ecosystems rather than the expansion of cultivable land. The Green Revolution in the late 1960s reduced widespread hunger, malnutrition, and extreme poverty, transformed agriculture into an industrial system which incorporated modern machinery, synthetic agro-chemicals and genetically modified organisms to the agriculture (Urra et al., 2019). Unfortunately, a large portion of the world's cultivable lands are now unproductive due to soil acidity brought on by excessive chemical apply, human interference, and shortage of water (Diacono and Montemurro, 2015). Therefore, it is imperative to shift towards organic farming for a better future.

The soil becomes friable and productive with the addition of recycled organic amendments, allowing it to produce safe and high-quality food products without depleting the environment's resources (Oliveira et al., 2017). Animal manures are traditionally regarded as good soil conditioners that improve nutrient circulation in agro-ecosystems, increase microbiological activity, and support soil structural development (Bertagnoli et al., 2020). Animal manure application aids copiotrophs whereas chemical fertilizations encourage the quantity of oligotrophs in rhizosphere soil. The copiotrophic abundance in soil in turn influences the rate of SOM turnover (Chaudhry et al., 2012), while oligotrophs are *k*-strategist and slow growing that thrive in nutrient-poor environments and are typically found ample to soil microaggregates (<250 μm diameter) having reduced SOM (Lin et al., 2019). On the other hand, long-term animal manure fertilization increased the percentage of firm soil macro-aggregates, representing diameters >250 μm and facilitated physical protection to soil organic carbon (SOC) ensuing a favorable environment for copiotrophic growth (Sheoran et al., 2019). According to Koch (2001), the possible reasons for oligotrophs to accede during too high nutrition condition: (a) production of inactive or variable but uncultivable cells, (b) sudden availability of too many non-metabolic transportable substances, (c) prevention of DNA synthesis due to inappropriate SOS response, and (d) cell death cause osmotic swelling. Copiotrophs are nutritionally opposed to oligotrophic bacteria, which promote the breakdown of organic matter and enable nutrient recycling to endure long-term productivity of an agro-ecosystem. Copiotrophs are indicators of healthy soils. Surprisingly, the stability of soil aggregates is highly influenced by the type of fertilizer used and related soil binding agents like SOC (Guo et al., 2018). Sihi et al. (2017) documented enhanced water-holding capacity of Indian soils upon the addition of recycled organic amendment to soils. Simultaneously, the activities of urease, alkaline phosphatase, and β-glucosidase found higher in organically treated fields that play a central role in soil nutrient dynamics (Liang et al., 2014). The phosphatase enzyme breaks down the phospho-ester linkages and transforms organic phosphate into a form that is readily available to plants, while the urease enzyme hydrolyzes fertilizer urea into ammonia. Cellobiose is changed into glucose by the enzyme β-glucosidase, which is related to the carbon geochemical cycle. The enzyme is regarded as a potential soil quality indicator because of its sensitive nature (Urra et al., 2020). According to Das and Varma (2011), soil enzyme levels can be altered because each soil type represents diverse organic matter profile, composition and activity of existing microorganisms, and intensity of native bio-chemical reactions. Such enzymatic activities could represent quality of any soil. With the addition of animal manure vermicompost to native soils, Antonious et al. (2020) recorded a notable increase in soil invertase activity which catalyses the conversion of sucrose to glucose and fructose. Apart from their benefits, animal-derived organic amendments have certain disadvantages, such as presence of heavy metals, pathogens, antibiotic resistant genes, and emerging contaminants if the wastes are not perfectly processed (Urra et al., 2019). However, efficacy of such amendments is profoundly dependent on the feedstock composition and process of recycling (Bhunia et al., 2022). When applying fertilizer, nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in agricultural produce should need to be taken under consideration because they have a substantial impact on human health. The plant body cannot process the extra given N, therefore it stores as nitrate in their tissues, primarily in the stem and leaves. According to Gupta et al. (2017), a higher nitrate and nitrite concentration in food is linked to a higher risk of gastrointestinal cancer and methemoglobinemia in young infants. Therefore, to attain higher productivity, healthy soils, and better fruit quality fertilizer dose calculation is necessary. This approach will also be helpful in reduction of associated pollutions like leaching, greenhouse gas (GHG) emissions etc.

Need of recycling organic waste in agriculture

Slaughterhouses are the largest part of the meat-generating sector (Bustillo-Lecompte and Mehrvar, 2015) produces huge amount of organic wastes every day through killing of bovine animals to meet the consumers' demand for meat which could pose serious environmental hazards if not managed properly. In India, there are about 32,000 informal slaughterhouses, the bulk of which do not adhere to any scientific waste disposal procedures (Kennedy et al., 2018). The global meat consumption has seen a 40% increase in the last decade. In 2020, it was reported that approximately 252.6 million metric tons (Mt) of meat were produced globally, including 99.1 million Mt chicken, 95.8 million Mt pork, and 57.7 million Mt beef. On an average each kg of meat consumes 4850 L of water for its processing and produces wastewater. Due to the extensive rise in demand for meat, the number of slaughterhouses is also increasing and thereby increasing the production of biodegradable organic waste (Ragasri and Sabumon, 2023). According to Roy et al. (2016), an Indian rural abattoir kills 20 buffaloes (on average) per day which results in massive amount of animal excrement being dumped directly into municipal or local sewage systems without any treatment (see Figure A.1) (Bhowmik et al., 2021a). According to the Ministry of Food Processing, India, for the year of 2019, a 7.4 Mt of meat was produced. This also makes India the highest producer of meat (buffalo), consisting of 38.92% of the global share. In India, the states of Uttar Pradesh, Maharashtra, West Bengal, Andhra Pradesh, and Telangana together contribute to 57.2% of the total meat produced in which Uttar Pradesh alone contributes to 15.1% of the total meat production (Ragasri and Sabumon, 2023). In the report 'Livestock and Poultry: World Markets and Trade', India has also ranked as the second-largest exporter of beef and the fourth-largest producer of beef worldwide as reported by United States Department of Agriculture- Foreign Agricultural Service (2020). On the other hand, global solid waste generation is estimated to be about 11.2 billion tons per year, which is projected to increase by 19 billion tons per year by 2025. It is estimated that the global population is projected to increase to 9.9 billion by

2050 which is approximately 26.9% increase than the present population during which the generation of solid waste is estimated to increase approximately by 70%, i.e., 3.4 billion tons per year (Mozhiarasi and Natarajan, 2022). According to Franke-Whittle and Insam (2013), inedible body parts of the slaughtered animals comprised up to 45% of their total body weight among which bovine blood and rumen digesta have no utility or resale value (Bhowmik et al., 2021a). Recent investigation of Mozhiarasi and Natarajan (2022) documented that about 50–54% of each cow, 52% of each sheep or goat, 60–62% of each pig, 68–72% of each chicken, and 78% of each turkey is utilized for meat and the remaining is disposed of as waste. The World Bank highlighted the global waste generation trend along with the projection from the year 2016–2050, which demonstrated that most of the world's waste is generated from South East Asia and the Pacific region particularly from India, China, Pakistan, and Bangladesh (Kaza et al., 2018). To recycle the slaughterhouse waste, European Union (EU) recommended anaerobic digestion, alkaline hydrolysis, incineration, composting, and rendering like advanced technologies (Franke-Whittle and Insam, 2013; Adhikari et al., 2018), although these multi-unit systems demand massive labor employment and huge capital investment. Informal slaughterhouses in India and other underdeveloped countries like Kenya (Cook et al., 2017) were unable to implement such advanced and expensive treatment techniques due to a lack of infrastructure and financial constraints, therefore rural abattoirs preferred landfilling and open dumping of waste as simplest option. Landfills release a variety of hazardous substances into the environment. Leachate is produced as a result of microbial decomposition of organic waste in landfills, which also releases methane and carbon dioxide like GHGs (IPCC, 2002; Ximenes et al., 2008) together with annoyances from the bad odors (Domingo and Nadal, 2009). Furthermore, as reported by Bhunia et al. (2022), improper disposal of such organic wastes poses significant risks to public health because they serve as latent reservoirs for infectious pathogens like *Salmonella*, *Clostridium*, *Campylobacter*, *Bacillus*, *Brucella*, *Erysipelothrix,* *Staphylococcus*, and bovine spongiform encephalopathy (BSE) that can cause diseases. The burial and burning of slaughterhouse waste also carry similar risks (Franke-Whittle and Insam, 2013). Due to incomplete combustion of cattle carcasses, extremely carcinogenic dioxins and furans are released. Such emissions have the potential to negatively impact human immunological and reproductive systems (Rier, 2008). In addition, nutrient loss due to waste landfilling leading to large economic defeat recently has become a subject of concern for the developing nations (Bhunia et al., 2022).

On the other side, release of toxic organic contaminants into the agro-ecosystem and increase of antibiotic-resistance in soil bacterial communities might be attributed through fertilizations with untreated animal waste (Urra et al., 2019). Horizontal gene transfer (HGT) is the primary mechanism driving the spread of antibiotic resistance genes in bacteria, and rhizosphere soil has been identified as a key HGT hotspot (van Elsasa and Bailey, 2002). According to Bondarczuk et al. (2016), antibiotic resistance in native bacterial population might be pronounced by the accumulation of heavy metals in agricultural field due to such practices. However, Urra et al. (2019) elaborated the possible mechanism behind the occurrence and propagation of antibiotic resistant genes in agricultural soils: the theory of co-selection where resistance is offered either by different genes of the same genetic loci (drives co-resistance) or same gene provides resistance to both antibiotic and heavy metals (allowing cross selection). According to O'Neill (2016), an estimated 10 million fatalities per year may occur by 2050 due to the incorporation of antibiotic resistance genes in indigenous soil bacteria. Therefore, to overcome the situation and to ensure biosecurity, waste recycling is necessary before reuse them in agriculture.

Among the different important issues in South Asian agriculture during recent decades is GHG emissions from crop land and their effect analysis on global climate change. Globally, the production of CH_4, N_2O, and CO_2 from the agricultural soils has increased primarily due to changes in land use land cover, decomposition of stubble residues, and fertilizer overuse (Verge et al., 2007), although landfills were the third-largest emitter of anthropogenic methane as conveyed by Chakraborty et al. (2011). According to Smith et al. (2008), world grain yield almost doubled between 1970 and 2010 because of Green Revolution and use of inorganic fertilizers, and this agronomic escalation was possible only through the boost (around 331%) in global fertilizer usage, from 32 to 106 Mt yr^{-1}. Although the application of N is imperative for higher crop yield, they generally exhibited N_2O emissions (Cai et al., 2007), and South Asia attained 36% increase in N_2O emission between 2000 to 2015 as of Crippa et al. (2019). According to United Nations Environmental Programme (UNEP, 2019), additions of N in the form of fertilizers to the soils reinforce the greenhouse effects, and around 60% of the N_2O is emitted from the agricultural field. It is well documented that the emissions N_2O were triggered by synthetic fertilization and excessive N supply while long-term manure applications encouraged CH_4 production, especially organic fertilizers derived from animal sources (Skinner et al., 2019). Recycling organic waste in agriculture, this 'waste to fertilizer conversion' strategy will also be helpful in achieving the world's 2050 Net Zero vision reducing CH_4 and N_2O emissions from waste and agriculture. However, as recycled slaughterhouse waste applied as an organic fertilizer, therefore, it was decided to measure CH_4 emissions in the current investigation. Surprisingly, Meijide et al. (2010) did not get any significant change in air-soil methane flux during barley production with digested pig slurry and synthetic fertilizer. Although, the emission fluxes can be varied with soil temperature and annual rainfall (Skinner et al., 2019) apart from the effects of soil edaphic factors.

In developing countries like India, (a) reutilization of huge organic waste generated, (b) restoration of soil health damaged by the rigorous chemical application, (c) production of safe, affordable, and healthy food, (d) reduction in GHG emissions from waste and agriculture sectors, and (e) absence of alternative economy to support country's GDP (Gross Domestic

Product), are the emerging problems that clearly appeared. Therefore, agriculture needs to be organic and more productive in order to address the future climatic and socio-economic difficulties. Adoption of Organic Agriculture 3.0 could provide a solution to the issues related to food security and soil health aiming to shift organic cultivation from its current niche to mainstream, and this initiative may provide an opportunity to grow organic market rapidly (Arbenz et al., 2017). According to Rahmann et al. (2017), this version is advantageous over the previous versions, Organic Agriculture 2.0 and 1.0 which may intensify agro-ecological sustainability, encourage socio-economic empowerment, and innovation in food production embracing novel ethics and habits.

Recycling organic waste into more valuable goods helps to advance the bioeconomy, prevents materials from ending up in landfills or other disposal sites, and adds them to a circular value chain for future reuse. The fundamental tenets of "circular" and "bioeconomy" are solely complementary, never entirely assimilating into one another, and both should promote the reuse and recycling of waste materials (Maina et al., 2017). According to European Commission (2012), production of diversified value-added products, including fuels, fertilizer, feed, food, and bioenergy converting renewable bio-based resources promoted bioeconomy, while the circular economy attempts to extend the life cycle of feedstock used, try to minimize waste generation and greenhouse gas emissions (European Commission, 2015). Sheridan (2016) and Stegmann et al. (2020) considered the bioeconomy to be circular by nature. This study may promote bioeconomy aiming a better future.

Treatment alternatives of slaughterhouse wastes

Incineration

In the European Union (EU) countries where the landfilling of livestock mortalities is banned following the EU Landfill Directive 1999/31/EC (Salminen and Rintala, 2002) resulted into more wastes being directed towards either on or off-farm incineration (EU Animal Byproduct Regulation (ABPR) 2002/1774/EC) than towards recycling management. About 90% incinerations were done in European countries alone (Mubeen and Buekens, 2019).

Incineration is an alternative method of waste disposal where slaughterhouse byproducts are converted into inorganic ash at >850 °C temperature. Industrial incineration plants use the process of waste combustion to produce electricity (Billen et al., 2015) and destroy all infective agents more effectively (NABC, 2004; Gwyther et al., 2011). Bovine spongiform encephalopathy (BSE) should survive the process if not conducted at 850 °C temperature for at least 2 seconds (Franke-Whittle and Insam, 2013). The effectiveness of incineration is debatable. Waste incineration mainly causes gaseous emissions and fly ash production. Rural abattoirs generally carried out burning of carcasses instead of high temperature incineration. Chen et al. (2003) reported higher polyaromatic hydrocarbon emissions from animal waste combustion. The increasing distance from incinerator plants can significantly reduce dioxin and furans levels in the atmosphere (Yan et al., 2008). Ash byproducts resulting from the process represented 5% of the initial volume (Chen et al., 2003) and especially in EU countries these are highly recommended for the landfilling according to the ABPR (EC 1774/2002). Outside of the EU, conventional ash land-spreading is practiced to improve soil fertility (Billen et al., 2015). Such practices can increase the risk of furans and dioxin entering into the human food and animal feed supply chains via bioaccumulation (Gwyther et al., 2011). In addition, Chen et al. (2004) found an increased metal contamination in flue gas. Both land-filling and land-spreading can potentially damage the environment through heavy metal pollution (Gwyther et al., 2011). Simultaneously, it may be the cause of surface and groundwater contaminations. Moreover, some non-governmental organizations (NGOs) and societies have protested against the waste incineration because they doubted its sustainability.

Rendering

Rendering is another way of slaughterhouse waste disposal resulting into separation of fat from the bone and proteinaceous solids. Rendering industries typically produce low-value products such as animal or pet food from the obtained protein (Adhikari et al., 2018). Separated fat can be used for biodiesel production and in making of grease, soap, candles and other household items (Kalbasi-Ashtari et al., 2008). Melted fat or tallow was found effective in steel rolling industry (Franke-Whittle and Insam, 2013). The rendered meal/cake was also used as organic fertilizer in agriculture (Salminen and Rintala, 2002). Rendering practices provide an additional source of income to the slaughterhouse owners. The technology refers to various processes including mechanical (mixing, pressing, decanting and separation), thermal (cooking and drying) and sequential chemical extraction (NABC, 2004). EU Directive 1990/667/EC suggests rendering of high-risk waste materials at 133 °C for 20 min (at 300 kPa pressure) to obtain pathogen free end-product. In recent years, the feeding of rendered meals to fish and farm animals is strictly prohibited in EU countries (Franke-Whittle and Insam, 2013) because of the problems with transmissible spongiform encephalopathy contamination (Gwyther et al., 2011). They are now subjected to either landfilling or incineration in the EU, and may also be used as fuel source. Rendering failed to stop the BSE and particularly *Salmonella* infections (NABC, 2004). Recontamination can occur during the handling of the final product (Gwyther et al., 2011). Commercial rendering poses fewer environmental concerns including issues of biosecurity, release of process effluent having high chemical oxygen demand and emissions of gas together with bad odours. The efficient filtering and multi-step bio-chemical treatments can reduce the risk of pollution from rendering plant effluents. In addition, Kalbasi-Ashtari et al. (2008) reported that cold water treatment to the feedstock removed 90% odours. The negative issues of biosecurity especially re-survival of infectious *Salmonella* and TSE challenges its worldwide acceptance today as it had in the past.

Composting

Composting is an aerobic process of organic waste stabilization and has been reported extensively along with anaerobic digestion in previous years. Composting of slaughterhouse waste provides an inexpensive and environment-friendly recycling alternative. Composting undergoes four consecutive phases namely mesophilic, thermophilic, cooling and maturation. The positive effects of composted slaughterhouse waste as organic fertilizer have been studied by Coria-Cayupaan et al. (2009), Ragályi and Kádár (2012) and Nunes et al. (2015) who found increased productivity and greater copiotrophic diversity in soil.

Successive groups of aerobic microorganisms were found abundant during the composting process but, their precise roles are still unclear. The recent use of molecular biology techniques helps researchers to explore more about the diversity and ecology of compost communities. Two types of composting systems have already been established for the recycling of livestock mortalities including windrow and in-vessel composting (NABC, 2004). The substrate composition and compost pile temperature affected the process significantly (Fuchs, 2010). In addition, microbial competitions also influenced the composting process. Organic composting aimed to achieve favourable environment for the beneficial microbial communities. The final stage of composting, the maturation phase was characterized by fungal dominancy instead of initial bacterial abundance as reported by Fuchs (2010). The thermophilic phase minimized the numbers of pathogens (Gwyther et al., 2011) and made the waste suitable for agricultural applications. Reuter et al. (2011) reported that pH >8.0 contributed towards pathogen inactivation. However, some opportunistic pathogens such as spore-forming *Clostridium* and *Bacillus* and particularly the strains of *Salmonella* may recolonize the compost if not managed properly (NABC, 2004). It is possible that composting temperature is not sufficient to sterilize the end product completely (Franke-Whittle and Insam, 2013). Small-scale composting of livestock mortalities can contaminate the underlying soil due to release of leachate under the periods of high rainfall (Glanville et al., 2006). On the other hand, windrow composting has several drawbacks including requirement of large space, higher emissions of GHGs to the atmosphere and relatively low process efficiency (Tritt and Schuchardt, 1992; Salminen and Rintala, 2003), although it can reduce the pathogenic load better than in-vessel composting system (Cekmecelioglu et al., 2005).

The compost fertilizer produced from cattle, pig and poultry waste recycling has greater legislative restriction in the EU (Gwyther et al., 2011), although it is still preferable for slaughterhouse waste management in developing countries like Bangladesh, India, Nepal, Bhutan and Pakistan. We feel additional treatments to the end-product like anaerobic digestion, drying and controlled thermal management would be necessary for complete removal of pathogens.

Anaerobic digestion

Currently, the practice of anaerobic digestion and co-digestion (also called bio-digestion) of Type 1 slaughterhouse wastes are not allowed in the EU without proper feedstock pre-treatment (Gwyther et al., 2011). For example, biogas plants require 70 °C exposure for 60 min pasteurization of animal waste prior to anaerobic digestion according to the European Commission Regulation (EC) No. 1774/2002 which may facilitate complete pathogen inactivation (Sahlström et al., 2008). On the other hand, Salminen et al. (2002) reported aerobic post-treatment of anaerobically digested poultry slaughterhouse waste to reduce its phytotoxic effects and to obtain pathogen-free compost.

Anaerobic digestion and co-digestion involve the degradation of organic matter biologically under favourable environmental conditions to produce biogas which can be used as alternative source of energy to replace fossil fuels. The other end-products have some potential fertilizer applications. Anaerobic digestion typically occurs in four successive stages namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. Syntrophic co-operations and temperature alterations both have adverse effects on the methanogens (Franke-Whittle and Insam, 2013), the methane producing microorganisms. Generally, anaerobic digestion of dead livestock is carried out either in mesophilic (at 35 °C for 15-30 days) or following thermophilic conditions at 55 °C for 12-14 days (Cantrell et al., 2008). According to Salminen and Rintala (2002), the time-temperature combinations can significantly affect the system physico-chemical environment. However, thermophilic digestion is more effective than mesophilic in terms of higher methane yield and better pathogen inactivation (NABC, 2004). For example, Monteith et al. (1986) reported that enterovirus and parvovirus in cattle manure were destroyed just after 30 min of thermophilic anaerobic digestion, whereas mesophilic digestion required 8-13 successive days. Spillmann et al. (1987) showed faster rotavirus elimination by thermophilic digestion. In addition, Côté et al. (2006) confirmed *Salmonella*, *Cryptosporidium* and *Giardia* inactivation following the same process, while Viau and Peccia (2009) failed to eradicate slaughterhouse pathogens by the mesophilic anaerobic digestion. Brown et al. (2000) reported that BSEs survived both mesophilic and thermophilic mode of anaerobic digestion. Slaughterhouse wastes had greater methane generation potential, although their high fat and protein contents caused serious stratification problems as reported by Cuetos et al. (2010). Authors also found higher accumulation of unionised ammonia during anaerobic digestion of animal carcasses. Edström et al. (2003) suggested the use of ammonium-rich medium for better acclimatization of methanogens prior to digestion. On the other hand, poultry feather digestion was difficult due to its complex composition - mainly tight packing of the protein chains (Ozdemir et al., 2019).

Alkaline hydrolysis

Alkaline hydrolysis is relatively young compared to the existing waste valorization alternatives as it was a 1990s technology and holds more environmental potential in terms of limited gaseous emissions and odour formation, complete pathogen inactivation and high quality hydrolyzate production as stated by Arias et al. (2018). El-Thaher et al. (2013) reported 150 °C exposure for 180 min (at 180 kPa pressure) was required for alkaline hydrolysis of hazardous livestock waste. The same treatment was recommended by the Canadian legislation for disposal of slaughterhouse specified risk materials (Gwyther et al., 2011). Alkaline hydrolysis generally used sodium or potassium hydroxides to significantly accelerate the process. Hydrolysis end-product i.e. sterile hydrolyzate either can be land-spread as an effective fertilizer or can be used as animal feed (NABC, 2004), although protein feeding is strictly restricted in the EU countries. Gousterova et al. (2003) showed 90% protein recovery from the hydrolysis of sheep wool at 120 °C for 20 min at 200 kPa pressure. Jiang et al. (2008) investigated chicken feather hydrolysis at 120°C for 120 min under specified alkaline conditions. According to Adhikari et al. (2018), conventional acid hydrolysis using HCl and alkaline digestion (hydrolysis without heating the feedstock using sodium hydroxide of wool and chicken feather waste was truly difficult due to the presence of keratin like proteins and their complex compositions. Zhu et al. (2010) recorded higher biogas production from corn stover anaerobic digestion when an alkaline pre-treatment was done with 5% Sodium hydroxide. On the other hand, high effluent alkalinity and their rich nutritional profile made problems when they entered into the wastewater purification systems.

Few post-treatment options have been adopted by the Scientific Steering Committee of the European Commission (EC SSC, 2003) before its discharge in to the sanitary sewers in the EU. Alkaline hydrolysis has proven effective for slaughterhouse pathogen eradication. Pollard et al. (2008) reported Influenza H5N1 virus inactivation by the alkaline hydrolysis of poultry carcasses. Murphy et al. (2009) confirmed destruction of prions through alkaline digestion.

Similarly, El-Thaher et al. (2013) demonstrated inactivation of thermally resistant *Geobacillus stearothermophilus*. In recent days, alkaline hydrolysis of slaughterhouse waste is allowed both within and outside the EU.

Enzymatic management

Enzymatic hydrolysis also showed promise in meeting industrial needs. The technology was first patented by Eckmayer et al. (1980) who showed effective hydrolysis of protein from slaughterhouse waste employing typical protease under the specified mild conditions. Webster et al. (1982) also studied protein recovery from animal waste by using pepsin (at pH 3.0), papain (at pH 5.5) and alcalase (at pH 8.5) enzymes. Enzymatic hydrolysis of slaughterhouse waste converted them into more useful products. Due to their high fat and protein contents, they can be hydrolyzed for various industrial applications. Gómez-Juárez et al. (1999) recovered 75% protein from abattoir blood waste using papain at a pH of 7.5. Bhaskar et al. (2007) utilized fungal protease (at pH 7.1) to obtain 34% protein from sheep stomach and intestine waste. Ramani et al. (2010) recorded highest hydrolytic activity of acidic lipase towards beef tallow waste, produced from *Pseudomonas gessardii* strain. Recently, Pfeuti et al. (2019) hydrolyzed poultry feather waste applying 0.5% commercial Savinase (an alkaline serine protease) to improve the nutritional quality of feather meal. Enzymatic hydrolysis has no effects on pathogen inactivation as other recycling alternatives have. Composition of the feedstock and hydrolytic conditions significantly affect the hydrolyzate recovery (Adhikari et al., 2018). The long processing times, enzyme specificity in certain pH ranges and their high cost are the major disadvantages of enzymatic waste management (Bhaskar et al., 2007).

An additional heat treatment at the end of hydrolysis is required for quick enzyme inactivation. For example, Adhikari et al. (2018) recommended 95 °C exposure for 15 min to inactivate the enzyme. Bhaskar et al. (2007) reported overall 6% higher protein recovery yields from the hydrolyzate of sheep visceral waste.

Recycling abattoir waste into fertilizer by conductive drying

As slaughterhouse wastes have enough fertilizer potential due to presence of considerable amounts of growth-supporting plant nutrients (Ragályi and Kádár, 2012) and rural abattoirs failed to adopt above mentioned sophisticated and capital-intensive technologies for their waste management, therefore, Roy et al. (2013) developed a cost-effective eco-friendly alternative for reusing abattoir waste in rural agriculture. Sufficient heat treatment ensures availability of nutrients, reduces high waste moisture content and eliminates most of the pathogens from the final product. Hazardous slaughterhouse wastes are either recycled into animal feed or to an organic fertilizer practicing drying technology. This method was first adopted by Roy et al. (2013) for recycling of rural slaughterhouse waste in India. Majority of the rural slaughterhouses especially in developing countries dumped their solid wastes and wastewater directly into local water bodies or be land-spreaded as we found in case of our working sites in India. Drying technology may help them to recycle such wastes easily instead of their open dumping and to earn money from the recycled waste. The process included cook-drying of rumen digesta and waste blood in different ratios to obtain 'bovine-blood-rumen-digesta-mixture' (BBRDM), a non-hazardous abattoir-derived organic product. Recently, fabrication of a newly designed helical-ribbon mixer dryer was illustrated by our group (Bhowmik et al., 2021a) for transition to equipment-driven pilot-scale production from current small-scale household cooking. Earlier, Ragályi and Kádár (2012) found an increased productivity of maize, mustard and triticale in Hungary upon the application of dried slaughterhouse compost. They also recorded higher residual fertility of soil even after three to four years of application. Roy et al. (2016) followed the same recycling strategy to reuse the rural slaughterhouse waste in field cultivation of tomato. Produced BBRDM is devoid of extra chemicals and slaughterhouse pathogens whereas other available waste management technologies do not guarantee absence of residual chemicals used during waste processing and pathogen free by-product (Bhowmik et al., 2021a). For example, as the method vermicomposting lacks thermophilic phase, therefore complete eradication of pathogen in the final produce cannot be assured (Tognetti et al. 2005). On the other hand, re-contamination of *Clostridium*, *Salmonella*, and BSE may be possible in composted fertilizer when temperature starts to decrease during subsequent cooling stage (NABC, 2004). Interestingly, Roy et al. (2015) showed that waste cook-drying effectively destroyed slaughterhouse pathogens including *Bacillus*, *Brucella*, *Salmonella*, *Clostridium*, *Mycobacterium* and strains of *E. coli*.

Aim and objectives

A great number of studies have been performed to evaluate agronomic efficacy of abattoir-derived organic amendments and their possible impacts on soil biology and fertility. Very recently, Yetilmezsoy et al. (2022) demonstrated the positive effects of sheep-derived organic amendment on onion, carrot, and black bean. Ozdemir et al. (2019; 2021) applied recycled poultry waste as nutrient source for walnut and chickpea. Doyeni et al. (2021) documented the effects of animal-derived fertilization on soil health, GHG emissions, and spring wheat yield in loam and sandy loam field. Frazão et al. (2021) fertilized soybean with poultry litter to study its agronomic effectiveness. In order to produce okra, Sankar et al. (2021) constructed organic briquettes using rumen digestion and blood from cows. Applying the same fertilizer, Roy et al. (2016) cultivated organic tomatoes. Upon the addition of poultry manure, Adeyemo et al. (2019) evidenced significant changes in soil structure, organic matter turnover, and maize yield in Nigeria. At the same year, Lin et al. (2019) noticed increased SOM percentage and aggregation stability, and positive alteration in native microbial community due to long-term pig manure application, and Lupwayi et al. (2019) linked enzyme activities and soil microbial abundance to long-term animal manure applications. Oliveira et al. (2017) obtained highest radish yield in animal-manured soils. Ragályi and Kádár (2012) applied abattoir waste for triticale, maize, and mustard in Hungary, while Nunes et al. (2015) used it for soybean and corn production in Brazil. In addition, Llaven et al. (2008) previously reported better fruit quality of bell pepper when soils were fertilized with vermicompost made from animal waste.

The present investigation may resolve two major socio-economic issues of the developing nations: (a) need for a cheap, good quality organic fertilizer for marginalized farmers and (b) hygienic waste management through reuse leading to development of a green business. In addition, BBRDM application could provide double benefits to farmers due to strong residual fertility of organic fertilizers as reported by Ragályi and Kádár (2012) earlier. To the best of our knowledge, no research on the agricultural applications of rural slaughterhouse waste has yet been published in which the application dose calculation, fertilization frequency determination, and impact analysis on plant and soil health taking farmer's economy into consideration were reported in a holistic manner. Therefore, pot and field-scale cultivations of seasonal vegetables were performed to address this gap as well as conducted social surveys in seven districts of West Bengal (India) to study stakeholder willingness for the product. This work is advantageous over our previous studies of Roy et al. (2013; 2016) where dissimilar levels of N was supplied to agricultural soils.

Recycling organic waste in agriculture is the primary purpose of the study and objectives were threefold as;

(a) Characterization of recycled slaughterhouse waste as fertilizer,

(b) Dose calculation, application frequency determination, and effect analysis on agro-ecosystem health, and

(c) Stakeholder willingness assessment for the product, and development of bio-economy through waste to fertilizer conversion.

In this study, a previously unreported spectral analysis of BBRDM (recycled slaughterhouse waste) was carried out. The residual fertilizer effects of BBRDM were also evaluated with Amaranthaceae plants. Additionally, comparative analysis of methane emissions among the fertilizer treatments was performed, and finally the composition, abundance, and diversity of soil bacterial communities in response to BBRDM addition was obtained through amplicon-based metagenomics. To study the possible correlations, multivariate principal component analysis (PCA) was also performed between soil physico-chemical parameters and microbial communities under different soil treatments. Furthermore, economic feasibility of using BBRDM as fertilizer was investigated along with its effect on fruit nitrate/ nitrite concentrations. This research may be helpful in transforming chemical farming into sustainable green agriculture promoting circular economy for a better future.

2.

Materials and methods

2.1. Preparation of ‘bovine-blood-rumen-digesta-mixture’

To prepare BBRDM, bovine blood and rumen content were collected from the abattoirs of Magrahat II block in South 24 Parganas district of West Bengal state, India immediately after butchering of animals. About 60 L fresh blood and 20 kg rumen digesta were mixed (in 3:1 ratio) following Roy et al. (2013), and heated at 90-110 °C for 2-3 hours using a novel helical-ribbon mixer dryer to obtain 14 kg of BBRDM in one batch. BBRDM was a coarse granular non-hazardous organic material and olive brown (2.5Y 4/3) in color (Roy et al., 2013). The recycling machine consists of three sub-units: (a) a cylindrical drying container, (b) helical ribbon-shaped mixing spindle, and (c) a burner (see Figure A.2), and installed at Magrahat slaughterhouse for pilot-scale manufacturing of BBRDM (see Figure A.3). Three batches were produced per day, and BBRDM was kept at room temperature during the study time. An Indian Patent was granted to us (Bhowmik et al., 2021b) in 2021 (number 370 569) on this equipment. According to Roy (2018), BBRDM retained a shelf life of 12 weeks, and showed best results when applied after two weeks of manufacturing

2.2. Characterization of BBRDM as fertilizer

A digital pH meter (LMPH - 10, Labman Scientific Instruments Ltd. Chennai, India) was used to measure pH of the final produce. Ammonical nitrogen (NH_4-N), total Kjeldahl nitrogen (TKN), and nitrate nitrogen (NO_3-N) were evaluated applying Kjeldahl method. Vanadomolybdophosphoric acid colorimetric and flame photometric methods were followed to determine total phosphorous (TP) and potassium (K) contents in BBRDM (Radojevic and Bashkin, 1999) respectively. Organic carbon (SOC) percentage was calculated according to the methodology of Walkley and Black (1934). To assess C/N ratio of the final produce, OC of BBRDM was divided by the TKN content. Similar methodologies were applied to characterize commonly available vermicompost for comparison with BBRDM.

Spectral characterization was done using scanning electron microscopy (SEM) combined with energy dispersive spectroscopy (EDS) and powder X-ray diffraction (XRD). To study surface morphology and elemental composition of BBRDM, SEM-EDS analysis was performed (Martins et al., 2002), and molecular structure of presented crystal or any other material was studied using XRD crystallography (Manohara and Belagali, 2017). Sample (BBRDM) was dried on oven first, crushed into fine particles, and then spread onto double-sided carbon tape. Micrographs were recorded on ZEISS EVO 18 (Jena, Germany) SEM, and Bruker XFlash 6I30 (Billerica, United States) energy dispersive X-ray detector was applied for elemental identification of BBRDM (Yao et al., 2011). Working distance was around 9.0 mm and electron high tension (EHT) was 20 kV for the run. A Philips PAN analytical (Almelo, Netherlands) powder X-ray diffractometer was used to obtain XRD spectrum at 2θ angle where the anode material was copper (Sharma et al., 2019).

2.3. Assessment of BBRDM as fertilizer

2.3.1. Pot cultivation

To investigate the effect of BBRDM on plant health and productivity, successive cultivation of cabbage-spinach (during August to November, 2018) and bell pepper-amaranth (during November, 2018 to March, 2019) was conducted in pot at the rooftop of the School of Environmental Studies, Jadavpur University, India (22° 33' 42" N, 88° 24' 46" E, 9.9 m MSL). Crop selection was done according to the biological soil disinfection potential of the sole or residual crop. A total eighteen tubs (diameter 18 cm × depth 22 cm) were poured with (a) soil of Magrahat village as control (S), (b) soil + N/P/K=10:26:26+urea (as chemical fertilizer) generally applied for vegetable production in West Bengal (CF), (c) soil + vermicompost containing N/P/K=4:1:2 (VC), (d) soil + 6 g BBRDM kg^{-1} of soil (80 kg N ha^{-1}, low dose) (BL), (e) soil + 9 g BBRDM kg^{-1} of soil (120 kg N ha^{-1}, recommended dose of BBRDM which is similar to CF and VC) (BR), and (f) soil + 13 g BBRDM kg^{-1} of soil (180 kg N ha^{-1}, high dose) (BH). Soil was taken from top 30 cm of an agricultural field (22° 13' 48" N, 88° 22' 12" E, 2.71 m MSL) located in Magrahat II block of South 24 Parganas. Each pot was filled with 4 kg of soil having 17 % moisture. The standard Munsell Soil Color Charts by Torrent and Barron (1993) were used to define soil color, hydrometric measurements were applied to determine particle size distribution, casagrande methods were followed to measure moisture content and water holding capacity (Radojevic and Bashkin, 1999). Soil pH was measured potentiometrically. Particle density and bulk density were examined according to the ASTM D7263 active standard, and the US Bureau of Soils and Chemical Systems' Triangular Classification Chart was used to study the kind of soil (Murthy, 1992). Before cultivation, soil N, P, K, and SOC contents were determined using the techniques outlined in section 2.2. A completely randomized block design (RBD) with three replications was followed for each six treatments. Fertilization was done through side-dressing following a total 120:80:80 kg NPK ha^{-1} of soil (Manjunath et al., 2018) which is recommended for hybrid cabbage (var. Hari Rani F1 hybrid) and bell pepper (var. Arka Basant) in the state of West Bengal, India. Fertilization dose for BBRDM was determined based on N content of the fertilizer. In order to keep nitrogen levels constant among treatments, the fertilizer dose of the CF treatment (N/P/K=10:26:26) was first calculated while accounting for the P and K contents of the fertilizer. Then, the required quantity of N was subsequently given via urea (N/P/K=46:0:0) fertilization. After seedling transplantation, pots were watered thrice a week. Infestations of common pests including bollworms, aphids, leaf miners, whiteflies, spider mites, thrips, and nematodes as well as diseases like clubroot, blight, wilt, leaf spots, mildews, and white mould were not remarkably seen during the study time. As a result, no insecticides were used. Every two weeks, plant growth data were evaluated to measure physiognomic changes.

Residual fertilizer effect was studied on the same soil after removing plant heads. On the previously fertilised pots, approximately same numbers of seeds were sown, and no fertilizer was added during the cultivation period. After 30 days of cultivation, growth parameters were recorded. When necessary, the soil was irrigated and no serious incidences of diseases or pest attacks were noticed during the study time.

2.3.2. Field study

After successful pot trial, cabbage-spinach sequence was transferred to field considering plant growth response, rate of disease infestation, and seasonal characteristics. The field cultivation was carried out over two successive years in Magrahat village of South 24 Parganas district (22° 13' 48" N, 88° 22' 12" E, 2.71 m MSL) during August to November of 2019 as season 1 and in 2020 as season 2. For at least ten years, no organic amendments had been applied to the soil. For this experiment, a randomized block design strategy was applied. As *Brassicaceae* plants are widely used for soil biofumigation, thus spinach was cultivated in the residual soil after harvesting cabbage heads (Mowlick et al., 2013) to prevent major wilting problem. Following Roy et al. (2016), the cultivation plot was divided into twelve sub-plots: six were fertilized with well-prepared BBRDM, and the remaining six were treated with N/P/K = 10:26:26+urea as a control. Each sub-plot attained a soil surface of 15 m^2. To provide 120 kg N ha^{-1} of soil, fertilization was made on dry matter basis (see section 2.3.1 of the previous chapter for fertilizer dose calculation) as suggested by Manjunath et al. (2018) for the cabbages of Indo-Gangetic plains. At the time of field preparation, half of the fertilizer was supplied as basal dose, while the remaining quantity was delivered through side-dressing method in two equivalent splits according to Tiwari et al. (2003), after 21 and 40 days of transplanting. Approximately same height (21 days old) of cabbage seedlings were transferred to field maintaining 45 cm × 60 cm crop space. Every sub-plot contained 48 plants. Crop was irrigated adequately with 15 days interval. Once a month, neem oil was applied as biocontrol agent against common pest attacks. Hand-weeding method was followed to eradicate weed infestation. Over the whole cultivation period, no chemical fungicides, insecticides, or even herbicides were used. Cabbage plants were harvested after 8 weeks, and the yield parameters were then examined. During the study period, the cultivation site received an average 222 mm rainfall in season 1 and 196 mm in season 2 while the mean seasonal temperatures varied between 36 °C and 16 °C, and 38 °C and 14 °C, respectively. Data on climatic variations were recorded from the website of India Meteorological Department (IMD) of Government of India for the experimental region. However, after removing cabbage heads soil samples from BBRDM and chemical (N/P/K=10:26:26+urea) fertilized sub-plots were collected at 5-10 cm soil depth, and then combined them together to form two composite samples as directed by Sengupta and Dick (2015) earlier: one denoting the BBRDM-fertilized soil (BR) while the other representing chemically cultivated soil (CF). Upon arrival at the laboratory, soils were divided into two fractions: one for soil physico-chemical analysis (dried at 30 °C and then sieved to <2 mm) and the other for determining soil biological properties (stored at 4 °C after screening through <2 mm mesh). Samples for metagenomic study were kept at -20 °C. Elliott's (1986) wet sieving technique was followed to study the size distribution of the soil aggregates, and Walkley and Black's (1934) method was applied to calculate the SOC content. Activity of soil enzymes like urease, alkaline phosphatase, and β-glucosidase were measured according to Tabatabai and Bremner (1972), Tabatabai and Bremner (1969), and Eivazi and Tabatabai (1988), respectively as cited in Dick et al. (1997).

Residual fertilizer effect was investigated on the same soil cultivated spinach (*Spinacia oleracea* L., which belongs to the family Amaranthaceae) as test crop. The yield characteristics were recorded after 30 days of cultivation as mentioned in section 2.3.1. For both seasons, approximately same numbers of seeds were sown over the previously fertilized beds, and no additional fertilization was done during the cultivation period. When necessary, the soil was irrigated, and during the study, no significant cases of illnesses or pest attacks on spinach were noticed. Following the recommendations of European Directive (EC) No. 1881/2006, plant leaves from the main and residual plots were taken for nitrate and nitrite analysis, with the determination being made in accordance with ISO 6635: 1984 (Stachniuk et al., 2018). For comparison, cabbage and spinach leaves were also obtained from a local vegetable market.

2.4. Status of available soil N during the cultivation

The amount of available soil nitrogen (NH_4^+-N) was determined at the beginning of cultivation (day 1) and every two weeks throughout the cultivation period using Subbiah and Asija's (1956) alkaline permanganate method. This technique is appropriate for Indian soils in general but does not quantify soil nitrate (Roy et al., 2016). P and K dynamics were not investigated as fertilization was done maintaining equal amount of N instead of P and K.

2.5. Microbiological analysis of soil under different treatments

Amplicon sequencing of the variable V3-V4 region of 16S rRNA gene was studied on llumina platform to examine the diversity, abundance, and composition of bacterial communities under organic and inorganic fertilizer regimes. Metagenomic DNA (from both pot and field soils as S, CF, BR) was extracted using Nucleospin Soil Kit (TaKaRa Bio Ltd, Japan) . Following Kowalchuk et al. (1997), prior to DNA isolation soil samples were washed carefully with 120 mM K_2PO_4. A ND-2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA) was applied to evaluate the quality of extracted DNA. By synthesizing 16S rRNA Forward (5'-CCTACGGGNBGCASCAG-3') and 16S rRNA Reverse (5'-GACTACNVGGGTATCTAATCC-3') primers amplification of the hypervariable 16S rRNA V3-V4 regions was done, and developed amplicon libraries applying Nextera XT Index Kit (Illumina Inc., USA) in accordance with the standard protocol for 16 S Metagenomic Sequencing Library preparation. The targeted bands were removed for purification after amplified PCR products were studied on 2% agarose gel at 120 V for 60 min. The purified amplicons were then quantified using a Qubit fluorometer (Thermo Fischer Scientific, USA), sequenced (2 × 250 bp) on an Illumina platform (Illumina, San Diego, USA), and analyzed using Quantitative Insights Into Microbial Ecology (QIIME) bioinformatic pipeline following Caporaso et al. (2010). With the accession number PRJNA593705, obtained raw sequences were deposited to NCBI- Sequence Read Archive (SRA) database. In order to further investigate the possible relationship between soil characteristics and the relative abundance of bacterial phyla under various field treatments, Principal Component Analysis (PCA) was performed using the R software.

2.6. Benefit-cost analysis of cabbage-spinach cultivation

Cost-benefit analysis was performed to study the economic feasibility of reusing slaughterhouse waste in agriculture as an organic fertilizer. Land preparation, seedling purchase and transplantation, irrigation, fertilization, weed and pest control, harvesting and rental value of the land all were included as cultivation cost. BBRDM was purchased at INR 26 kg^{-1}, while the chemical fertilizer (N/P/K=10:26:26+urea) costed INR 34 kg^{-1}. Labors were provided with INR 350 per head per day during the field cultivation. An average market price of INR 20 kg^{-1} for spinach and INR 30 kg^{-1} for cabbage was considered for profit calculation. Then, in accordance with Tiwari et al. (2003), based on net return from multi-year cabbage-spinach field over a hectare of soil the benefit-cost ratio was evaluated.

2.7. Quantification of air-soil methane flux

Soil samples taken from the experimental site (both pot and field) and waste disposal site (WDS) were placed in 40 millilitre borosilicate screw cap vials, which were then kept in the dark for three days (Chan and Parkin, 2001). With a Systronics GC-8205 (India) gas chromatograph linked with a flame ionization detector (GC-FID), methane concentrations (in 5 ml aerobic headspace) were measured where the carrier gas was H_2 having a flow of 30 mL/min, the column temperature was 50 °C, and temperature of the detector was set at 140 °C.

With some minor adjustments, the air-soil methane flow was computed in accordance with Khoiyangbam et al. (2004).

2.8. SWOT analysis for BBRDM commercialization

Our investigation followed three steps (a) visit of the study locations for exploratory/ participatory observations, (b) open-ended questions followed by face to face semi-structured interviews, and (c) review of documented evidence with allied literature. A multiple-choice questionnaire embedding twenty questions (see Annexure II) prepared in English was followed for data collection. A total 50 rural abattoirs from 7 districts of West Bengal namely Malda, Murshidabad, Howrah, Hooghly, Nadia, West Medinipur, and South 24 Parganas participated during the survey (between March and May, 2023), and both slaughterhouse owners (50 individuals) and local farmers (a total 500 individuals, 10 from each site) were interviewed to understand their mind-set. The recorded interviews were transcribed using grounded method, and barriers and enablers were shortlisted by Strength, Weakness, Opportunity, and Threat (SWOT) matrix following Paes et al. (2019) for wide-scale implementation of the model.

2.9. Statistical analyses

Statistics was established by SPSS package (SPSS Inc., USA) for Windows version 16.0, and all experiments (except the metagenomic study, spectrum characterisation of BBRDM, and SWOT analysis) were performed in triplicates. Standard deviations are shown with mean values. While Student's *t*-test compared field applications, *post hoc* Tukey's test was applied to contrast pot treatments and nitrate/nitrite concentrations in cultivated vegetables. Differences made significant at 0.5% cut-off level.

3.

Findings

BBRDM characterization and pot cultivation

Highlights

- BBRDM (bovine-blood-rumen-digesta-mixture) produced as an organic fertilizer
- Spectral characterization of BBRDM done by SEM-EDS & XRD analysis
- BBRDM application dose was calculated
- Application of BBRDM produced high yield in pot
- Residual fertilization effect was evident in BBRDM treated soils
- CH_4 emissions from abattoir dumping site higher than the BBRDM fertilized pots
- Copiotrophs abundant in soil amended with BBRDM

3.1.1. Physico-chemical analysis of BBRDM and spectral characteristics

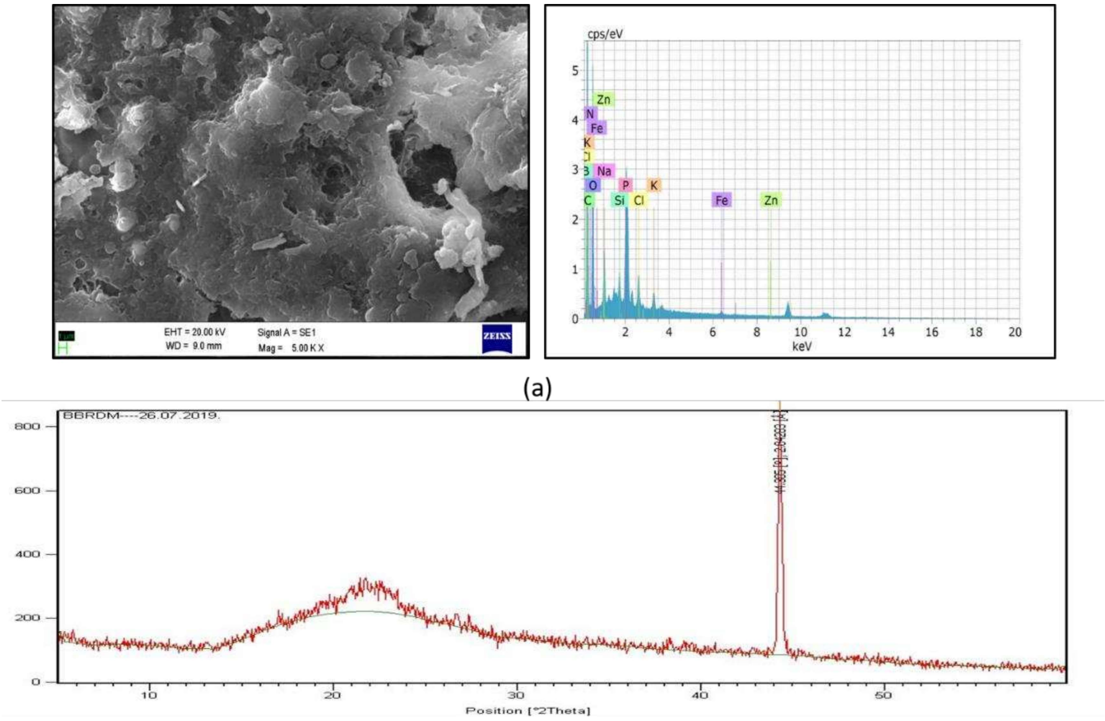
BBRDM was brown olive (2.5Y 4/3) in color, the pH was recorded as 7.5, and the moisture content was around 16.95% (w/w). For BBRDM, the following were noted: NH_4-N 518.51±28.60 mg kg^{-1}, TKN 5977.75±184.04 mg kg^{-1}, NO_3-N 1232.99±66.28 mg kg^{-1}, TP 783.14±30.46 mg kg^{-1}, and K 911.28±59.81 mg kg^{-1}. The NPK content of BBRDM was approximately 7.63:1:1.16. As shown in Figure 1a, the SEM-EDS analysis confirmed the presence of eleven different elements, namely carbon, oxygen, nitrogen, phosphorus, sodium, potassium, boron, zinc, iron, calcium, and silicon in BBRDM. Concentrations of OC (57.01%) and N (12.16%) were relatively higher in BBRDM compared to the other existing elements as of SEM-EDS analysis, and carbon to nitrogen (C/N) ratio of BBRDM was measured around 4.68. It was determined by X-ray powder diffraction that ammonium-potassium nitrate complex salt required for better plant development was also present in BBRDM (Figure 1b). The applied vermicompost contained a 348.88±10.26 mg kg^{-1} NH_4-N, 2264.02±88.64 mg kg^{-1} TKN, 619.36±42.90 mg kg^{-1} NO_3-N, 1016.57±38.20 mg kg^{-1} TP, and 454.36±18.62 mg kg^{-1} K (Table 1). The said fertilizer was dark brown in color (10YR 2/2), having pH 6.0, and contained 39.54% (w/w) moisture.

3.1.2. Pre-cropping soil properties

According to the standard Munsell Soil Color Charts, the soil was light brownish grey (10YR 6/2) in color and its pH ranged from 6.0 and 7.0. The water holding capacity was 70 mL L^{-1} and the moisture content was 17% (w/w). Particle size distribution was as follows: sand 44%, silt 39% and clay 17%. The soil had a specific gravity of 1.07, particle size density of 2.27 g cm^{-3}, and a bulk density of 1.19 g cm^{-3}. Soil type was loamy. Soil N 1086.24±39.16 mg kg^{-1}, P 143.65±2.83 mg kg^{-1}, K 38.46±2.08 mg kg^{-1}, and SOC 07.32±0.61 mg g^{-1} were obtained. Table 1 shows the physico-chemical characteristics of soil and different organic fertilizers used during the cultivation.

Table 1. Physico-chemical characteristics of soil and organic fertilizers used

Treatments	Nutritional status					
	pH	**Moisture (%)**	**N (mg kg^{-1})**	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Org C (mg g^{-1})**
Soil	6.5	17	1086.24	143.65	38.46	7.32
Vermicompost	6.0	39.54	2264.02	1016.57	454.36	14
BBRDM	7.5	16.95	5977.75	783.14	911.28	29.97



(a)

(b)

Figure 1. (a) Morphological and elemental composition of BBRDM was revealed by SEM-EDS analysis, and (b) XRD confirmed the presence of ammonium-potassium nitrate complex in the final produce. See section 2.2 for details

3.1.3. Plant growth and residual yield

During pot study, application of BBRDM at rate of 6 g kg^{-1} and 9 g kg^{-1} of soil offered significantly higher yield of cabbage and bell pepper compared to the vermicompost and chemical fertilizer (N/P/K=10:26:26+urea) applied (Figure 2; A.3). In contrast, plants did not survive at higher BBRDM dose (13 g kg^{-1} of soil) due to the presence of labile carbon fractions in BBRDM. Lesser disease infestation, early flowering and fruiting, and well-developed root system were noticed when soils fertilized with recommended dose of BBRDM, and the availability of soil N was found to be greater in BBRDM fertilized pots, as shown in Figure 3. The residual effects of BBRDM were also tasted on spinach and amaranth (see Figure A.4). Comparative study of growth parameters among all the soil treatments after 30 days of cultivation as displayed in Figure 2 revealed that the yield and development of residual crops was greater in soils treated with recommended dose of BBRDM. On the other hand, plants cultivated in residual pots of BH treatment perished after 15 days of seed sowing. During this investigation, compared to chemical fertilization soils amended with vermicompost attained higher residual values.

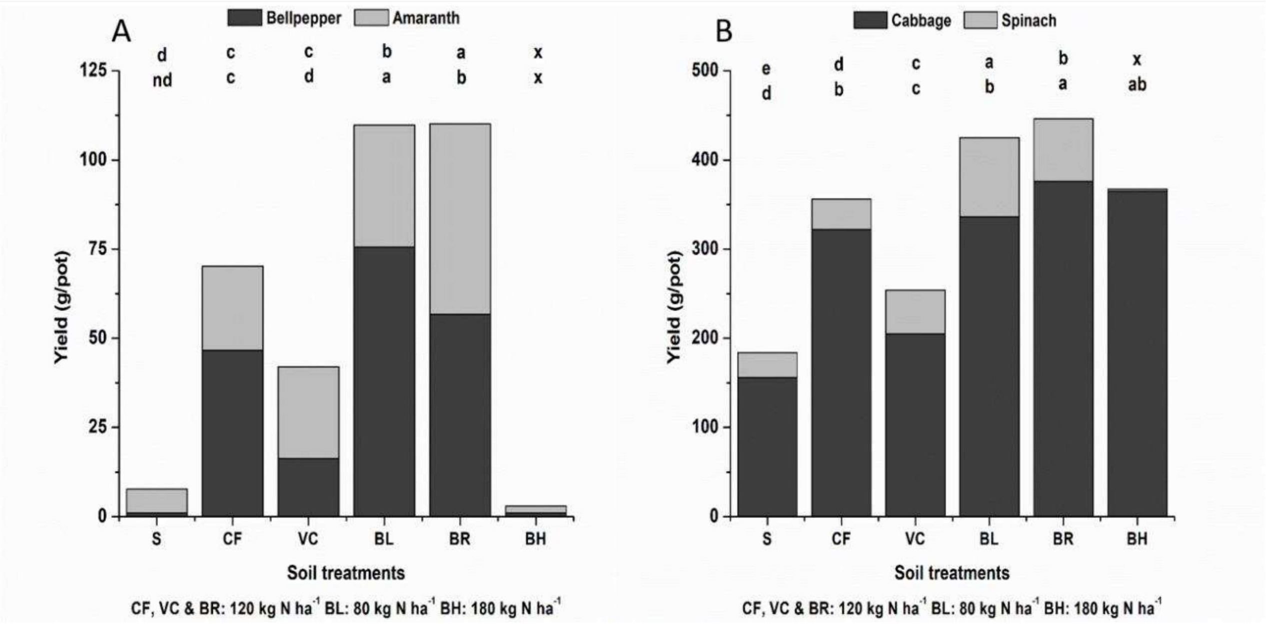


Figure 2. Crop yield from (a) bell pepper-amaranth and (b) cabbage-spinach rotation in pot. S: soil, CF: chemical fertilizer, VC: vermicompost, BL: low dose of BBRDM, BR:

recommended dose of BBRDM, and BH: high dose of BBRDM. Pairwise comparison was performed by Tukey's *post hoc* analysis, and superscripts indicate significant differences among pot treatments at 5% cut-off level. X: plants died

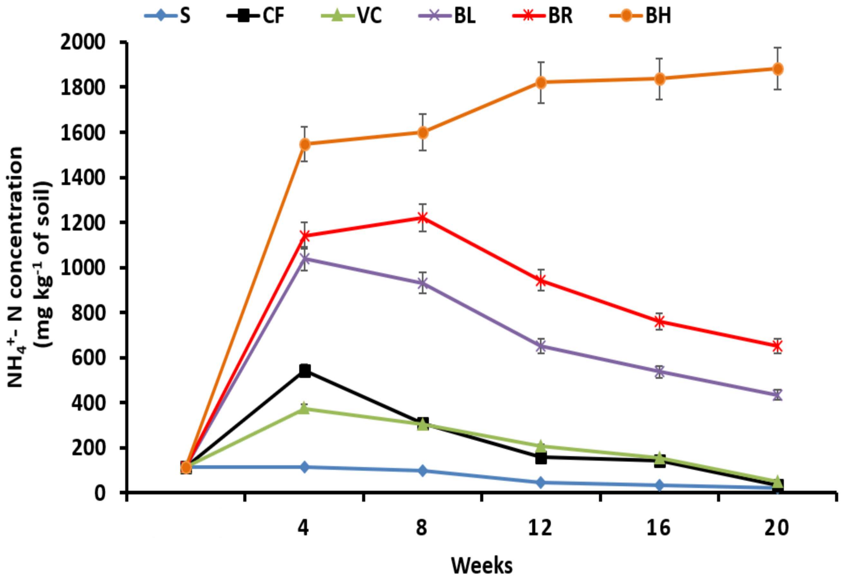


Figure 3. N dynamics in pot soil. Subbiah and Asija's (1956) method was followed to determine available soil N. See section 2.4 for details. S: soil, CF: chemical fertilizer, VC: vermicompost, BL: low dose of BBRDM, BR: recommended dose of BBRDM, and BH: high dose of BBRDM

3.1.4. Fertilizer effects on soil microbial abundance and diversity

At 3% dissimilarity threshold, all sequences obtained through 16S V3-V4 metagenomics were clustered into operational taxonomic units (OTUs). The study found phyla *Proteobacteria* (35.22% in BR=BBRDM fertilized soil and 32.87% in CF=chemically cultivated soil), *Planctomycetes* (16.55% in BR and 10.23% in CF), *Bacteroidetes* (15.26% in BR and 13.06% in CF), *Chloroflexi* (11.77% in BR and 9.77% in CF), *Firmicutes* (5.12% in BR and 4.2% in CF), and *Verrucomicrobia* (1.5% in BR and 1.4% in CF) abundant in soils treated with BBRDM. On the other hand, chemically cultivated soil had a predominance of *Actinobacteria*, 12.54% in BR and 19.44% in CF respectively. Among the identified bacterial classes, *Alphaproteobacteria* (23.6%), *Actinobacteria* (11.8%), *Cytophagia* (6.3%), *Flavobacteriia* (4.6%), *Bacilli* (2.9%), and *Saprospirae* (1.3%) were notable in CF treatment while soils fertilized with BBRDM attained higher abundance of *Planctomycetia* (16.4%), *Gammaproteobacteria* (13.8%), *Sphingobacteriia* (8.9%), and *Betaproteobacteria* (2.2%). Fertilizer impact on the composition and diversity of soil bacterial communities are represented in Figure 4. However, *Planctomyces* was the most prevalent genus found from the study accounting for 12.63% and 7.11%, respectively of the total sequences for BBRDM and CF treatments. Interestingly, the Shannon alpha diversity index (8.96) was higher in chemically treated soils in comparison with the BBRDM fertilized soils (7.52), considering the species-level phylogeny. Any of the slaughterhouse pathogens was not found as documented by Franke-Whittle and Insam (2013) and Roy et al. (2015) earlier, in BBRDM fertilized soils.

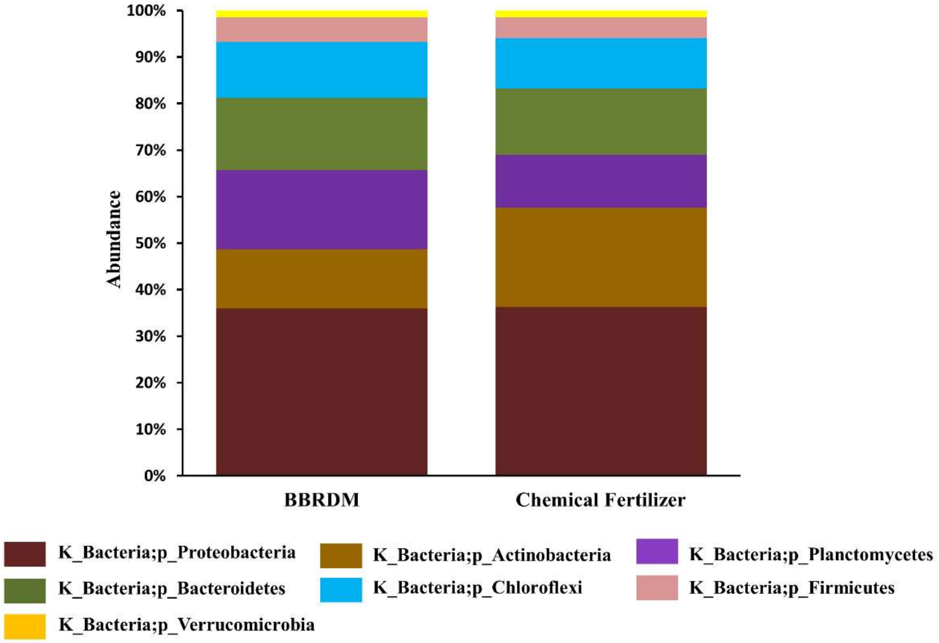


Figure 4. Predominance of bacterial phyla, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes* and *Verrucomicrobia* under chemical (N/P/K=10:26:26+urea) and organic (BBRDM) treatment was studied on Illumina platform.

Quantitative Insights into Microbial Ecology (QIIME) was applied to analyze the sequences. Color code (read L to R) indicates their abundance. See section 2.5 for details

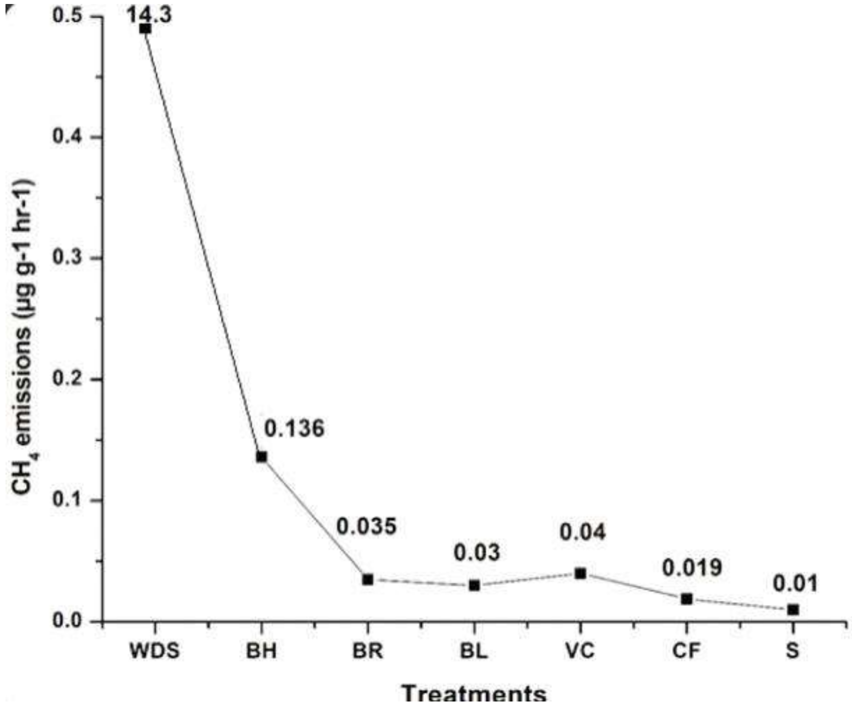


Figure 5. Air-soil methane flux from waste dumping site and cultivation tub. Gas chromatograph equipped with flame ionization detector (GC-FID) was to quantify. S: soil, CF: chemical fertilizer, VC: vermicompost, BL: BBRDM (low dose), BR: BBRDM (recommended dose), BH: BBRDM (high dose), and WDS: waste dumping site.

3.1.5. Air-soil methane flux from cultivation tub

The mean air-soil methane flux (in 5 mL headspace) was 0.01 µg g^{-1} hr^{-1} in treatment S, 0.019 µg g^{-1} hr^{-1} in treatment CF, 0.04 µg g^{-1} hr^{-1} in treatment VC, 0.03 µg g^{-1} hr^{-1} in treatment BL, 0.035 µg g^{-1} hr^{-1} in treatment BR, and 0.136 µg g^{-1} hr^{-1} in treatment BH. As shown in Figure 5, methane emissions from the soils of abattoir dumping sites varied between 13.13 to 28.55 µg g^{-1} hr^{-1} (in 1 mL headspace) which was calculated 150 times higher than that emitted from the soils of BBRDM fertilized pots.

Field application and effects on agro-ecosystem health

Highlights

- BBRDM applied for field cultivation of cabbage and spinach
- Cabbage yield higher with BBRDM treatment compared with chemical fertilization
- BBRDM had stronger residual effect on spinach growth
- Economically, BBRDM advantageous over commercially available fertilizers
- Nitrate/ nitrite concentration was lowest in fruits fertilized with BBRDM
- Methane emissions lower in BBRDM soils than the waste dumping site
- Changes in soil parameters noticed under different fertilization
- BBRDM amended soils showed copiotrophic dominance in field

3.2.1. Cabbage yield and fertilizer response

The cultivation plot was divided into twelve sub-plots: six were tasted with recommended dose of BBRDM, and another six were fertilized with N/P/K=10:26:26+urea, as shown in Figure 6. During the field study, Highest cabbage yield was obtained with BBRDM application. The yield was 27% higher in season 1 and 38% in season 2 compared to N/P/K=10:26:26+urea. Figure 7 represent the meteorological changes during the cultivation period, and Figure 8 depict yield differences (at $p < 0.05$) between the treatments for both years. With adding the prescribed amount of BBRDM (9 g kg^{-1} of soil) to the field soils, reduced disease infestation, compact head formation, and a well-developed plant root system (see Figure A.5) were observed. Although equal amount of N was supplied to both the field, the concentration of soil available N that was found higher in BBRDM fertilized plots. This increase, as shown in Figure 9, may be due to the delayed mineralization of the organic N present in BBRDM which attributed in lower nitrate/nitrite accumulation in plant body as discussed in section 3.2.7. For CF, highest NH_4^+ peak was observed at 4th week and for BR on 8th week, the highest NH_4^+ peak was seen.

3.2.2. Residual fertilizer effects on spinach growth

Assessment of residual fertilizer effects for BBRDM was also crucial to plant health. Highest residual yield was recorded in BBRDM-fertilized soils for both seasons (see Figure A.5), while the yield obtained from residual CF plots was not satisfactory. A farmer can get double benefit over the BBRDM fertilization as the amendment hold strong positive residual effect on plant growth. Between the yields achieved from two residual fields, Figure 8 showed clear variations at $p < 0.05$. Vermicompost was not tested during the field cultivation as the yield from VC treatment was relatively lower in pot than the BBRDM and N/P/K=10:26:26+urea.

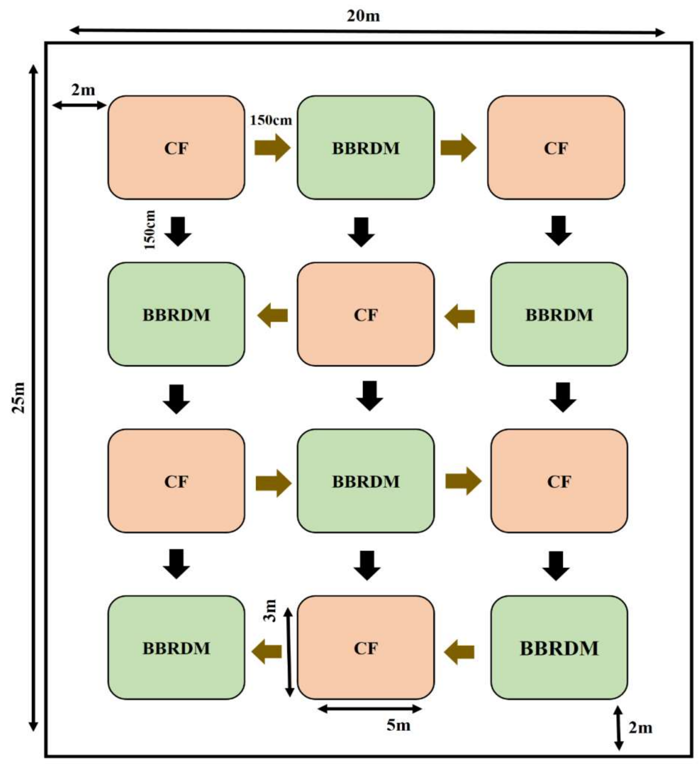


Figure 6. Layout of the experimental field designed according to Roy et al. (2016). See section 2.3.2 for details

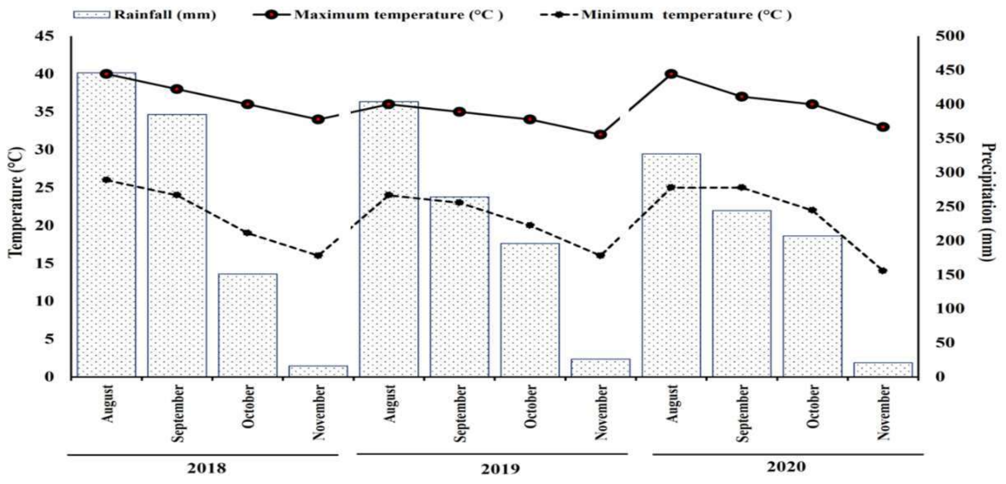


Figure 7. Climatic variations including monthly rainfall, maximum and minimum temperatures during the period of cultivation. Data were recorded from the website of India Meteorological Department (IMD) of Government of India for the experimental region

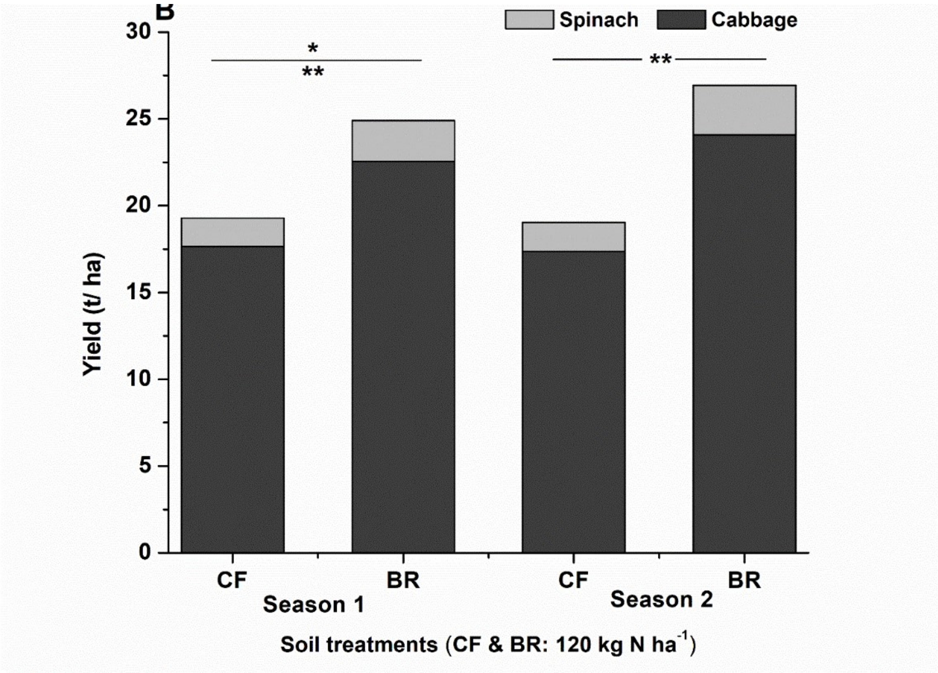


Figure 8. Cabbage-spinach yield in field. CF: chemical fertilizer and BR: recommended dose of BBRDM. Student's *t*-test compared field values. ** means $p < 0.01$ and * denotes $p < 0.05$

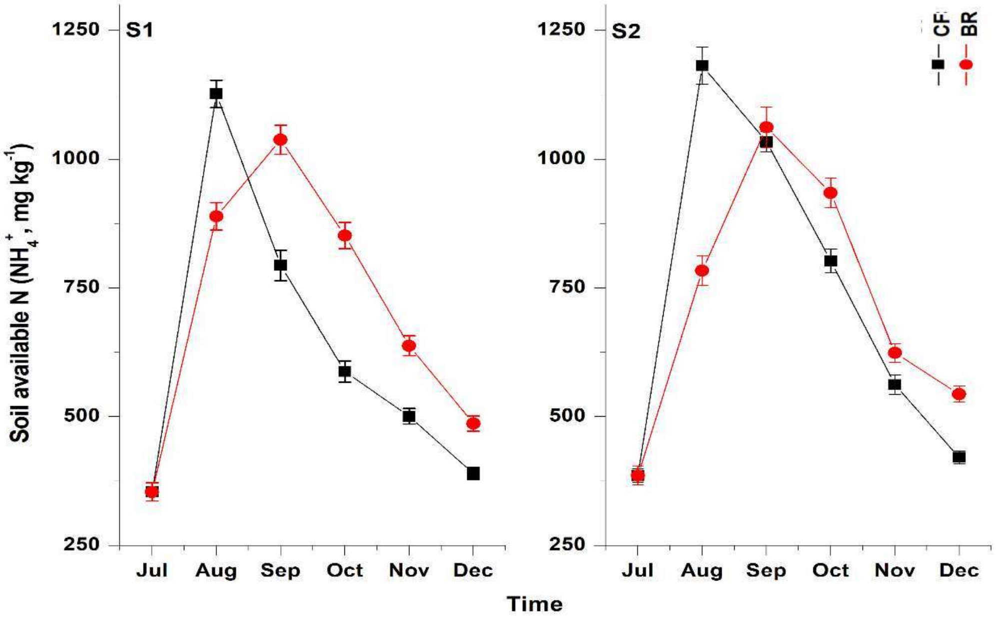


Figure 9. N dynamics in field soil. Subbiah and Asija's (1956) method was followed to

determine available soil N. See section 2.4 for details. CF: chemical fertilizer and BR: recommended dose of BBRDM; S_1: season 1 and S_2: season 2

3.2.3. Microbial abundance under different fertilizer regimes

Gel electrophoresis of the PCR-amplified products revealed distinct bands for sample S, BR and CF that seemed to co-migrate on the gel against lane L. Reads obtained through amplicon-based 16S V3-V4 metagenomics were then assembled into operational taxonomic units (OTUs) maintaining similarity level at 97% in order to evaluate the abundance, composition and diversity of soil microbial communities under different field treatments. Among the field treatments, phyla *Proteobacteria* (23.85% in BR = BBRDM amended soils, 21.59% in CF = chemically treated soils, and 20.86% in S = completely unfertilized soils), *Chloroflexi* (15.58% in BR, 16.94% in CF, and 18.65% in S), *Actinobacteria* (11.75% in BR, 13.77% in CF, and 15.21% in S), *Firmicutes* (11.49% in BR, 8.89% in CF, and 8.16% in S), *Acidobacteria* (8.51% in BR, 8.81% in CF, and 9.56% in S), *Planctomycetes* (8.81% in BR, 7.59% in CF and 6.47% in S), *Bacteroidetes* (5.57% in BR, 3.41% in CF, and 3.16% in S), *Verrucomicrobia* (4.72% in BR, 4.41% in CF, and 2.74% in S), and *Gemmatimonadetes* (2.49% in BR, 2.44% in CF, and 2.35% in S) were found to be dominant. Figure 10 illustrated the impact of fertilization on the variety of microbes. Interestingly, the dominance of oligotrophic *Chloroflexi*, *Actinobacteria*, and *Acidobacteria* were more pronounced either in soils treated with chemical or in unfertilized regime while soils treated with BBRDM attained higher copiotrophic abundance such as *Proteobacteria*, *Firmicutes*, *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia* and *Gemmatimonadetes*, as well demonstrated in Figures 10. Compared to BBRDM application, the Chao1 index which accounts for richness was greater in chemical treatment indicating decreased bacterial richness in BBRDM fertilized soils while a significantly larger Shannon Index value that means both species richness and evenness was obtained for BBRDM fertilisation regime. Soils fertilized with well-prepared BBRDM did not attain any of the slaughterhouse pathogens as described by Franke-Whittle and Insam (2013) and Roy et al. (2015). As visualized in Figure 11, this study also revealed possible correlations between soil properties and microbial abundance under various fertilization conditions.

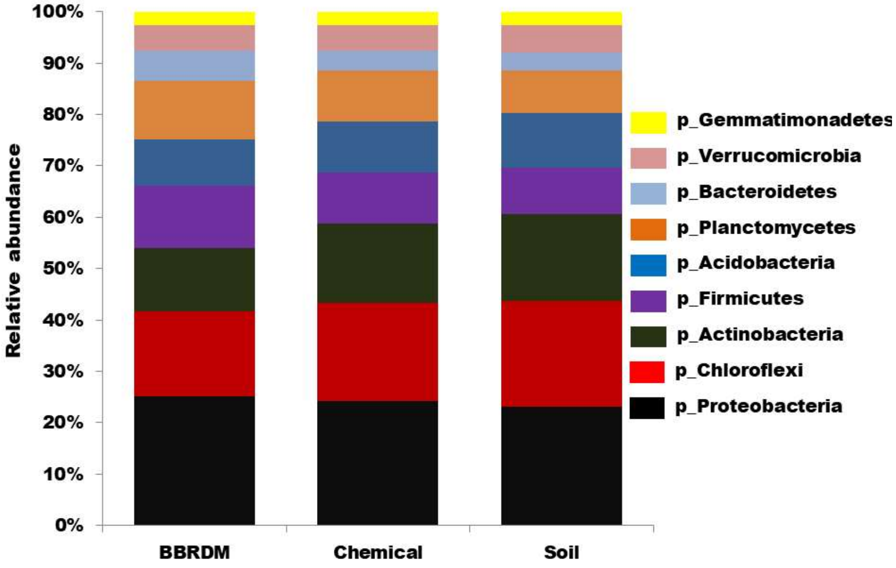


Figure 10. Relative abundance of bacterial phyla *Proteobacteria*, *Chloroflexi, Actinobacteria*, *Firmicutes, Acidobacteria*, *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia*, *and Gemmatimonadetes* was studied on Illumina platform from organic (BR), chemical (CF), and unfertilized soils (S), and Quantitative Insights into Microbial Ecology (QIIME) was used to analyze the sequences. See section 2.5 for details. Color code (read bottom-to-top) indicates their abundance

3.2.4. Changes in soil properties, and possible correlation with microbial abundance

As shown in Table 2, judicious fertilizer application and adequate N supply enhanced the structural, physico-chemical, and biological qualities of the soil. During field cultivation, BBRDM fertilization achieved higher SOC concentration, enzymatic activity, and improved aggregate size distribution compared to the application of N/P/K=10:26:26+urea. When soils were treated with the prescribed amount of BBRDM (9 g kg^{-1} of soil), a considerable increase in macroaggregate formation was observed; around 17% in season 1 and 19% in season 2 while CF treatment dramatically increased the percentage of soil microaggregates. On the other hand, the distribution of clay+silt having diameters <50 μm did not differ substantially across the treatments at $p < 0.05$. Our investigation also showed that the concentration of SOC was comparatively lower in unfertilized soils, while the BBRDM fertilization regime had the maximum indicating highest soil binding potential (Table 2). Under BBRDM treatment, SOC displayed a significant positive correlation with the percentage of macroaggregate formation (PCA=0.84). In comparison, the uses of N/P/K=10:26:26+urea produced a negative association between the SOC turnover and microaggregate development (PCA= -0.95) as shown in Figure 11. In addition, the field application of BBRDM increased the activities of alkaline phosphatase and β-glucosidase which reflected higher organic matter turnover and better soil profile under organic farming system. On the other hand, as demonstrated in Table 2, soil urease activity was higher in CF treatment. The increase was around 18% and 68% when compared to BBRDM and control plot possibly due to direct application of urea to the field soils. Interestingly, under BBRDM fertilization, activity of β-glucosidase established a strong positive correlation with the soil SOC content (PCA=1.0) (Figure 11). However, no correlation was made between the SOC and concentration of alkaline phosphatase and urease enzyme as SOC was considered as principal biological binding agent instead of total N (TN) or P (TP) in this study.

Multivariate PCA analysis revealed that copiotrophic abundance was positively shaped by the SOC content and macroaggregate formation in BBRDM fertilization, whereas microaggregate having relatively lower SOC allowed faster proliferation of oligotrophs establishing a strong negative correlation with SOC, as was observed in CF or in unfertilized soils (Figure 11). Most importantly, even after given same amount of N to both fertilization regimes and found SOC as key influencer on microbial abundance.

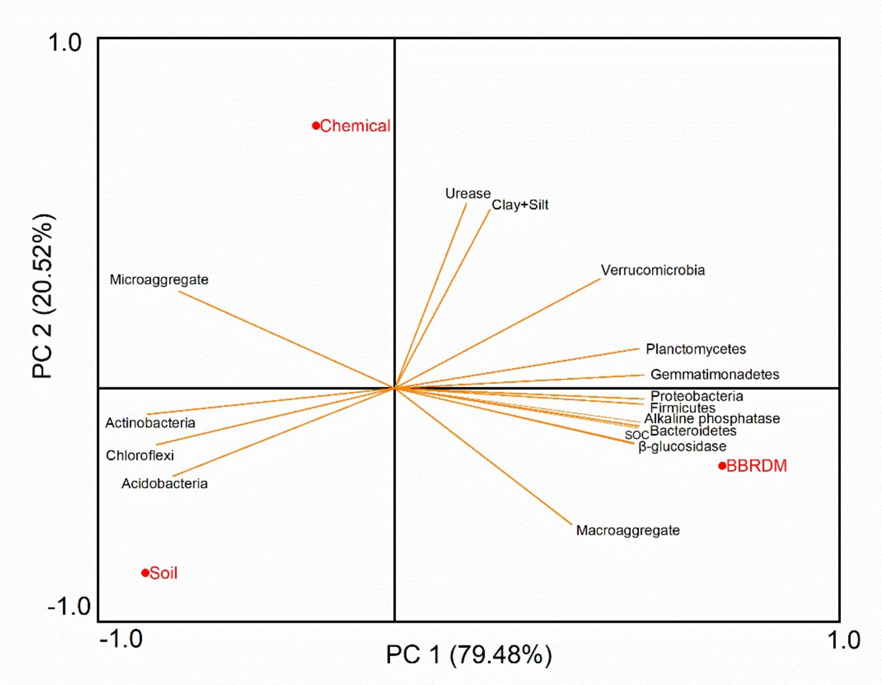


Figure 11. Biplot of the multivariate principal component analysis (PCA) carried out on soil physico-chemical changes and respective microbial abundance under various fertilizer treatments. Arrows indicate correlation coefficient

Table 2. Fertilizer effects on soil structural, physico-chemical and biological health

			Treatments		
			Before cultivation	After cultivation	
	Soil parameters		**Soil**	**Soil + CF**	**Soil + BBRDM**
Aggregate size distribution (%)	Macroaggregate	S_1	36.15 ± 3.02	$29.36 \pm 4.65^{*}$	$42.39 \pm 13.23^{*}$
		S_2	37.93 ± 6.42	$32.64 \pm 4.00^{*}$	$45.23 \pm 7.30^{*}$
	Microaggregate	S_1	53.75 ± 4.78	$58.10 \pm 2.03^{*}$	$46.05 \pm 10.56^{**}$
		S_2	53.02 ± 6.58	54.21 ± 5.32^{ns}	$43.35 \pm 4.17^{**}$
	Clay+Silt	S_1	10.11 ± 0.41	12.54 ± 2.68^{ns}	11.56 ± 5.01^{ns}
		S_2	09.05 ± 2.09	13.15 ± 4.83^{ns}	11.42 ± 2.56^{ns}
Soil binding potential (g kg^{-1})	SOC content	S_1	7.40 ± 0.35	8.24 ± 0.32^{ns}	$17.14 \pm 0.22^{**}$
		S_2	6.09 ± 0.24	8.49 ± 0.07^{ns}	$18.25 \pm 0.45^{**}$
Enzymatic response (mg kg^{-1} hr^{-1})	Alkaline phosphatase	S_1	143.06 ± 7.76	156.47 ± 9.88^{ns}	$211.23 \pm 15.50^{**}$
		S_2	148.37 ± 12.47	146.19 ± 8.99^{ns}	$236.38 \pm 21.48^{**}$
	Urease	S_1	91.42 ± 3.44	$161.64 \pm 2.01^{**}$	124.88 ± 17.69^{ns}
		S_2	89.48 ± 4.55	$143.17 \pm 4.30^{**}$	$132.45 \pm 5.27^{**}$
	β-glucosidase	S_1	62.38 ± 9.43	$68.33 \pm 5.08^{**}$	$87.96 \pm 10.22^{**}$
		S_2	59.65 ± 4.14	67.38 ± 9.82^{ns}	$94.39 \pm 13.40^{*}$

Differences made with Student's *t*-test. Values represented as mean of replicates for each treatment ($n=3$) with standard deviations. *: $p < 0.05$; **: $p < 0.01$ and ns: not significant. Macroaggregates: >250 µm diameter, microaggregates: 50 to 250 µm, and clay+silt: <50 µm. β-glucosidase and alkaline phosphatase were measured as mg *p*-nitrophenol kg^{-1} soil hr^{-1}, while urease activity was expressed as mg NH_4 –N kg^{-1} soil hr^{-1}. SOC: soil organic carbon; CF: chemical fertilizer, and BBRDM: recommended dose of BBRDM. S_1: season 1 and S_2: season 2.

3.2.5. Air-soil methane flux from cultivation field

In order to assess the methane emission potential of BBRDM and its contribution to global greenhouse budget, measurement of air-soil methane flux from amended agricultural soils was essential. The mean air-soil methane flux (in 5 ml headspace) was recorded as 0.008 in BBRDM cultivated soils, 0.005 in chemical treatment, and 0.002 $\mu g\ g^{-1}\ hr^{-1}$ in unfertilized soil, while the abattoir dumping sites emitted around 14.30 $\mu g\ g^{-1}$ methane per hour which was around 1787 times higher than that produced from BBRDM fertilized plots, as shown in Figure 12. Therefore, it seemed more sensible to convert waste into fertilizer rather than put it in a landfill or in the open. Methane emitted from CF and BBRDM treatments were not differ statistically at $p < 0.05$.

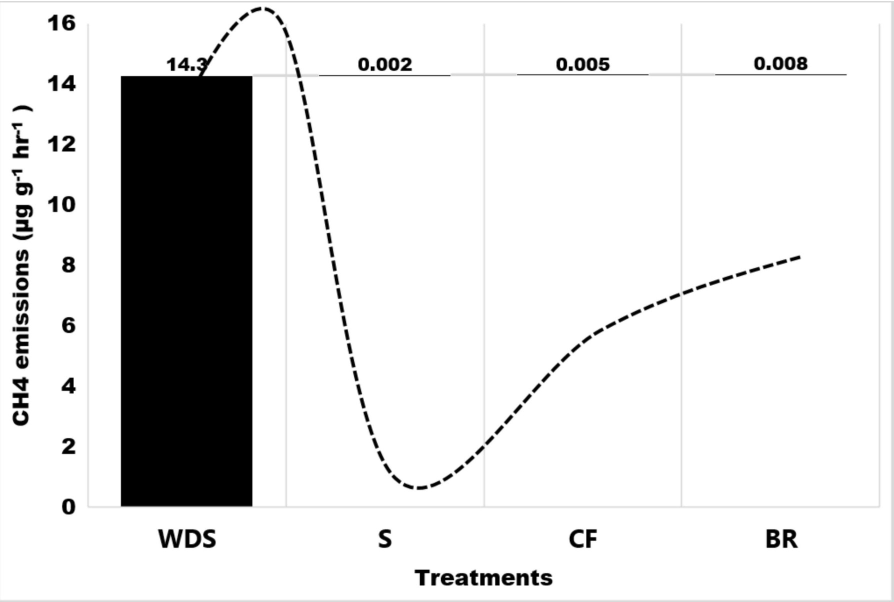


Figure 12. Air-soil methane flux from waste dumping site and cultivation field. See section 2.7 for details. S: soil, CF: chemical fertilizer, BR: BBRDM (recommended dose), and WDS: waste dumping site.

Table 3. Parameters used to calculate cost-benefit ratio for cabbage-spinach rotation

	Parameters	**Average value (INR ha^{-1})**
Cabbage cultivation **(market price INR 30 per kg)**	Seedling purchase	16,000
	Price of chemical fertilizer	38,400
	BBRDM cost	39,000
	Labor charge for transplantation, fertilizer application and harvesting	33,600
	Ploughing and irrigational purpose	10,400
	Rental value of the land	8,000
	Benefit-cost ratio for	CF = 3.56 BBRDM = 4.71
Spinach cultivation **(market price INR 20 per kg)**	**Parameters**	**Average value (INR ha^{-1})**
	Price for spinach seed	2,800
	Ploughing and irrigational cost	3,200
	Labor involvement	5,600
	Benefit-cost ratio for	CF = 1.6 BBRDM = 2.8

3.2.6. Economics of cabbage-spinach sequence

The benefit-cost outcome from cabbage and spinach rotation on a one-hectare plot of land with chemical and organic treatments was calculated. During the multi-season field trial with cabbage, and found that BBRDM fertilization had the highest net return, followed by chemical treatment. However, due to greater input of BBRDM (on a dry matter basis) the cost of cultivation was found to be higher. In terms of residual profit from spinach growth, BBRDM plots produced a healthy margin while chemically cultivated spinach fell short of our expectations. The prices of various inputs, labor costs, and net returns as described before in section 2.6 are summarised in Table 3. The average benefit-cost ratios for BBRDM and N/P/K=10:26:26+urea was around 3.75 and 2.58, respectively, which was found to be greater than value 1 that indicated better economic profit for use BBRDM as fertilizer in commercial agriculture.

3.2.7. Concentration of nitrate/ nitrite in cultivated vegetables

In order to compare our study results with the concentration of nitrate/ nitrite in the market yield vegetables, we bought cabbage and spinach leaves from a neighbourhood vegetable market located at Jadavpur (West Bengal) during the course of this investigation. As documented in Table 4, vegetables collected from the nearby fruit market had a greatest level of nitrate and nitrite, while cabbage grown under the BBRDM system attained lower values even compared to the product obtained from the N/P/K=10:26:26+urea treatment, although both fertilization regimes were supplied with an equal quantity of nitrogen. Results thus indicated unsystematic use of chemical fertilizers by farmers of the studied region, South 24 Parganas district. On the other hand, because there was more available residual nitrogen in the soil, the nitrate/nitrite content in organic spinach was higher. Values from Table 4 represented statistical variations between the soil treatments at $p < 0.05$.

Table 4. Concentrations of nitrate/nitrite (mg kg^{-1} fresh weight) in vegetables at final harvest

Cultivar		**NO_3^-**			**NO_2^-**		
		Organic	**Chemical**	**Market**	**Organic**	**Chemical**	**Market**
Cabbage	S_1	586.04 ± 27.51 ^{a}	742.66 ± 31.08 ^{b}	1019.41 ± 38.04 ^{c}	3.40 ± 0.13 ^{a}	5.17 ± 0.34 ^{b}	5.56 ± 0.66 ^{b}
	S_2	617.55 ± 18.63 ^{a}	694.19 ± 16.54 ^{a}	1228.72 ± 46.19 ^{b}	4.61 ± 0.50 ^{a}	4.83 ± 0.19 ^{a}	5.72 ± 0.51 ^{b}
Spinach	S_1	162.66 ± 12.43 ^{a}	119.84 ± 22.31 ^{a}	452.06 ± 24.8 ^{b}	0.08 ± 0.06 ^{b}	0.03 ± 0.01 ^{a}	0.23 ± 0.07 ^{c}
	S_2	156.51 ± 06.19 ^{b}	104.56 ±18.06 ^{a}	477.23 ± 27.55 ^{c}	0.09 ± 0.03 ^{ab}	0.06 ± 0.04 ^{a}	0.17 ± 0.08 ^{b}

Tukey's *post hoc* analysis was done to compare soil treatments. With standard deviations, values represented mean of replicates for each treatment. Significant differences between the treatments at 0.05 level are indicated by the superscripts. chemical: N/P/K=10:26:26+urea, organic: recommended dose of BBRDM, and market: samples obtained from local vegetable market; S_1: season 1 and S_2: season 2.

Commercialization of BBRDM: identification of drivers and barriers

Highlights

- Survey conducted in seven districts of West Bengal state
- We assessed stakeholder willingness for the product, BBRDM
- SWOT measured opportunity for circular bio-nutrient economy in rural abattoirs

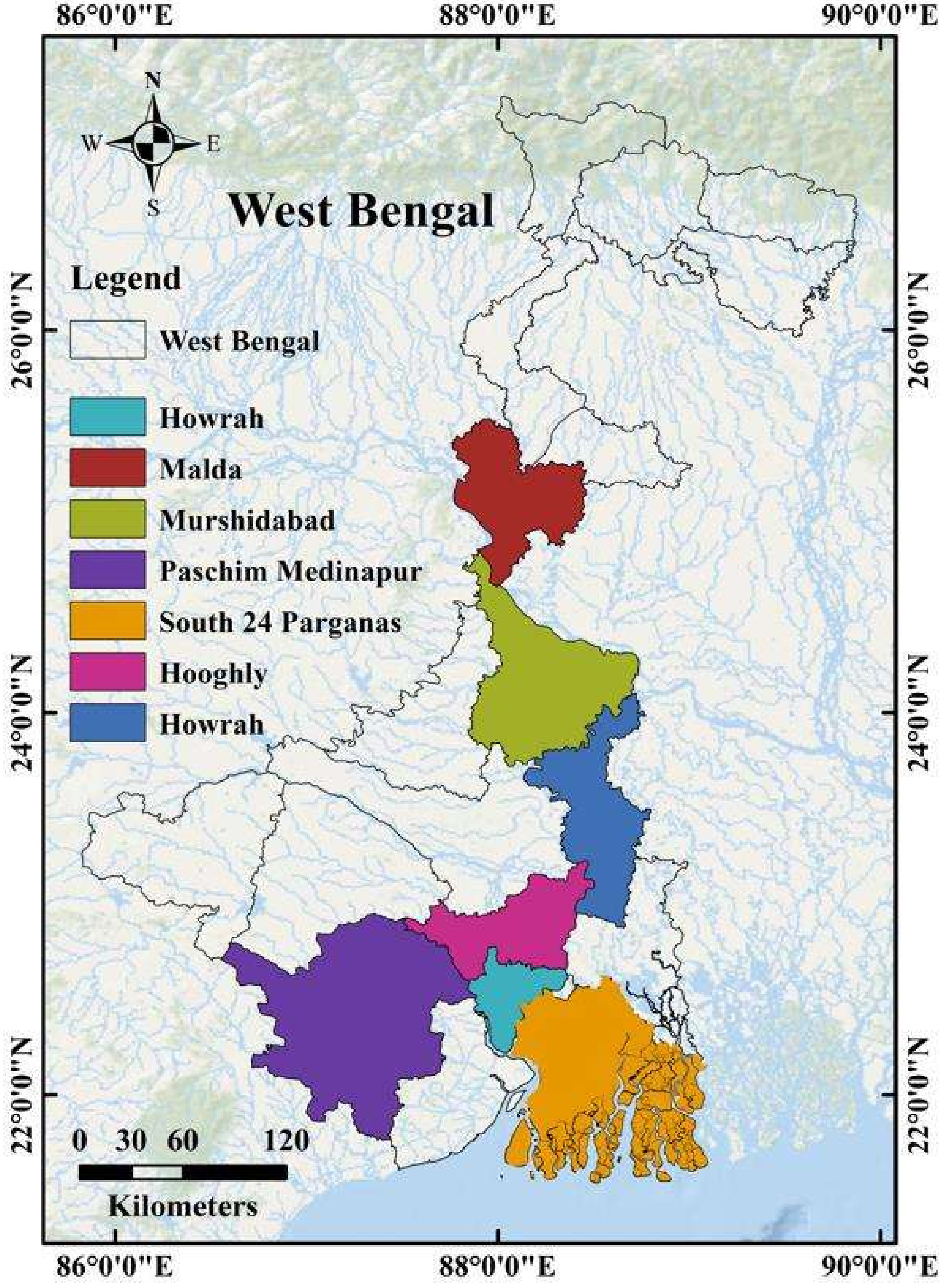


Figure 13. Map of the study area

To identify the potential barriers and enablers for commercialization of the product BBRDM, a social survey of 50 slaughterhouses and 500 farmers was conducted in seven districts of West Bengal namely Malda, Murshidabad, Howrah, Hooghly, Nadia, West Medinipur, and South 24 Parganas (see Figure 13; A.5) followed by SWOT analysis of the recorded interviews. As shown in Figure 14, the major threats for circular economy of rural abattoirs were recorded as: lack of awareness regarding the bio-based products (92% of the total respondents), lack of expertise, infrastructure and huge recycling cost (72%), no demand for organic food (98%), and low support from the local government (97.9%), while low fertilizer cost (56.3%), secondary income to rural abattoir owners (93.9%), stakeholder willingness for the product and technology (4.8% Very high, 18.6% High, and 38% Moderated), and the possibility to turn waste streams into valuable resources (66%) were identified as key strengths. Although the survey found: low volume of waste generation (80%), regular biomass availability (46.9%), huge dependency on chemical farming (89.6%), and doubts regarding the effectiveness of organic amendments (72.4%) as major weaknesses, the prime opportunities were possibility of new employment (54%), development of waste reuse green business model (1% Very high, 1% High, and 40% Moderated), advancement through organic farming (75.4%), and reduction in waste generation and associated environmental pollution (55%) for valorising abattoir waste in agriculture (Figure 14). The descriptive SWOT can be an effective tool for commercialization of BBRDM, for development of climate entrepreneurship, and for further research on policy intervention on regional and country scale.

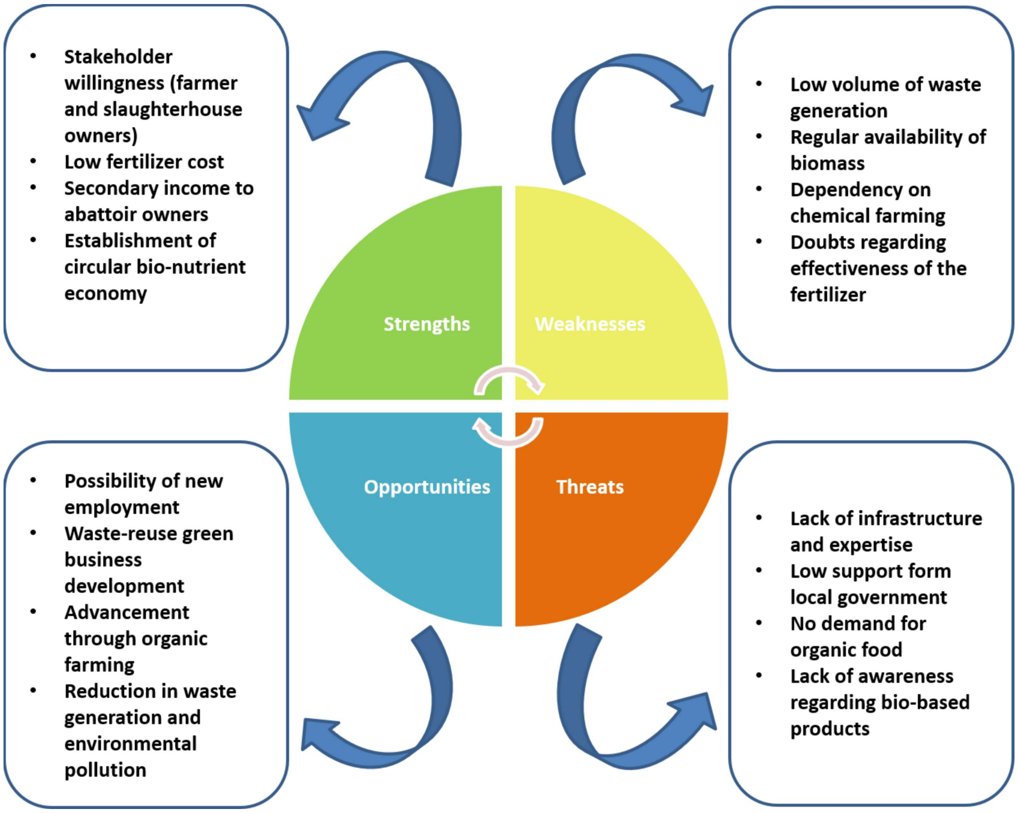


Figure 14. Strengths, weaknesses, opportunities, and threats examination of the concept "circular bio-nutrient economy" for sustainable management of rural abattoir wastes in agriculture

4.

Discussion

Effects on plant growth and fruit quality

Recent agronomic application of recycled slaughterhouse waste in the form of BBRDM increased crop yield, soil fertility, and fruit quality compared to the traditional N/P/K=10:26:26+urea fertilizer converting waste to economy towards a circular future. During this investigation, the crop yield was highest at fertilization rate of 6 and 9 g BBRDM kg^{-1} of soil (see Figure A.4 and A.5). In support of our work, the study of Yetilmezsoy et al. (2022) reported 70% higher vegetable yield upon the addition of recycled slaughterhouse waste as fertilizer. An increase in okra production was demonstrated by Sankar et al. (2021) when soils fertilized with abattoir-derived organic briquettes. Outcome from Roy et al. (2016) established better yield of tomato applying dried abattoir waste instead of synthetic agro-chemicals. Nunes et al. (2015) used the same fertilizer for soybean and corn cultivation in Brazil and Ragályi and Kádár (2012) for maize, mustard, and triticale in Hungary, and both evidenced greater productivity. Arancon et al. (2005) and Llaven et al. (2008) also found an enhanced productivity as well as better fruit characteristics when they applied animal manure for their crop production. However, McAndrews et al. (2006), Ragalyi and Kadar (2012), and Sieling et al. (2014) observed proper application of animal manures how improved soil health as well as introduced residual fertility to soils. Highest residual yield for amaranth and spinach was recorded at a rate of 9 g BBRDM kg^{-1} of soil comparing chemical treatment, as displayed in Figure 2 and 8. Although, plants fertilized with high dose of BBRDM (13 g kg^{-1} of soil) did not survive after second dose of fertilization due to the presence of huge labile carbon fractions in animal waste.as explained by Bonanomi et al. (2020). Earlier, Roy et al. (2013) and Grzyb et al. (2013) also experienced similar results (plant mortality) in organically managed plots. According to Bonanomi et al. (2020), excessive supply of organic nitrogen may arrest plant growth initiating phytotoxicity, therefore dose calculation is necessary before the use of organic amendment in agriculture, as performed in section 2.2.1.

The recycled slaughterhouse wastes supplied nitrogen as a major source of nutrient for the cultivation of seasonal vegetables. For plant growth, development and reproduction, nitrogen is unavoidable. In higher plants, about 75% of the total accumulated nitrogen is needed for chloroplast development (Del Amor, 2007). Inorganic forms of nitrogen that are available to plants include nitrate (NO_3^-) and ammonium (NH_4^+), which are produced when organic nitrogen is mineralized by soil microbes and made available to plant roots. Considering NH_4^+ to be the first mineralized product of nitrogen, Takakai et al. (2010; 2017) quantified NH_4^+ as soil available N from soybean and paddy fields under various histories of manure application. Roy et al. (2013; 2016) also measured NH_4^+ during the pot as well as field cultivations of solanaceous vegetables in India. Sradnick and Feller (2020) found that commercial organic fertilizers made from animal sources gave more readily available N to plants instead of higher P and K. In terms of N content, BBRDM was relatively greater than wood ash, sugarcane bagasse, and bovine manure, and it was roughly equivalent to cotton meal (Lima et al., 2011). BBRDM had a C/N ratio of 4.68 (Table 1) which categorizing it aa a Class I (>2.5% N) organic fertilizer following Gentile et al. (2011). According to Nyberg et al. (2002), lower C/N ratios indicate high fertilizer quality which is in support of our product. BBRDM also contained considerable amount of P (783.14±30.46 mg kg^{-1}) and K (911.28±59.81 mg kg^{-1}) in its account (see Table 1) that may help plants to combat disease infestation and water stress. Being the first mineralized product, NH_4^+ was considered as soil available N, and measured it every 2 weeks after BBRDM fertilization. As shown in Figure 3 and 9, with the progression of time, NH_4-N was found to be more readily available in BBRDM fertilised soils than the chemical. This finding may be explained by an increase in organic matter decomposition (Takakai et al., 2010; 2017) brought on by copiotrophic abundance and higher enzymatic activity (Yuan et al., 2013; Dong et al., 2014), which was also a sign of health agro-ecosystem. Although, due to plant mortality concentration of NH_4^+ in BH treated pots increased over the time (Figure 3). According to Yuichi et al. (1992), side-dressing management enhanced soil N use efficacy to some extent. With conventional farming practices, Indian farmers mostly utilize cow dung as base manure and then top-dress their paddy fields applying urea either with N/P/K=10:26:26 or with diammonium phosphate (DAP, N/P/K=18:46:0) to maximize crop yield. In case of vegetable production, side-dressing or ring-dressing method was generally followed by the rural farmers, while Sexton et al. (2006) assured that top dressing with urea added more N to soils. To expedite soil nutrient availability and to avoid phytotoxicity events, Ozdemir et al. (2019) suggested farmers to adopt side-dressing method for vegetable cultivation. Previously, Zhang et al. (2016a) proven lesser N runoff with the side-dressing fertilization. However, organic fertilization has a pivotal role in micronutrients delivery to crops. Most of these essential micronutrients are present in agricultural soils. They ensure healthy plant growth and have crucial role in crop nutrition. In higher plants, deficiencies with Zn and Fe affect protein and chlorophyll synthesis, Ca structing cells and contributes in upholding of physical barriers against pathogens, and B is found to be responsible for sugar transportation and early flowering (Rajamani et al., 1990). As represented in Figure 1, SEM-EDS analysis confirmed these elements in BBRDM that are essential for plant growth and crop development.

Soil health and food security are the principal components of Organic Agriculture 3.0. To fulfil the rising demand for quality food, it is imperative to deal agro-ecosystem health in proper way. As shown in Table 4, in comparison with vegetables purchased from the local market, concentration of nitrate/ nitrite was lower in cultivated products which indicated indiscriminate use of synthetic agro-chemicals by the farmers during cultivation. BBRDM treated vegetables accumulated considerably lower amount of nitrite/ nitrate even after providing similar quantity of N to the experimental plots which is in harmony with the findings of Mogren et al. (2008) and Roy et al (2016). Similar experimental outcomes were reported by Liu et al. (2014) and Hallmann et al. (2017) who confirmed that conventionally grown vegetables had a greater concentration of nitrate/ nitrite than the organic application where N input was constant across fertilization regimes. According to Roy et al. (2015) and Kyriacou et al. (2019), greater availability of N in agricultural soil was the probable cause of highest nitrate/ nitrite concentration in fruit, although Uddin et al. (2021) proved that the variety of crop, cultivation time, and mode of fertilization affected the ratio significantly. As visualized in Figure 9, lower nitrate/ nitrite concentration in BBRDM-treated vegetables may be due to the delayed mineralization of the organic N present in BBRDM and slow nutrient release. Nitrates are converted into nitrites inside the body which causes methemoglobinemia especially in young infants and increase the percentage of free oxygen radicals in the body leading to permanent cell damage. According to Gupta et al. (2017), greater nitrate/ nitrite consumption may also worsen the immune system and cause mutagenicity, teratogenicity, birth abnormalities, recurrent diarrhoea, recurrent stomatitis, histological changes in heart muscles, pulmonary alveoli, and adrenal glands. Although increasing nitrate and nitrite consumption has negative health effects, a current study by Bondonno et al. (2021) confirmed that dietary intake of nitrate-containing foods may reduce the risk of cardiovascular disease. Therefore, proper fertilization and judicious N supply is recommended for safe and quality food production. However, as majority of the vegetable supply comes from the district (South 24 Parganas) where our field experiment was conducted, and it was believed that characteristics relating to the structure and composition of the soil (edaphic factors) would be similar, otherwise it is very difficult to measure edaphic factors of farmer's cultivation. During this experiment, as microbial abundance and diversity were found to be highest in the top soil, and declined with depth in parallel with SOM content and prey availability (Frey, 2015), the soil sampling depth was maintained at 5-10 cm.

Effects on biology and fertility of soil

Due to intensive agricultural management, SOM content reduced significantly in arable soils. It is well documented that the indiscriminate use of synthetic agro-chemicals has been shown to have a negative impact on soil health, primarily through lowering SOM concentration as of Ali et al. (2017). In order to foster SOM content and increase soil fertility, organic farming is recommended by the scientific community in next-generation agriculture. According to Liang et al. (2018), animal-derived organic fertilizers replenished more SOM than they lost. Tian et al. (2017) reported that the SOM content and quality was altered mostly by the type of fertilization and activity of associated microbial communities in soil. In addition to having a significant impact on soil aggregation, SOM promotes a robust uptake of nutrients, provides food for native microbes, and leads to increased C sequestration in agro-environment (Osman, 2013; Li et al., 2017). Interestingly, an increase in SOM level allowed water retention, air exchange and root proliferation decreasing soil bulk density as reported by Smith et al. (2012). However, according to Brust (2019), the C/N ratios between 1 to 15 of any organic substrate encourage quick N release into the soil for prompt plant consumption, whereas immobilization of N takes place in organic material when C/N ratio is more than 35. Thus C:N ratio has a strong impact on soil microflora and plant nutrient availability. BBRDM attained a C/N ratio of 4.68. During this investigation, it was found that BBRDM-fertilized soil had increased copiotrophic abundance (Figure 4 and 10) which accelerated OM decomposition and facilitated appropriate nutrient circulation, making the majority of nutrients more accessible in the rhizosphere. As a result, it was ascertained that BBRDM was more beneficial for plant development than both vermicompost and chemical fertilizers (see Figure 2 and 8). In a current study by Ozdemir et al. (2021), it was shown that application of poultry abattoir waste was more favourable for chickpeas growth than the experimental soil. Moreover, SOM protects soil health preventing the leaching of herbicide and heavy metals like toxic elements (Fageria, 2012). Turp et al. (2021) also confined that application of biowastes improvised soil structural, physico-chemical and biological properties, thereby improving plant health and yield. Our study confirmed that, in contrast to CF treatment, BBRDM fertilized soils attained more macroaggregate percentage, while microaggregates formation was promoted by the CF treatment in soils having relatively lower SOC (Table 2) which is consistent with the earlier findings of Guo et al. (2018) and Lin et al. (2019) who found higher SOC in macroaggregates under long-term animal manure fertilization. As the study found, better soil aggregation in BBRDM treatment which in turn, accelerated copiotrophic communities for faster proliferation (see Figure 4 and 10), may be due to higher SOC content. A positive correlation between the soil aggregation stability and associated binding agents (SOC) was established earlier by Zhang et al. (2012; 2014) as noticed during our study (Figure 11).

The ability of soil microorganisms to secrete enzymes is crucial for organic matter breakdown in soil (Sayara et al., 2020). Chaudhry et al. (2012), Guo et al. (2018), and Lin et al. (2019) claimed that because indigenous microbial communities are extremely sensitive to soil physico-chemical changes, different fertilizations represented diverse microbial diversity in an agro-ecosystem. In support, Trivedi et al. (2015) proved that due to varied substrate infirmity and heterogeneity of biological niches alteration of soil microbial structure had occurred in aggregates. According to Davinic et al. (2012), soil copiotrophs including *Proteobacteria*, *Firmicutes*, *Planctomycetes*, *Bacteroidetes*, and *Verrucomicrobia* were found to be abundant in macroaggregates with a high SOC percentage, while microaggregates having lower SOC favoured *Chloroflexi*, *Acidobacteria*, and *Actinobacteria* like soil oligotrophs (Lin et al., 2019). However, majority of the farmers in India utilize chemical fertilizers to grow their seasonal crops like vegetables. Our study showed that application of recycled slaughterhouses waste could be an alternative of synthetic fertilizers for vegetable production in India. Therefore, these two treatments (CF and BBRDM) was considered for the metagenomic study, although the unfertilized soil (as S) was also measured for making fair comparison. Higher copiotrophic abundance in BBRDM treatment compared to CF (see Figure 4 and 10), an outcome of the study, corroborated the findings of Newton and McMahon (2011), Davinic et al. (2012), and Lin et al. (2019). According to Dong et al. (2014), addition of organic amendment to soils increased SOC which in turn influenced soil pH, and altered native microbial diversity. Similar to our findings, an inverse correlation between the abundance of oligotrophs (especially *Acidobacteria* and *Actinobacteria*) and organic substitution ratio was reported by Ji et al. (2018), where two major phyla *Bacteroidetes* and *Proteobacteria* showed strong positive correlation with the ratio as displayed in Figure 11. According to Wang et al. (2018), phyla *Chloroflexi* and *Acidobacteria* contained many acidogenic bacteria and their presence indicated intense soil acidification. In the current study, increased copiotrophic activity in response to BBRDM addition helped to neutralize soil acidity via supply of alkaline matter (Figure 4 and 10). It was established that the members of phyla *Verrucomicrobia* and *Planctomycetes* promoted C biogeochemical cycle utilizing xylan, cellulose, pectin and sugars like various C-based compounds (Gu et al., 2019), while members of the phyla *Verrucomicrobia* contributed to biological soil N fixation as reported by Khadem et al. (2010). Corroborative to the research of Lupwayi et al. (2019) and Urra et al. (2020), upon the addition of well-prepared BBRDM, increased β-glucosidase and alkaline phosphatase activity was evidenced, while urease activity was pronounced in soils directly fertilized with N/P/K=10:26:26+urea (Table 2) which was already mentioned by Sun et al., (2019) in his study few years back. According to Frankerberger and Dick (1983), there is an inverse relationship between the soil urease activity and N release from fertilizer. However, the study represented a strong positive correlation between β-glucosidase activity and SOC content (as displayed in Figure 11), which was parallel to the findings of Zhang et al. (2015) who positively correlated the activity of β-glucosidase with SOC/TN content.

Environmental benefits of BBRDM application

It has been acknowledged that due to massive variations in land uses, improper residue management, and uncontrolled N fertilization to agricultural fields greenhouse gas (GHGs) emissions in South Asia were multiplied (Verge et al., 2007). According to Leggett et al. (2011), India and China, produced around 20% of the global GHGs, the largest agricultural producers in Asian sub-continent. Among the major greenhouse gases (considering CO_2, CH_4, and N_2O), CH_4 alone contributes to 18% of the total global greenhouse budget (Forster et al., 2007), and in India, majority of the CH_4 and N_2O emitted from the waste and agriculture sector (Pathak et al., 2010). Methane emissions and waste generation are directly correlated with each other, and is proportionate with the growing population and urbanization. After appropriate heat treatment of abattoir waste, a significant decrease was noticed in the air-soil methane flux, which was around 150 times lower in pot and 1787 times in filed (Figure 5 and 12). According to Majumdar et al. (2006), due to lack of appropriate moisture in recycled waste CH_4 emission decreased significantly. Earlier, Salminen et al. (2003) claimed that because metabolism of long-chain fatty acids is rapid in animal waste therefore they showed higher methane yield potential which might be lowered by applying proper treatment. However, according to Student's *t*-test, CF and BR treatment do not differ statistically at $p < 0.05$ in terms of air-soil methane flux (0.008 $\mu g\ g^{-1}\ hr^{-1}$ in BR and 0.005 $\mu g\ g^{-1}\ hr^{-1}$ in CF), where during the pot study CH_4 emission was found to be higher in BH and VC treatments (0.04 $\mu g\ g^{-1}\ hr^{-1}$ in VC and 0.136 $\mu g\ g^{-1}\ hr^{-1}$ in BH). In favour of our study, Zhang et al. (2016b) evidenced reduced GHG index with judicious organic fertilization, and it was established that application of BBRDM as an organic fertilizer seems to be preferable than landfilling and or open dumping in terms of emitted methane. According to Le Mer and Roger (2001) and Amin et al. (2013), SOC stores in soils reduces GHG emissions by sequestering carbon, while Cai et al. (2007) established that N supply vastly affected air-soil CH_4 flux, therefore, to achieve 2050 Net Zero vision sensible fertilizer application and proper N supply is recommended.

Fertilizer from abattoir for a circular future

People believes manure application may incur additional costs to their agriculture. According to Archer et al. (2007), the cost may vary greatly depending on the method used to produce the fertilizer and the length of the delivery route. As the local farmers were our intended beneficiaries, therefore it is necessary to demonstrate them how BBRDM (priced at INR 26 kg^{-1}) was more advantageous economically than the chemical as well as other organic fertilizers available on the local market. Cow dung (INR 30 kg^{-1}) was the most affordable alternative while horn dust was the most expensive (INR 150 kg^{-1}) in the local market of the South 24 Parganas district, where the study was conducted. An INR 32 kg^{-1} was paid for urea and N/P/K=10:26:26, while neem cake, bone meal, and vermicompost manure were often sold for INR 80 kg^{-1} (Bhowmik et al., 2021a). However, compared to N/P/K=10:26:26+urea treatment, a larger net return with BBRDM application was recorded during the multi-season field trials (see Table 3). Such a higher return and considerably lower fertilization budget should be alluring to farmers' economy. For owners of rural abattoirs, simultaneously, BBRDM manufacture concurrent with the meat trade was found to be more profitable which also encourage them to recycle instead of open-dumping or landfilling of waste. To shift the economy from linear to circular track and to encourage hygienic waste disposal in rural abattoirs, adoption of strategies like waste to fertilizer conversion might be helpful as suggested by the current investigations of Donner et al. (2021) and Bhowmik et al. (2021a). Through recycling organic waste in agriculture, our research evidenced development of circular bio-nutrient economy where recirculation of nutrients occurred along with economy in each step of a loop providing socio-environmental benefits, as shown in Figure 15. According to Valve et al. (2020), this transformation involved the efficient use of nutrients, produced a need for organic fertilizers, and assured safe and profitable production and consumption of recycled nutrients. Bhunia et al. (2022) also suggested the bio-based circular economy model for biorefinery systems. Under the framework of circular bio-economy, commercial application of BBRDM was taken under consideration for the first time for sustainable agricultural development. Moreover, our investigation demonstrated utilization of animal-derived organic fertilizers considering promotion of agro-ecosystem health, quality food production, more economic return, and lower GHG footprint should be the mainstay for next generation agriculture. As best of our knowledge, probably this is the first report to support the agricultural application of recycled slaughterhouse wastes as organic fertilizer vouching a variety of multiple benefits. An in-depth economic analysis of slaughterhouse waste conversion was carried out previously by Bhowmik et al. (2021a), our research group, therefore the present study preferred cost-benefit analysis of BBRDM application and assessment of potential barriers and enablers for wide-spread implementation of circular bio-nutrient economy.

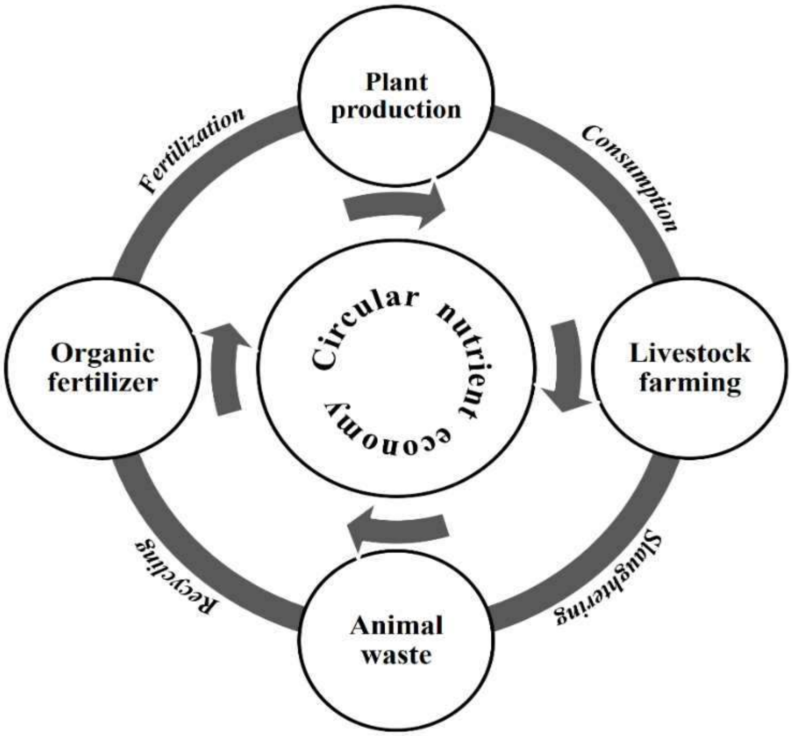


Figure 15. Recirculation of nutrients along with economy in a circular bio-nutrient economy system

However, data obtained from the survey (as shown in Figure 14) revealed that irregularity in biomass availability, lack of knowledge regarding the bio-based products, required infrastructure and expertise for waste recycling, and huge dependency on synthetic agro-chemicals could be the possible barriers for wide-scale implementation of the model, although stakeholder willingness, possibility of new employment, opportunity of waste reuse green business development, and low fertilizer cost and alternative income to slaughterhouse owners were found as prime drivers towards ‘zero waste society’ through circular bio-nutrient economy transition. Outcome of the survey is corroborative with the findings of Paes et al. (2019) who performed SWOT of circular economy in the context of organic waste management to boost companies towards green solutions. Previously, Donner et al. (2021) experienced similar barriers for small-scale entrepreneurship development under the framework of circular economy. Valve et al. (2020) also stated that circular bio-nutrient economy cannot be possible without active participation of the respective stakeholders. These results may broadly support the transition to circular economy in organic waste management would require simultaneous changes to all of the following aspects: value chains, product design, new business/ market models, new ways of converting waste into a resource, and shifting consumer behavior. Figure A.6 documented the surveys of slaughterhouse owners and rural farmers for SWOT analysis.

5.

Conclusion and future scope

Most of the time, wastes from rural abattoirs is not properly disposed off which has a negative impact on the environment, society, and economy. Recycling organic waste in agriculture could make safe disposal of livestock without harming the environment. It is possible to recycle slaughterhouse wastes in a number of ways. Among these, rendering was the most prevalent. Although composting was also preferred, anaerobic digestion scored highest for animal waste valorization. The combination of anaerobic digestion and an alkaline or enzymatic pre-treatment has been proven to be the most effective for completely inactivating pathogens and removing contaminants while also enhancing the potential biogas generation. Rural slaughterhouses in developing nations like Bangladesh, India, Sri Lanka, Nepal, Pakistan etc. were unable to adopt such technology mostly because of their lack of infrastructure and financial constraints, which made open-dumping and landfilling of waste a common practise. For rural abattoirs, the conductive drying method is both reasonably affordable and environmentally safe. Recycling of rural abattoir waste (mostly cattle blood and rumen digesta) by the use of conductive drying resulted in a pathogen-free and low-moistured final produce that can be used in place of chemical fertilizer. At the same time, this practice facilitated clean and salubrious environment around the rural abattoirs. Application of recycled slaughterhouse wastes as an organic fertilizer vouched several advantages as follows;

(a) Crop yield was highest with BBRDM application compared to vermicompost and chemical fertilization.

(b) BBRDM had stronger residual impact on plant growth.

(c) BBRDM attained boron (B), zinc (Zn), Iron (Fe) as growth promoting micro-nutrients along with NPK essential for plant development.

(d) BBRDM treated fruits tasted with lower nitrate/ nitrite concentrations, and plants fertilized with BBRDM evidenced lesser disease infestation.

(e) Economically BBRDM is advantageous over the commercially available fertilizers.

(f) Soil health promoting Phyla *Proteobacteria*, *Planctomycetes*, *Bacteroidetes* was found abundant in BBRDM treatment.

(g) Methane emissions evidenced lower in BBRDM soils than the waste dumping site.

Through this investigation, it has been established that judicious use of recycled abattoir waste increased crop productivity, improved soil fertility, provided high-quality food, and preserved farmers' income. Reusing slaughterhouse wastes could curtail the habit of openly discarding solid organic wastes, minimize nutrient loss in the form of waste, and lesser the need for chemical fertilizers. More importantly, farmers could gain double benefit applying BBRDM as fertilizer due to its strong residual effect on crop yield. Concurrently, this strategy significantly reduced the footprint of GHGs from dumping sites towards sustainable future. Circular bio-nutrient economy, where nutrients recirculated along with economy, was also fostered over waste to fertilizer conversion. Although fertilizer availability and lack of public awareness regarding such bio-based products obtained through SWOT analysis of opinions taken from rural abattoir owners and local farmers could be the possible threats to circular economy of rural organic waste management. As a result, the transition to bio-circular economy principles demands modifications in business strategies and customer behavior. The strategy 'waste-to fertilizer conversion' not only offered sustainable agricultural development, but also beneficial to respective stakeholders (slaughterhouse owners and local farmers) in terms of income generated apart from creating local employment opportunities. This approach also be helpful in achieving the world's 2050 Net Zero vision via reducing emissions from waste and agriculture. Adoption of this methodology may be advantageous in other developing countries having scattered, unorganized rural abattoirs for transition towards circular bio-economy.

Future directions: The present research work has diverse facets with every facet having the prospect of being developed to a new arena of research. However, (a) organic certification and farmers' producer company formation, (b) scaling up of field trials with diverse crop varieties, and (c) effect analysis on fruit quality are recommended for future research. Author also suggested to explore (d) the possible mechanism of defending soil-borne phytopathogens by BBRDM as the study noticed lesser disease infestation upon BBRDM application during multi-season field cultivation of cabbage and spinach, and to evaluate (e) the abundance of antibiotic resistance gene in BBRDM fertilized soils if present as livestock husbandry nowadays received huge antibiotic supply. These findings could be helpful in converting other organic wastes as fertilizer for sustainable agricultural production.

6.

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Annexure I

Figure A.1. Abattoir waste disposal in rural settings

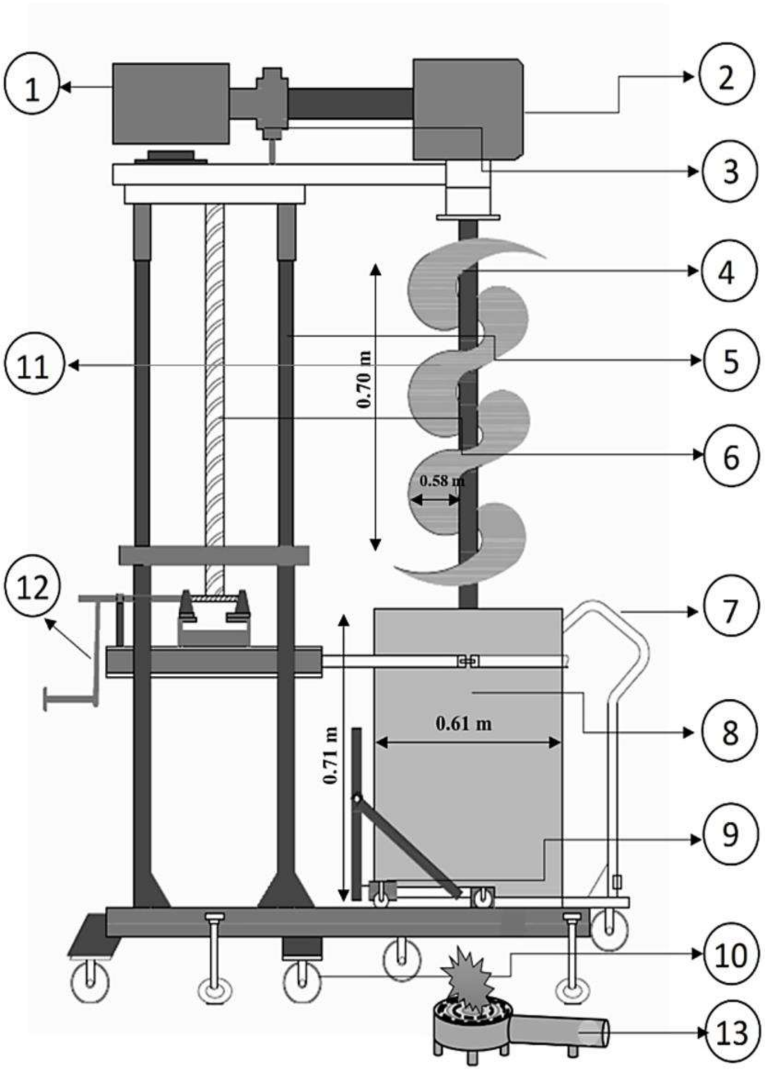

Figure A.2. Isometric view of whole drying equipment with raised up mixing spindle before loading. (1) 2 HP motor, (2) gearbox, (3) coupling of motor and spindle, (4) mixing spindle, (5) main support system of the equipment, (6) screw jack lever, (7) mobile cart (8) drying and mixing drum, (9) rail for drum wheel, (10) castor wheels, (11) helical mixing blade, (12) hand wheel handle for spindle operation, and (13) burner fuelled by LPG

Figure A.3. BBRDM preparation

Figure A.4. Successive pot cultivation of bell pepper-amaranth and cabbage-spinach using BBRDM

Figure A.5. Field cultivation of cabbage-spinach applying BBRDM, and effect analysis on plant health

Figure A.6. Surveying vegetable farmers and slaughterhouse owners

Annexure II

JADAVPUR UNIVERSITY
School of Environmental Studies

Recycling abattoir waste in agriculture: a survey of possible barriers and enablers

supported by
Indian Council of Social Science Research (ICSSR), New Delhi

Name of the place________________ Geo-coordinate________________ Date____________

Section A. Questionnaire for rural slaughterhouse owners

1. (a) Name of the respondent (optional):

   (b) Age: <25/ 25-50/ 50-75/ >75

   (c) Gender: Male/ Female/ Transgender

   (d) Education: secondary/ HS/ graduate/ post graduate/ none

   (e) Family size: <3/ 3-6/ >6

   (f) Primary source of income: cultivation/ service/ business

   (g) Any prolonged illness: skin infection/ respiratory disease/ coughing/ typhoid and paratyphoid/ tuberculosis/ others_______________

2. Type of animal you slaughtered: Pig/ cow/ buffalo/ goat/ sheep/ poultry

3. (a) Number of animal killed per day: <5/ 5-10/ 10-20/ >20

   (b) Amount of wastes generated: low/ medium/ high

   (c) Frequency of waste generation: occasional/ regular/ seldom

4. Method you followed to dispose off waste: open-dumping/ landfilling/ composting/ burial and burning/ cooking/ others_____

5. Any safety measure taken during waste handling: wearing mask/ gloves/ sanitization/ medicine/ none

6. (a) Do you know about the existing waste management technologies? yes/ no

(b) If yes, then what it is: incineration/ rendering/ composting/ anaerobic digestion/ others____

7. (a) Are you aware of hazards caused by unscientific abattoir waste disposal? yes/ no

   (b) If yes, then which one: emission of greenhouse gases/ disease occurrence/ leachate formation/ economic depletion/ nutrient loss/ soil health degradation

8. Why you have not taken recycling as an alternative? lack of expertise/ lack of infrastructure/ due to extra cost/ has no interest

9. (a) Would you like to convert slaughterhouse waste into an organic fertilizer: yes/ no

   (b) If yes, then why: to get extra income/ to ensure sustainable agriculture/ to reduce environmental pollution/ all of the above

   (c) Would you think it will reduce waste generation and associated pollution: yes/ no/ no idea

10. If you install a recycling unit, what will be the major problem: electricity/ biomass availability/ lack of skilled person/ others______________

11. (a) Is there any possibility of new employment: yes/ no/ no idea

    (b) Is here any demand for organic food: yes/ no

12. Have you received any support from government/ non-government agencies? Yes/ no

13. Willingness of rural abattoir owners to build waste-reuse green business: very high/ high/ moderate/ low/ very low

Section B. Questionnaire for local farmers

14. (a) Name of the respondent (optional):

    (b) Age: <25/ 25-50/ 50-75/ >75

    (c) Gender: Male/ Female/ Transgender

(d) Education: secondary/ HS/ graduate/ post graduate/ none

(e) Family size: <3/ 3-6/ >6

(f) Primary source of income: cultivation/ service/ business

15. (a) Type of crop you cultivated: vegetable/ cereals/ pulses/ all of them

    (b) Number of season you cultivated: one/ two/ three

16. (a) Type of fertilizer you used: chemical/ organic/ both

    (b) If organic, then which source: animal manure/ plant sourced/ others________________

    (c) Why you are not interested in organic manure: not working properly/ high cost/ unavailability

17. (a) Are you aware of advantages of organic farming: yes/ no

    (b) If yes, then what they are: increased soil fertility/ higher crop productivity/ lesser disease infestation/ better residual fertility

18. (a) Are you happy with your present income from agriculture? yes/ no

    (b) Would you like to use recycled slaughterhouse waste as an organic fertilizer: yes/ no

    (c) If yes, then why: low fertilizer cost/ better yield potential/ easy availability/ eco-friendly

    (d) Would you think it will help you to get more agronomic return: yes/ no / no idea

19. Have you ever trained for organic farming: yes/ no

20. Willingness of local farmers to pay for waste-derived organic amendment:

    very high/ high/ moderate/ low/ very low

Note: yes=1, no=0; very high=5, high=4, moderate=3, low=2, very low=1; regular=3, occasional=2; seldom=1

7. Publications, patent and conference

INTELLECTUAL
PROPERTY **INDIA**
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते

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(Rule 74 Of The Patents Rules)

पेटेंट सं. / Patent No. : 370569

आवेदन सं. / Application No. : 202031033116

फाइल करने की तारीख / Date of Filing : 01/08/2020

पेटेंटी / Patentee : 1.Ms. Ankita Bhowmik 2.Mr. Shantanu Bhunia 3.Dr. Joydeep Mukherjee

प्रमाणित किया जाता है कि पेटेंटी को उपरोक्त आवेदन में यथाप्रकटित AN APPARATUS FOR RECYCLING SLAUGHTERHOUSE WASTE AND METHOD THEREOF नामक आविष्कार के लिए, पेटेंट अधिनियम, १९७० के उपबंधों के अनुसार आज तारीख 1st day of August 2020 से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled AN APPARATUS FOR RECYCLING SLAUGHTERHOUSE WASTE AND METHOD THEREOF as disclosed in the above mentioned application for the term of 20 years from the 1st day of August 2020 in accordance with the provisions of the Patents Act,1970.

अनुदान की तारीख : 29/06/2021
Date of Grant :

पेटेंट नियंत्रक
Controller of Patent

टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, **1st day of August 2022** को और उसके पश्चात प्रत्येक वर्ष मे उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained will fall / has fallen due on 1st day of August 2022 and on the same day in every year thereafter.

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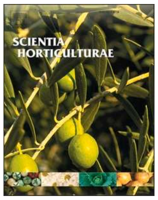

Application of recycled slaughterhouse wastes as an organic fertilizer for successive cultivations of bell pepper and amaranth

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ABSTRACT

The effects of 'bovine-blood-rumen-digesta-mixture' (BBRDM), an organic fertilizer prepared from recycled rural slaughterhouse wastes on the yield of vegetable crops and soil health were studied. Sophisticated waste management technologies are difficult to apply in rural abattoirs due to poor infrastructure and low waste generation resulting in environmental as well as human health hazards. Bovine blood and rumen digesta (3:1) were mixed and dried at 100−120 °C for 6−8 hours using a tray dryer to obtain BBRDM (N/P/K = 7.63:1:1.16; C/N = 4.68). Application of 6 g BBRDM kg^{-1} of soil showed two-fold higher yields of bell pepper in comparison with the control as well as N/P/K = 10:26:26+urea treatment. Plants fertilized with 13 g BBRDM kg^{-1} of soil died after second phase of application due to the presence of labile C fraction in BBRDM. Crop yield was two-fold higher during residual cultivation of amaranth after 12 weeks of bell pepper cultivation in soils treated with BBRDM in comparison to the other treatments. The abundance, diversity and composition of bacterial communities were superior in soils fertilized with BBRDM compared to soils cultivated with chemical fertilizer as revealed by V3-V4 16S rRNA gene sequencing. Air-soil methane fluxes recorded in soils of the abattoir waste dumping sites ranged from 13.13–28.55 μg g^{-1} hr^{-1} which were around 150 times higher than those emitted from soils of BBRDM treated bell pepper pots. Recycled slaughterhouse wastes promoted healthy growth of plants and abundance of soil beneficial microbes. This recycling methodology may be advantageously adopted in rural slaughterhouses.

1. Introduction

Quality and yield of crops are highly influenced by the availability of nutrients and carbon storage in the soil. Inorganic fertilizers improve plant growth and crop yield within a short period of time. However, long-term use of synthetic fertilizers pollutes ground water sources, adversely affects the soil pH and jeopardizes the health of plant growth promoting microorganisms (Lin et al., 2019). Addition of organic fertilizers to soils increases the cohesion of aggregates and moisture holding capacity (Ferreras et al., 2006), improves crop productivity by supplying essential nutrients directly or indirectly, stimulates microbial abundance and diversity as well as encourages activities of the major soil enzymes involved in organic matter decomposition and nutrient recycling (Chang et al., 2007). Moreover, such fertilizers protect crops by suppressing plant diseases caused by several soil-borne pathogens such as *Fusarium*, *Verticillium*, *Rhizoctonia*, *Phytopthora*, *Pythium* and thus contribute towards a healthy root system (Bailey and Lazarovits, 2003; Bonanomi et al., 2020). The major nutrients supplied by the organic fertilizers are nitrogen (N), phosphorus (P) and potassium (K). Most vegetable crops need continuous supply of nitrogen compared to phosphorus and potassium. Nitrogen is essential for the growth, development and reproduction of plants. Approximately 75 % of the total absorbed N is required for chloroplast formation in higher plants (Del Amor, 2007). The nitrogen content of different animal manures is highly variable due to animal types, gender, age, diet of the animals and geoclimatic conditions. Organic fertilizers derived from animal wastes also provide micronutrients such as zinc (Zn), boron (B), iron (Fe), copper (Cu) that are essential for crop improvement (Adediran et al., 2005).

All body parts of the buffaloes are utilized following slaughtering of animals in rural abattoirs for some economic purpose except blood and

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undigested food also known as rumen digesta. Thus, organic wastes generated in rural slaughterhouses are mainly bovine blood and rumen digesta characterized by the presence of lipids and proteins and containing several infectious pathogens as well (Salminen and Rintala, 2002). Their safe disposal is an economical as well as an environmental challenge not only in India but also in other developing countries (Escudero et al., 2014). India's per capita meat consumption is directly proportional to the growing human population and urbanization. India is ranked as the second largest beef exporter and fourth largest beef producer in the world (United States Department of Agriculture- Foreign Agricultural Service, 2019). Improper disposal of such organic wastes through open dumping creates human health and environmental hazards (Franke-Whittle and Insam, 2013). Generally, slaughterhouse wastes are treated through anaerobic digestion, acid hydrolysis, rendering, incineration and composting methods. These sophisticated waste management technologies are not adopted by the small rural Indian slaughterhouses due to lack of infrastructure and low volume of wastes generated.

Previous works by Roy et al. (2013; 2015; 2016) demonstrated transformation of blood and rumen digesta to a non-hazardous organic product through heat treatment followed by sun drying. The recycled slaughterhouse wastes supplied nitrogen as a major source of nutrient for the cultivation of solanaceous vegetables. Application of this organic fertilizer during field cultivation of tomato in India produced better growth and higher yield in comparison with the chemical fertilizer diammonium phosphate (Roy et al., 2016). As another example, agricultural application of composted slaughterhouse wastes (20−50 t ha^{-1} of soil) enhanced the quality and productivity of maize, mustard and triticale in Hungary as well as residual soil fertility which was evidenced even after three to four years of cultivation (Ragalyi and Kadar, 2012). Thus, recycling of rural slaughterhouse wastes to fertilize crops may be an environment-friendly alternative strategy for abattoir waste management.

Conservation of soil health is pivotal to sustainable agriculture and a reflection of agricultural productivity. Soil microbes are an integral part of terrestrial ecosystems and constitute 60 % of the earth's biomass (Bar-On et al., 2018). They play vital roles in ecological recycling, energy flow, plant growth and carbon sequestration. The changes in structure, abundance and composition of plant associated soil microbial communities are induced by the changing climate (Dubey et al., 2019). Actinobacteria, bacteria, mycorrhizae, protozoa and algae are the major soil microbial communities significant to agricultural ecosystems in terms of residue decomposition, nutrient mineralization and availability (Fierer, 2017), growth promoting substance production, nitrogen fixation (Jacoby et al., 2017) as well as contributing to plant disease suppression (Garbeva et al., 2004; Gomez Exposito et al., 2017). Soil microbial communities are indicators of soil health and are profoundly affected by the application of inorganic as well as organic fertilizers. For example, Belay (2002) reported that soil organic carbon (SOC) was reduced by the long-term use of chemical fertilizers, while microbial community composition was significantly influenced by the total nitrogen (TN) and SOC contents (Yuan et al., 2013; Dong et al., 2014).

Among the different important issues in Asia's agricultural sustainability during recent decades is greenhouse gas (GHG) emissions and their effect on climate change. Globally, emissions of CO_2, N_2O and CH_4 from the agricultural soils increased mainly due to changes in land use, decomposition of crop residues and organic fertilization (Verge et al., 2007). Although N fertilizers are imperative for higher crop yields, they promote methane as well as nitrous oxide emissions when additional organic carbon (OC) is supplied to soils (Cai et al., 2007). Both CO_2 and CH_4 fluxes were found to be higher during barley cultivation fertilized with digested pig slurry and inorganic supplements (Meijide et al., 2010). The emission fluxes depended on the soil temperature as well as annual rainfall. Therefore, we considered it worthwhile to record CH_4 emissions in the current investigation.

The objectives of the present research were (1) to characterize the mineral composition of BBRDM, (2) to evaluate the effects of BBRDM on bacterial community composition of soil, growth as well as yield of bell pepper plants and (3) to evaluate the residual effects of BBRDM on the growth and yield of amaranth. This work is advancement over our previous studies (Roy et al., 2013, 2016). Nitrogen concentration was maintained constant for the three fertilizers applied during the cultivation of bell pepper in contrast to the earlier application of dissimilar levels of N. Additionally, spectral characterization of BBRDM was performed which was not carried out earlier. Furthermore, comparative study of methane emissions among different fertilization treatments was done and finally the abundance and composition of bacterial communities in response to BBRDM fertilization was obtained through metagenomics.

2. Materials and methods

2.1. Recycling of blood and rumen digesta

Waste blood and rumen content were collected after the buffaloes were butchered at the slaughterhouses of Magrahat II block, South 24 Parganas district of West Bengal state, India. Bovine blood and rumen digesta were mixed in 3:1 ratio as determined previously by Roy et al. (2013) and dried in a tray dryer. A dryer housing 10 trays, each tray having 60 cm × 60 cm × 2 cm dimensions, was used for the production of BBRDM. Spacing between the trays was 8 cm. Four heating coils were used in the dryer each having heating capacity of 2 kW. Temperature was maintained at 100−120 °C for 6−8 hours to produce completely dried BBRDM product. About 36 L blood and 12 kg rumen digesta were taken to produce 8 kg of BBRDM in one batch.

2.2. Characterization of BBRDM and vermicompost

The pH of the final product was measured using a digital pH meter (LMPH - 10, Labman Scientific Instruments Ltd. Chennai, India). Ammonical nitrogen (NH_4-N), total Kjeldahl nitrogen (TKN) and nitrate nitrogen (NO_3-N) were determined by the Kjeldahl method. Total phosphorous (TP) and potassium (K) contents were measured following vanadomolybdophosphoric acid colorimetric and flame photometric methods (Radojevic and Bashkin, 1999) respectively. Organic carbon (OC) was estimated according to the methodology of Walkley and Black (1934). The C/N ratio was obtained by dividing OC of BBRDM by TKN. Commonly used organic fertilizer vermicompost was purchased from a local supplier located in Jadavpur, Kolkata (India) for comparison with BBRDM. The vermicompost was characterized by evaluating its pH, NH_4-N, TKN, NO_3-N, TP and K contents.

Spectral characterization of the product (BBRDM) was done using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and powder X-ray diffraction (XRD) analysis. The surface morphology of the BBRDM was studied using SEM imaging combined with EDS at the same surface location to identify elemental composition of the product (Martins et al., 2002). XRD analysis was performed to examine atomic as well as molecular structure of a crystal or any other material by measuring the angles and intensities of diffracted beams (Manohara and Belagali, 2017). The oven dried sample was crushed into fine particles and spread onto double sided carbon tape. Micrographs were recorded on ZEISS EVO 18 (Jena, Germany) scanning electron microscope. Elemental identification was done using Bruker XFlash 6I30 (Billerica, United States) energy dispersive X-ray detector (Yao et al., 2011). The electron high tension (EHT) was 20 kV with a working distance of 9.0 mm. Crushed sample (200 mg) was compressed and the XRD spectrum was obtained with a Philips PAN analytical (Almelo, Netherlands) powder X-ray diffractometer. The angle was 2θ and the anode material was copper (Sharma et al., 2019).

2.3. Soil analysis before cultivation

Soil was obtained from an agricultural field in Magrahat II block. Parameters analyzed included soil color identified with the standard Munsell Soil Color Charts (Torrent and Barron, 1993), particle size distribution determined by the hydrometric method, soil pH which was measured potentiometrically, moisture content and water holding capacity which were determined according to the ASTM D, 2216 active standard and Casagrande method respectively. Particle density and bulk density were quantified following the ASTM D, 7263 active standard and soil type was evaluated using the Triangular Classification Chart of the US Bureau of Soils and Chemistry Systems (Murthy, 1992). Soil N, P, K and SOC contents were also determined before cultivation applying the methods described in section 2.2.

2.4. Pot cultivation and assessment of BBRDM as a fertilizer

Pot cultivation of bell pepper (*Capsicum annuum* L. var. Arka Basant) belonging to the family Solanaceae was carried out in the winter season of November, 2018 to March, 2019. Eighteen pots (22 cm depth × 18 cm diameter) were prepared with (a) soil of Magrahat (S), (b) soil + chemical fertilizer (N/P/K = 10:26:26+urea) generally applied for capsicum cultivation in Magrahat (CF), (c) soil + vermicompost (VC), (d) soil + 6 g BBRDM kg^{-1} of soil (low dose) (BL), (e) soil + 9 g BBRDM kg^{-1} of soil (recommended dose of BBRDM which is similar to that of N/P/K = 10:26:26 + urea) (BR) and (f) soil + 13 g BBRDM kg^{-1} of soil (high dose) (BH). A completely randomized design with three replications for each treatment was followed. Fertilization was done on second, sixth and tenth weeks during a 20 week cultivation period following total 120:80:80 kg NPK ha^{-1} which is recommended for hybrid bell pepper cultivation in the state of West Bengal, India. A total amount of 120 kg N ha^{-1} was kept constant for the CF, VC and BR applications, while the concentration of N in BL and BH fertilization was maintained at 80 and 180 kg ha^{-1} respectively. If the commonly used chemical fertilizer for the cultivation of vegetables in India (N/P/K = 10:26:26) was applied singly then plants of the treatment would be fertilized with higher amounts of phosphate and potassium. Initially, application dose for chemical fertilizer (NPK = 10:26:26) was ascertained based on the amount of P as well as K; next the required amount of nitrogen was added to the soils by supplying measured amount of urea (N/P/K = 46:0:0) at 0.1 g kg^{-1} of soil to maintain equal nitrogen levels among the different fertilizer treatments. During this experiment, we followed side-dressing strategy for fertilizer application. Capsicum seedlings were taken from a local nursery and one was planted in each pot. The pots were placed side by side under a rooftop shed to ensure uniform environmental conditions and watered periodically after 2–3 days for the entire cultivation duration. Neem oil was applied once a month to control pest and weeds. The rooftop daily mean temperature varied from 16 °C to 28 °C during the investigation.

Cultivation of amaranth (*Amaranthus viridis* L. belonging to family Amaranthaceae) was started at the end of the bell pepper cultivation after 12 weeks for assessment of residual soil fertility. Approximately same numbers of seeds were sown over the soil of the previously fertilized pots. No fertilizer was added during the cultivation period and growth parameters of amaranth were recorded after 30 days of cultivation.

2.5. Status of available N and SOC during cultivation

Soil samples were collected at 3−5 cm soil depth from three replications of each treatment before starting the plantation (day 1) and after the 4th, 8th, 12th, 16th and 20th weeks during bell pepper cultivation. After the samples were air-dried and sieved using a 2-mm mesh, available soil N (NH_4^+) and SOC were measured following the methodologies of Subbiah and Asija (1956) and Walkely and Black (1934) respectively.

2.6. Soil bacterial community study by metagenomics

Collected soil samples (see previous section) from the chemical (N/P/K = 10:26:26+urea) and BBRDM treatments were pooled together to make two composite samples (Sengupta and Dick, 2015), one representing the chemically cultivated soil (C) while the other denoting BBRDM-fertilized soil (B). The abundance of nitrogen-fixing *Azotobacter*, phosphate-solubilizing bacteria, actinobacteria and fungi were recorded every 2 weeks after fertilization during bell pepper cultivation following the serial dilution agar plating method. Differential bacterial growth media applied are mentioned in Supplementary Table S.1. Amplicon sequencing of variable V3-V4 region of the 16S rRNA gene was performed on Illumina MiSeq platform to study the abundance, composition and diversity of bacterial communities under organic and inorganic fertilizer regimes. Metagenomic DNA was extracted from samples B and C using Nucleospin Soil Kit (TaKaRa Bio Ltd, Japan) following the manufacturer’s instructions. The isolated DNA was quantified using a ND-2000 UV–vis spectrophotometer. The amplicon libraries were prepared using Nextera XT index kit (Illumina, Inc. United States) according to the 16S Metagenomic Sequencing Library preparation method (Part # 15044223 Rev. B). Variable region V3-V4 of the 16S rRNA bacterial gene was amplified using 16S rRNA Forward (GCCTACGGGNGGCWGCAG) and Reverse (ACTACHVGGGTATCTAATCC) primers. Amplified products were resolved on 1.2 % agarose gel at 120 V for approximately 60 min. The amplicons were purified by AMPure XP beads and quantified using Qubit Fluorometer. Sequencing was performed on Illumina MiSeq platform using 2 × 300 bp chemistry and analyzed by the Quantitative Insights Into Microbial Ecology (QIIME) bioinformatic pipeline (Caporaso et al., 2010). The representative sequences of bacteria were classified by a naive Bayesian RDP classifier (Wang et al., 2007) and identified using the Greengenes database (16S/Archaea database) v.13_8 (McDonald et al., 2012). All the representative sequences of bacteria have been submitted in National Center for Biotechnology Information (NCBI)/Sequence Read Archive (SRA) database. The SRA accession number is PRJNA593705.

2.7. Analysis of fruit and leaf quality

Total carbohydrate and protein contents of mature fruits obtained from bell pepper plants cultivated with different fertilizer treatments were determined by the phenol sulphuric acid (Dubois et al., 1956) and Lowry’s (Lowry et al., 1951) methods respectively. Additionally, fruits were examined visually and tasted by three individuals. Leaf chlorophyll concentration was measured at 652 nm in an Ultrospec 1100 pro UV spectrophotometer following Arnon (1949).

2.8. Soil methane emissions

Moist soils were collected after three days of fertilization from the pots treated with the different amendments: S, CF, VC, BL, BR and BH (see Section 2.4). Forty milliliter borosilicate glass vials were filled up to 40 % with the different soil samples and kept in dark for 3 days. Methane concentration in the aerobic headspace was quantified with a SYS GC-8205 (India) gas chromatograph equipped with flame ionization detector (GC-FID). The column temperature was 50 °C and H_2 was used as the carrier gas having flow rate 30 mL/min (Chan and Parkin, 2001). Soil samples from abattoir waste dumping sites of Magrahat were also collected and analyzed following the same methodology. The air-soil methane flux was calculated following Khoiyangbam et al. (2004) with modifications.

2.9. Statistics

Experiments described in sections 2.2 (excluding spectral characterization of BBRDM), 2.3 and 2.7 were repeated thrice in duplicate sets, while experiments in sections 2.5 and 2.8 were carried out in duplicates for each replication. The experimental data were combined for the statistical analysis. Mean values are presented with standard deviations ($n = 6$). The one-way analysis of variance (ANOVA) in SPSS version 16.0 for Windows was performed to determine significant differences between soil treatments and pairwise comparison was done using Tukey's *post hoc* analysis. Differences were significant at 0.05 cut-off level.

3. Results

3.1. Physico-chemical analysis of organic amendments and spectral characterization of BBRDM

BBRDM was a coarse organic granular material and olive brown (2.5Y 4/3) in color. The moisture content was 16.95 % (w/w) and the pH was recorded as 7.5. The following were recorded for BBRDM: NH_4-N 518.51 ± 28.60 mg kg^{-1}, TKN 5977.75 ± 184.04 mg kg^{-1}, NO_3-N 1232.99 ± 66.28 mg kg^{-1}, TP 783.14 ± 30.46 mg kg^{-1} and K 911.28 ± 59.81 mg kg^{-1}. NPK ratio was approximately 7.63:1:1.16. The SEM-EDS analysis showed the presence of eleven elements namely carbon, oxygen, nitrogen, boron, phosphorus, sodium, calcium, silicon, zinc, iron and potassium in BBRDM (Fig. 1). Concentrations of OC and N were 57.01 % and 12.16 % respectively which were relatively higher compared to the other available elements. The carbon to nitrogen (C/N) ratio of BBRDM was 4.68. X-ray powder diffraction confirmed the presence of ammonium-potassium nitrate complex salt (Supplementary Figure S.1). Relatedly, applied vermicompost was dark brown in color (10YR 2/2), having pH 6.0, 348.88 ± 10.26 mg kg^{-1} NH_4-N, 2264.02 ± 88.64 mg kg^{-1} TKN, 619.36 ± 42.90 mg kg^{-1} NO_3-N, 1016.57 ± 38.20 mg kg^{-1} TP, 454.36 ± 18.62 mg kg^{-1} K and contained 39.54 % (w/w) of moisture.

3.2. Soil characteristics

Soil color was light brownish grey (10YR 6/2) according to the standard Munsell Soil Color Charts and soil pH ranged between 6.0 and 7.0. Water content was 17 % (w/w) while water holding capacity was 70 mL L^{-1}. Particle size distribution was as follows: sand 44 %, silt 39 % and clay 17 %. The particle density, bulk density and specific gravity of soil were 2.27 g cm^{-3}, 1.19 g cm^{-3} and 1.07 respectively. Soil type was loamy. The following were recorded for soil: N 1086.24 ± 39.16 mg kg^{-1}, P 143.65 ± 2.83 mg kg^{-1}, K 38.46 ± 2.08 mg kg^{-1} and SOC 07.32 ± 0.61 mg g^{-1}. Table 1 summarizes the various physico-chemical parameters of the soil and organic fertilizers used in this study.

Table 1
Physico-chemical analysis of the soil and organic fertilizers used in this study.

Treatments	Nutritional status					
	pH	Moisture (%)	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Org C (mg g^{-1})
Soil	6.5	17	1086.24	143.65	38.46	7.32
Vermicompost	6.0	39.54	2264.02	1016.57	454.36	14
BBRDM	7.5	16.95	5977.75	783.14	911.28	29.97

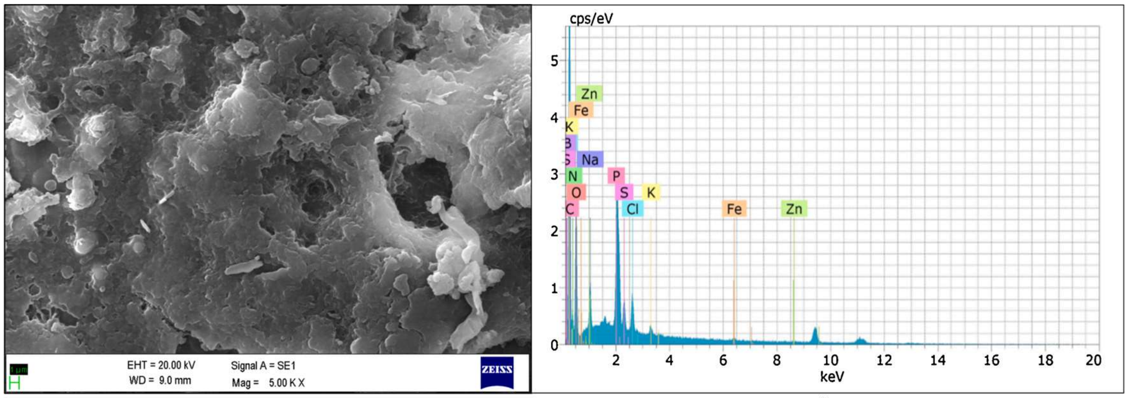
3.3. Growth of bell pepper plants and fruit yield

Addition of BBRDM to soils had major effects on productivity and quality of vegetable crops compared to chemical as well as organic vermicompost fertilization. Application of BBRDM at rate of 6 g kg^{-1} and 9 g kg^{-1} of soil produced significantly higher fruit number and fruit weight than the inorganic fertilizer and vermicompost (Table 2). In contrast, the highest BBRDM rate (13 g kg^{-1} of soil) had negative effects on the plants which did not survive after the second phase of application. Initiation of early flowering and fruiting was observed in plants fertilized with BBRDM implying an increased harvest index (HI). Leaf chlorophyll concentration of plants grown in unfertilized soils was significantly lower than the other treatments, and the pigment concentration was highest in plants cultivated in BBRDM-fertilized soils. Utilization of soil available N by plants with progression of time was rapid in soils fertilized with low and recommended doses of BBRDM compared to the other treatments as shown in Fig. 2. SOC levels remained more or

Table 2
Growth and yield parameters of bell pepper under organic and inorganic fertilization regimes.

Growth/yield parameters	Soil treatments					
	S	CF	VC	BL	BR	BH
Plant height (cm)	20.3^c	24.1^b	26.9^b	32.6^a	30.8^a	×
Number of leaves	17.0^d	28.0^{bc}	24.3^c	39.6^a	31.0^b	×
Leaf surface area (cm^2)	24.5^d	30.4^c	25.1^d	48.6^a	36.7^b	×
Stem diameter (mm)	7.4^b	9.1^a	8.0^b	9.8^a	9.2^a	×
Number of flowers	2.6^c	4.3^{bc}	4.0^c	8.6^a	6.0^b	×
Number of fruits	nd	3.3^b	1.6^c	6.0^a	3.6^b	×
Fruit weight (g)	nd	40.6^c	16.2^d	75.6^a	56.8^b	×
Chlorophyll a + b (mg g^{-1})	0.7^d	1.4^{bc}	1.2^c	2.3^a	1.8^{ab}	×

Within each row, superscripts indicate significant differences among different soil treatments at 0.05 level. **S:** soil, **CF:** chemical fertilizer, **VC:** vermicompost, **BL**: BBRDM (low dose), **BR**: BBRDM (recommended dose) and **BH**: BBRDM (high dose). **X:** plants died, **nd:** not determined.



a

b

Fig. 1. (a) Scanning electron microscopy (SEM) combined with (b) energy-dispersive X-ray spectroscopy showing morphology and elemental composition of 'bovine-blood-rumen-digesta-mixture'. The electron high tension (EHT) was 20 kV with a working distance of 9.0 mm. Details provided in Section 2.2. X-axis: kilo-electron-volt (KeV), Y-axis: counts per second per electron-volt (cps/eV).

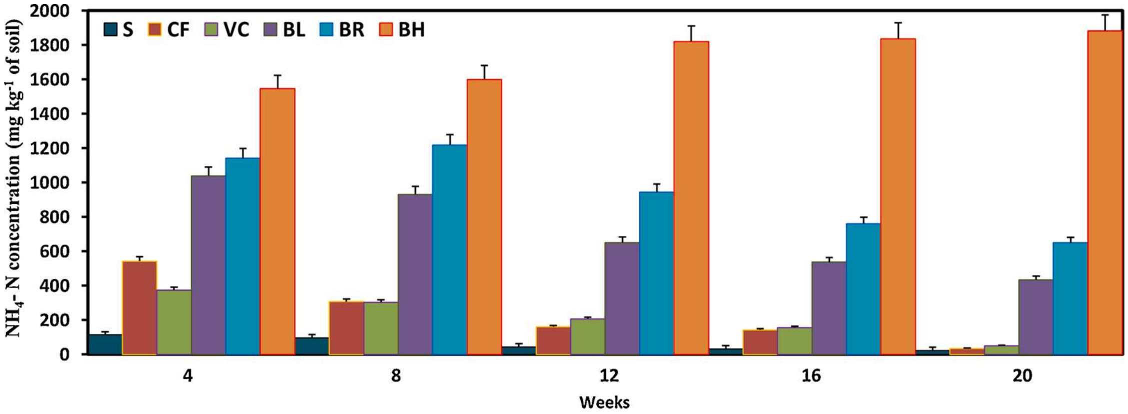


Fig. 2. Status of soil available N (NH_4^+) in different soil treatments: S (soil) as control, CF (chemical fertilizer), VC (vermicompost), BL (low dose of BBRDM), BR (recommended dose of BBRDM) and BH (high dose of BBRDM) during winter bell pepper cultivation. NH_4-N was measured following alkaline permanganate method of Subbiah and Asija (1956). Details provided in sub-section 2.5. Values represent the mean of replicates per treatment (n = 6) and error ranges one standard deviation from the mean.

less constant among the different soil treatments throughout the cultivation. The carbohydrate and protein contents of market available bell pepper (per 100 g) were 4.16 ± 0.32 g and 0.68 ± 0.24 g respectively, while BBRDM fertilized mature fruits contained 3.89 ± 0.43 g of carbohydrate and 0.76 ± 0.52 g of protein.

3.4. Residual fertilizer effects on amaranth cultivation

The residual effects of BBRDM were also valuable to plant health. Comparative study of growth parameters among all the soil amendments after 4 weeks of cultivation as shown in Table 3 revealed that the development and yield of amaranth attained higher values in soils treated with low and recommended doses of BBRDM in comparison to the other treatments. All plants cultivated in soils fertilized with high dose of BBRDM died even after 12 weeks of the bell pepper cultivation.

3.5. Quantification of emitted methane

The mean air-soil methane flux was 0.01 µg g^{-1} hr^{-1} in S, 0.019 µg g^{-1} hr^{-1} in CF, 0.04 µg g^{-1} hr^{-1} in VC, 0.03 µg g^{-1} hr^{-1} in BL, 0.035 µg g^{-1} hr^{-1} in BR and 0.136 µg g^{-1} hr^{-1} in BH treated soils (in 5 mL headspace). Methane emissions from the soils of abattoir waste dumping sites ranged from 13.13–28.55 µg g^{-1} hr^{-1} (in 1 mL headspace) which was around 150 times higher than that emitted from soils of BBRDM fertilized bell pepper pots (Supplementary Figure S.2).

3.6. Composition and diversity of bacterial communities

The abundance (c.f.u. mL^{-1}) of bacteria, nitrogen-fixing *Azotobacter*, phosphate-solubilizing bacteria as well as fungi was higher in soils treated with BBRDM, while actinobacterial growth was enhanced by the application of inorganic fertilizer (Supplementary Table S.1). Gel electrophoresis of the amplified products obtained after amplification using specific bacterial primers showed distinct bands for B and C which appeared to co-migrate on the gel (Supplementary Figure S.3). All sequences obtained through metagenomic analysis were assembled into operational taxonomic units (OTUs) at 97 % similarity cut-off level. *Proteobacteria* (35.22 % in B = BBRDM fertilized soil and 32.87 % in C = chemically cultivated soil), *Planctomycetes* (16.55 % in B and 10.23 % in C), *Bacteroidetes* (15.26 % in B and 13.06 % in C), *Chloroflexi* (11.77 % in B and 9.77 % in C), *Firmicutes* (5.12 % in B and 4.2 % in C) and *Verrucomicrobia* (1.5 % in B and 1.4 % in C) were found to be noticeably abundant in soils fertilized with BBRDM (Fig. 3). On the other hand, *Actinobacteria* (12.54 % in B and 19.44 % in C) was dominant in NPK = 10:26:26+urea treated soil. The data obtained from conventional differential plating was corroborative of the results of the metagenomic study. Among the identified bacterial classes, soils subjected to BBRDM treatment had higher abundance of *Planctomycetia* (16.4 %), *Gammaproteobacteria* (13.8 %), *Sphingobacteriia* (8.9 %) and *Betaproteobacteria* (2.2 %); CF had a relatively higher abundance of *Alphaproteobacteria* (23.6 %), *Actinobacteria* (11.8 %), *Cytophagia* (6.3 %), *Flavobacteriia* (4.6 %), *Bacilli* (2.9 %) and *Saprospirae* (1.3 %) presented in Supplementary Figure S.4. In this current investigation, *Planctomyces* was the most abundant genus which accounted for 12.63 % and 7.11 % of the total sequences for BBRDM and chemical fertilizer treatments respectively. The Shannon alpha diversity index (8.96) was higher in chemically fertilized soils in comparison with the BBRDM treated soils (7.52) considering the species-level phylogeny. Major slaughterhouse pathogens reported by Franke-Whittle and Insam (2013) and Roy et al. (2015) were not found in soils when fertilized organically with BBRDM.

Table 3
Residual effects of different fertilizers on growth and yield of amaranth.

Growth parameters	Residual fertility					
	S	CF	VC	BL	BR	BH
Plant height (cm)	10.7^d	12.6^c	12.8^c	14.6^b	17.4^a	×
Number of leaves	6.0^d	6.6^d	8.3^c	10.3^b	12.0^a	×
Leaf surface area (cm^2)	3.2^d	5.1^c	4.6^{cd}	8.9^b	10.2^a	×
Root length (cm)	3.5^d	4.8^c	4.6^c	6.0^b	6.8^a	×
Plant fresh weight (g/pot)	6.7^d	23.6^c	25.8^c	34.2^b	53.3^a	×
Chlorophyll a + b (mg g^{-1})	0.4^c	0.7^c	1.2^b	1.6^a	1.7^a	×

Within each row, superscripts indicate significant differences among different soil treatments at 0.05 level. **S:** soil, **CF:** chemical fertilizer, **VC:** vermicompost, **BL**: BBRDM (low dose), **BR**: BBRDM (recommended dose) and **BH**: BBRDM (high dose). **X:** plants died.

4. Discussion

The application of dried slaughterhouse wastes as an organic fertilizer to nutrient deficient and low organic matter containing soils increased crop productivity as well as abundance of plant growth promoting microbial communities relative to soils supplied with the equivalent amounts of inorganic N supplements. Arancon et al. (2005) and Llaven et al. (2008) found an increased productivity as well as better fruit characteristics of bell pepper when animal manure vermicompost was added to field soils in comparison to inorganic supplementation. Furthermore, composted cattle slaughterhouse wastes produced higher yield of lettuce cultivated in Argentina (Coria-Cayupaan et al., 2009). Ragalyi and Kadar (2012) applied composted slaughterhouse wastes as fertilizer for the cultivation of maize, mustard and triticale in Hungary

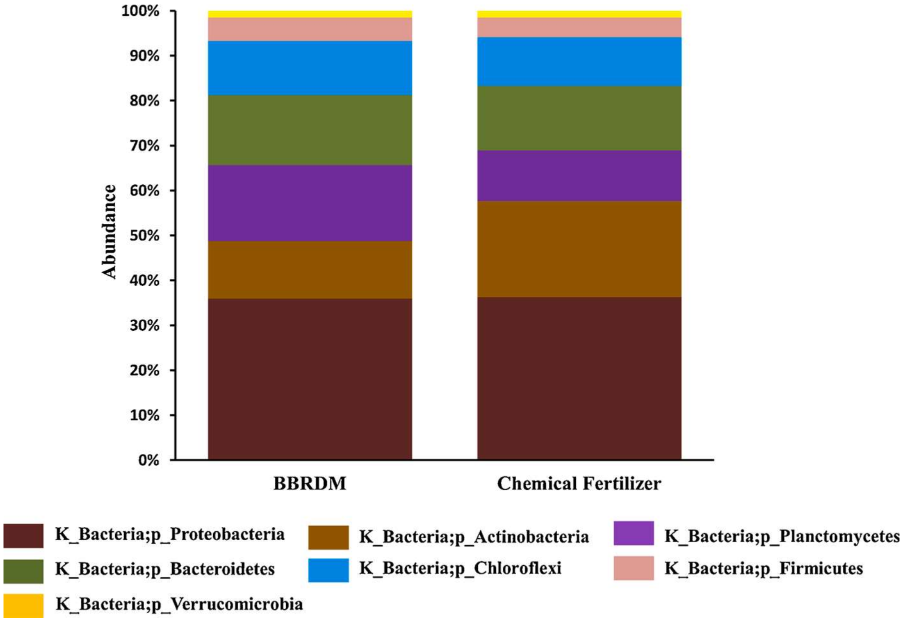


Fig. 3. Relative abundances of dominant bacterial phyla *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes* and *Verrucomicrobia* identified through 16S rRNA gene sequencing under organic (BBRDM) and inorganic (chemical fertilizer) fertilization regimes. Illumina sequencing was performed on MiSeq platform using 2 × 300 bp chemistry and analyzed by the Quantitative Insights into Microbial Ecology (QIIME) bioinformatics pipeline. Color code (read L to R) indicates the abundance of predominant phyla. Details provided in Section 2.6.

while Nunes et al. (2015) applied the same fertilizer for soybean and corn cultivation in Brazil.

Bell pepper plants fertilized with high dose of BBRDM did not yield satisfactory results (Table 2). Roy et al. (2013) and Grzyb et al. (2013) also found significantly high mortality of plants when cultivated with excessive supply of organic supplements. High mineralization rates require low application doses (Tejada and Gonzalez, 2003) while excessive supply of organic amendments to soils may arrest crop development and introduce phytotoxicity (Bonanomi et al., 2020). Several drawbacks of organic waste composting such as reduced C/N availability, high moisture content of the final product and emissions of major GHGs during this process were considered pertinent (Tritt and Schuchardt, 1992; Salminen and Rintala, 2002). On the other hand, the drying process applied in this investigation has no such unfavorable impacts on environment. The tray-drying method and the drying conditions maintained the quality of organic matter, ensured availability of nutrients and removed most of the pathogens from BBRDM. Slaughterhouse wastes are latent reservoirs of several infectious pathogens such as *Staphylococcus*, *Salmonella*, *Clostridium*, *Bacillus*, *Brucella*, *Mycobacterium*, *Erysipelothrix* and *Escherichia* that pose risks to human and animal health as cautioned by Franke-Whittle and Insam (2013). Satisfyingly, none of the pathogens were recovered through soil metagenomics and data of this investigation confirmed the absence of pathogens in BBRDM as previously reported by Roy et al. (2015). According to the Reuter et al. (2011), most of the livestock pathogens were destroyed at 77 °C which was higher than the inactivation temperature of most disease-causing microorganisms. The supply of biologically fixed N from the atmosphere is not sufficient to meet the crops' N demand and requires external application of different N sources. The amount of N in BBRDM was approximately equal to that in cotton meal and relatively higher than wood ash, sugarcane bagasse and bovine manure (Lima et al., 2011). The C/N ratio of BBRDM was 4.68 which is a Class I ($<$2.5 % N) organic fertilizer according to Gentile et al. (2011) and demonstrated faster mineralization of N. Lower C/N ratios indicate high quality fertilizer and rapid mineralization of nutrients (Nyberg et al., 2002; Roy et al., 2016). The demand for plant available P differs within crop species and soil microbial communities, although the higher uptake of P does not mean higher yields (Eichler et al., 2004; Bachmann et al., 2011). Plant available forms of N are inorganic including nitrate (NO_3^-) and ammonium (NH_4^+) which appear through the mineralization of organic nitrogen by soil microorganisms and becomes available to the plant roots. NH_4^+ is formed initially which is transformed to NO_3^-. Biological oxidation of NH_4^+ to NO_2^- followed by the oxidation of NO_2^- to NO_3^- involving both the ammonium and nitrite -oxidizing bacteria is known as nitrification. NO_3^- absorbed by plants must be reduced to NH_4^+ catalyzed by nitrate reductase in cytosol as well as nitrite reductase in chloroplast before its incorporation into amino acids. Ammonium taken in by plants is used directly in protein formation. Several studies suggested that plants generally prefer NH_4-N rather than NO_3-N because of low energy requirement (Britto and Kronzucker, 2002; Boudsocq et al., 2012). Takakai et al. (2010; 2017) quantified NH_4^+ as soil available N from soybean as well as rice paddy fields under different histories of manure application, considering NH_4^+ as the first mineralized product of N. Roy et al. (2013; 2016) also measured NH_4-N during the pot as well as field cultivations of solanaceous vegetables in India. According to Sradnick and Feller (2020), commercial organic fertilizers obtained from plant and animal sources provided a high amount of plant available N instead of higher P and K. Being the first mineralized product, we considered NH_4^+ as soil available N, and measured it every 2 weeks after fertilization. The availability of NH_4-N in BBRDM fertilized soils was found to be relatively higher as represented in Fig. 2 which may be attributed to increase in the organic matter decomposition (Takakai et al., 2010; 2017) due to copiotrophic abundance and greater enzymatic activity (Yuan et al., 2013; Dong et al., 2014), which was also an indicative of faster N mineralization. Soils fertilized with low (80 kg N ha^{-1}) and recommended (120 kg N ha^{-1}) doses of BBRDM displayed rapid utilization of available nitrogen as compared to other treatments, while concentration of NH_4-N increased gradually in BH treatment (180 kg N ha^{-1}) with the progression of time because bell peppers of BH-fertilized soils died after the second phase of fertilizer application. Similar experimental findings in BBRDM-treated solanaceous pots were reported by Roy et al. (2013) who found increased ammonical N availability in soils fertilized with BBRDM (3:1) compared to BBRDM (2:1) and (1:1). Chalhoub et al. (2013) demonstrated that regular organic fertilization enhanced N mineralization in soil similar to our observations on BBRDM application. According to Yuichi et al. (1992), side-dressing can effectively increase N use efficiency. Indian farmers mostly use cow dung manure as basal dressing under conventional farming system, and then apply urea either with N/P/K = 10:26:26 or with DAP (N/P/K = 18:46:0) in paddy cultivation through top dressing. Sexton et al. (2006) demonstrated top dressing with urea allowed faster N absorption. Farmers adopted side-dressing or ring-dressing fertilization in case of vegetable crops. Zhang et al. (2016) confirmed that side-dressing strategy reduced N leaching and may improve the N recovery efficiency. In addition, supply of micronutrients to crops is intensely affected by organic fertilization practices. Most of these essential micronutrients are generally found in soils. They ensure healthy plant growth and have crucial role in influencing crop nutrition. Zn and Fe deficiency affect protein and chlorophyll synthesis in higher plants, while B enhances sugar transportation and flowering (Rajamani et al., 1990). Early flowering and fruiting enhanced the productivity of bell pepper crops which led to higher HI (Richards et al., 2002). Allievi et al. (1993); Jeyabal and Kuppuswamy (2001) and Ragalyi and Kadar (2012) observed proper application of animal manures improved soil health as well as introduced residual fertility to soils. AdeOluwa et al. (2009) showed 60 % organic fertilizer + 40 % urine produced highest total fresh biomass during residual cultivation of green amaranth, which was supportive of our results. However, amaranth plants died at higher application rate of BBRDM (Table 3) due to the presence of labile C fraction in animal waste derived organic fertilizer (Bonanomi et al., 2020).

The CO_2, CH_4 and N_2O emissions adversely affect the environment and CH_4 alone contributes to 18 % of global climate change (Forster et al., 2007). Waste generation and methane emission are in a direct relationship which is proportionate with the growing population and urbanization. Poultry slaughterhouse wastes including blood, integument, organs, ligaments, bones and feathers showed higher methane yields due to rapid metabolism of long-chain fatty acids (Salminen et al., 2003). Likewise, abattoir waste dumping sites of Magrahat emitted substantial amounts of methane. Majumdar et al. (2006) reported that CH_4 emissions reduced significantly after sufficient heat treatment due to lack of moisture in organic wastes, which is corroborative of our research work (Supplementary Figure S.2). Thus, application of BBRDM as an organic fertilizer appears to be a better option than open dumping in terms of emitted methane. Concentrations of emitted methane were relatively higher in BBRDM and vermicompost fertilized soils which were directly or indirectly affected by the additional supply of soluble OC along with the continuous application of N (Chan and Parkin, 2001).

Most Indian farmers use chemical fertilizers for the production of vegetable and other seasonal crops. Our research demonstrated application of recycled slaughterhouse wastes may be an alternative to commercial fertilizers for the cultivation of vegetables and conservation of soil health. For this reason, we considered only these two treatments (CF and BBRDM) for metagenomic-based analysis. The bacterial community structure as revealed by Illumina sequencing of the 16S rRNA gene implied that dominant bacterial communities significant to an agricultural ecosystem were shifted dramatically towards organic fertilization and a drastic decrease in diversity and abundance of microbes was observed in chemically fertilized soils (Fig. 3). Our findings are in corroboration with the previous studies of Newton and McMahon (2011); Chaudhry et al. (2012) and Li et al. (2019) who found a dominance of fast-growing copiotrophs in organically cultivated soils. The decomposition of organic matter supplies nutrients to microorganisms. Addition of organic fertilizers to soils increased pH, SOC, TN and P content and influenced microbial community compositions (Yuan et al., 2013; Dong et al., 2014). High SOC and TN allowed fast proliferation of copiotrophs, although their population declined later when slow-growing oligotrophs started to appear over time with a concomitant decrease in SOC availability (Li et al., 2017). Copiotrophs stimulate organic matter decomposition and nutrient recycling, thus supporting plant growth and are indicators of soil health.

5. Conclusions

The reported drying process is a novel recycling method for the safe disposal of highly polluting slaughterhouse wastes. The recycled non-hazardous product can be utilized as an organic fertilizer in agriculture. Nutrient availability and abundance of growth-promoting microbial populations in soils indicated significant improvement of soil health after the application of the dried slaughterhouse wastes. Growth and yield of bell pepper as well as amaranth were higher compared to conventional chemical supplementation suggesting this methodology to be an alternative to chemical fertilizer application. Reutilization of slaughterhouse wastes may limit the practice of open-disposal of solid organic wastes, reduce methane emissions as well as use of chemical fertilizers. Additionally, recycling of highly polluting bovine blood and rumen digesta through drying may be considered as an environment-friendly inexpensive option for the abattoir waste management, which could sustain a clean and healthy environment around rural slaughterhouses. However, field cultivations are necessary to verify these results, which would be the subject of our next communication.

CRediT authorship contribution statement

Shantanu Bhunia: Formal analysis, Investigation, Methodology, Writing - original draft. **Ankita Bhowmik:** Formal analysis, Investigation, Methodology, Writing - original draft. **Rambilash Mallick:** Funding acquisition, Supervision, Visualization. **Anupam Debsarcar:** Conceptualization, Supervision. **Joydeep Mukherjee:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing - review & editing.

Declaration of Competing Interest

Authors do not declare any conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2021.109927.

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Successive cultivation of cabbage and spinach by land application of recycled slaughterhouse waste: Benefit to farmers and agro-ecosystem health

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ABSTRACT

An organic fertilizer derived from recycled rural slaughterhouse wastes, namely 'bovine-blood–rumen-digesta-mixture' (BBRDM) was applied to cabbage–spinach rotation conducted in pot and two field-scale cultivations. Highest cabbage yield was attained applying 9 g kg^{-1} of soil (recommended dose of BBRDM), while pots fertilized with 13 g BBRDM kg^{-1} of soil restricted plant growth. Spinach residual yield was significantly higher when soils were fertilized with low (6 g BBRDM kg^{-1} in pot) and recommended doses (9 g BBRDM kg^{-1} in field) of BBRDM compared to chemical treatment. Judicious application of BBRDM and proper N supply facilitated better accumulation of soil organic carbon, increased the proportion of macroaggregates formation in soil and promoted copiotrophic abundance which in turn influenced the activity of β-glucosidase that converted soil organic matter to plant available form. We evidenced delayed nitrogen mineralization and slow nutrient release from BBRDM during field cultivation that culminated in lower accumulation of nitrate/ nitrite in vegetables even after supplying equal amount of N to both fertilization regimes. Market available vegetables contained higher nitrate/ nitrite. Air–soil methane flux (0.008 $\mu g\ g^{-1}\ hr^{-1}$ in BBRDM-fertilized field) was approximately 1787 times lower than that emitted from the abattoir waste dumping sites (14.30 $\mu g\ g^{-1}$ methane emissions per hour). The average benefit–cost ratio was 3.75 for BBRDM treatment compared to 2.58 for NPK fertilization indicating greater socio-economic advantage of applying BBRDM in commercial agriculture. Circular bio-nutrient economy was promoted through waste to fertilizer conversion and animal-derived organic fertilizers may be considered for sustainable agriculture in the future.

1. Introduction

Overuse of chemical fertilizers and addition of excessive nitrogen (N) to agricultural soils may lead to soil acidification, decline of soil structure, loss of biodiversity and even disruption in several ecosystem functions therefore thwarting

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sustainable agricultural development (Guo et al., 2018; Urra et al., 2020; Bhunia et al., 2021a). On the other hand, sensible application of innocuous organic fertilizers rich in organic matter, humus and beneficial microorganisms positively affects agro-ecosystem health stimulating soil aggregate stability, soil organic matter (SOM) turnover, copiotrophic abundance and indigenous enzymatic activity as well as protects crops from soil-borne phytopathogen infections owing to formation of synergistic consortia at the root-microbiome interface (De Corato, 2020; Bhunia et al., 2021b). Recycling organic waste in agriculture can enhance soil fertility and replace chemical fertilizers (Liu et al., 2021). These organic fertilizers provide essential micronutrients such as manganese (Mn), boron (B), zinc (Zn), copper (Cu) and iron (Fe) along with NPK for plant growth (Zhang et al., 2020; Bhunia et al., 2021a), while chemical fertilizers mainly supply ammonium, nitrate, phosphate and potassium in salt forms. Majority of these agro-chemicals are persistent in nature that not only cause nonpoint water pollution via nutrient runoff but also puts an unbearable economic burden on the farmers (Tal, 2018). Additionally, long-term inorganic fertilization poses higher risk of pest and disease infestation in crops (Yatoo et al., 2021). Despite the advantages, animal-derived organic amendments have some drawbacks such as presence of pathogens, heavy metals, organic and emerging contaminants if the animal-derived wastes are not processed properly (Urra et al., 2020). More specifically, fertilizations with untreated animal waste may release toxic organic pollutants in the agro-environment and enhance the population of antibiotic-resistant bacteria in soil (Urra et al., 2019; Bhunia et al., 2021b). According to O'Neill (2016), an estimated 10 million deaths per year may occur by 2050 due to the emergence of antibiotic resistance genes in indigenous soil bacteria. Thus, it is necessary to adopt suitable and scientific waste management strategies to overcome such limitations and make animal wastes suitable for soil application.

There are approximately 32 000 informal slaughterhouses in India, majority of which do not follow any scientific waste disposal methodology (Kennedy et al., 2018). Roy et al. (2016) reported killing of 20 buffaloes (on an average) in an Indian rural abattoir every day, therefore implying daily discharge of vast quantities of organic wastes without any treatment. Anaerobic digestion, alkaline hydrolysis, incineration, enzymatic treatment, composting and rendering are major waste management technologies that are generally recommended for slaughterhouse organic waste treatment in the European Union (EU) countries (Franke-Whittle and Insam, 2013; Adhikari et al., 2018). However, these multi-unit systems require substantial capital investment and massive labor employment. Due to the economic restrictions and lack of proper governance, informal slaughterhouses in India and other developing nations like Kenya (Cook et al., 2017) failed to adopt such sophisticated and capital-intensive treatment procedures. Rural abattoirs thus preferred the simplest options: landfilling and open dumping of their waste. Unscientific management of such organic wastes can pose a serious threat to the environment as well as have calculable risks to human health as they are latent reservoirs of *Bacillus*, *Brucella*, *Erysipelothrix*, *Salmonella*, *Clostridium*, *Campylobacter*, *Staphylococcus* and bovine spongiform encephalopathy (BSE) like infectious pathogens (Bhunia et al., 2022). According to Etemadzadeh and Emtiazi (2020), such contaminants not only cause environmental pollution but also increase the cost for abattoir waste recycling.

Slaughterhouse waste comprises 45% of the animal body weight among which bovine blood and rumen digesta do not offer any reuse or resale value. However, these wastes present enough potential as organic fertilizer because they contain large amounts of growth-promoting plant nutrients (Ragályi and Kádár, 2012). Therefore, Roy et al. (2013) developed a cost-effective process for recycling abattoir waste in agriculture where bovine blood and rumen digesta were cook-dried in different ratios to obtain a non-hazardous organic product 'bovine-blood–rumen-digesta-mixture' (BBRDM). Very recently, Bhowmik et al. (2021a) described fabrication of a novel helical-ribbon mixer dryer for transition from the currently practiced small-scale rural household cooking to equipment-driven large-scale production. Produced BBRDM is free from slaughterhouse pathogens and any additional chemicals whereas alternative treatment technologies of slaughterhouse waste processing do not guarantee complete removal of pathogens and absence of residual chemicals applied during waste processing (Bhowmik et al., 2021a). For example, complete destruction of pathogens in the final vermi-produce is not ensured as the process does not have any thermophilic phase (Tognetti et al., 2005). Livestock pathogens such as *Salmonella*, *Clostridium* and BSE may also re-contaminate the composted product when the process enters subsequent cooling stage and temperature starts to decrease (NABC, 2004).

Addition of recycled organic amendments makes the soil friable and fertile that can produce safe and quality food products without compromising environmental resources (Oliveira et al., 2018; Sarkar and Rakshit, 2021). By tradition, animal manures are considered as excellent soil conditioners which facilitate better circulation of nutrients in agro-ecosystems, stimulate microbiological activity and promote structural development of the soil (Bertagnoli et al., 2020). While chemical fertilizations promote oligotrophic abundance in rhizosphere soil, animal manure application helps copiotrophs to proliferate fast which in turn influences SOM turnover via mineralization and utilization of organic substances (Chaudhry et al., 2012). Oligotrophs are slow growing and k-strategist that survive in nutrient-deprived conditions and generally found abundant in soil micro-aggregates (<250 μm diameter aggregates) having lower SOM (Lin et al., 2019). In contrast, long-term organic applications enhanced the proportion of stable macro-aggregates, presenting diameters >250 μm in soil and providing a favorable environment for copiotrophic growth and facilitating physical protection of soil organic carbon (SOC) (Sheoran et al., 2019). Copiotrophs are nutritionally opposite to oligotrophic microorganisms that invigorate organic matter decomposition and support recycling of nutrients to sustain long-term productivity of an agro-ecosystem. Copiotrophs are indicators of healthy soils. Interestingly, soil aggregation stability depends largely on the type of fertilizer applied and associated soil binding agents like SOC (Guo et al., 2018). Sihi et al. (2017) recorded higher water-holding capacity of Indian soils when fertilized with recycled organic amendment. Simultaneously, organically treated fields attained higher activities of β-glucosidase, urease and alkaline phosphatase enzymes that have a pivotal role in soil nutrient dynamics (Liang et al., 2014). Urease enzyme hydrolyzes fertilizer urea into ammonia, while phosphatase is involved in breakdown of phospho-ester bonds and converts organic phosphate to plant-available form. β-glucosidase is associated with the carbon geochemical cycle, and converts cellobiose into glucose. Due to its sensitive nature, the enzyme is considered as a potential soil quality indicator (Urra et al., 2020). According to Das and Varma (2010), enzyme levels in soil systems may vary mainly because each soil type contains diverse amounts of organic matter, composition and activity of its living components as well as intensity of indigenous biological processes. Such enzymatic activities represent potential of a soil to enable specific biochemical reactions to maintain soil quality. Antonious et al. (2020) found significant increase in urease and invertase (catalyzes conversion of sucrose to glucose and fructose) activity following the incorporation of animal manure vermicompost to native soils. According to Montemurro et al. (2015), the increasing cost of inorganic fertilizers may be another reason besides its adverse effects that favors organic fertilization as an alternative in commercial agricultural practices. However, efficacy of such organic amendments is highly dependent on the feedstock composition and the process adopted for recycling of waste (Bhunia et al., 2021b). Nitrate (NO_3^-) and nitrite (NO_2^-) contents in agricultural produce have significant impact on human health and should be reckoned during fertilizer application. The excessive amount of supplied N cannot be metabolized by the plant body and accumulates as nitrate in their tissues, mainly in stem and leaves. High nitrate and nitrite consumption may cause adverse health effects in humans (Gupta et al., 2017).

Many studies have been carried out on the agronomic potential of animal-derived organic fertilizers and their influence on biology and fertility of soil. Very recently, Sankar et al. (2021) developed organic briquettes utilizing bovine blood and rumen digestive content as feedstock material and applied it in seasonal okra production. Bhunia et al. (2021a) also used blood and rumen digesta in 3:1 ratio for successive cultivation of bell pepper and amaranth in India. Roy et al. (2016) grew organic tomato with the same amendment, while Oliveira et al. (2018) recorded satisfactory yield of radish and lettuce upon the application of composted abattoir waste. In addition, Llaven et al. (2008) documented better fruit quality when soils were fertilized with vermicomposted animal manure. However, no work has yet been published on agricultural applications of rural slaughterhouse waste where application dose calculation, fertilization frequency determination and effect analysis on plant and soil health considering farmer's economy were reported holistically.

Therefore, we conducted pot and two field-scale cultivations to address this gap with the primary objective of evaluating agronomic efficacy of BBRDM on mid-season cabbage in comparison with traditional chemical fertilization. Spinach was cultivated in the same soil as residual crop after harvesting cabbage heads. The other objectives of this work were to investigate the effects of BBRDM on soil physico-chemical and biological properties, analyze the correlation between soil parameters and microbial abundance under different fertilizer regimes as well as changes in microbial community structure, appraise the residual effects of BBRDM on growth and yield of winter spinach and to study the economic feasibility of using BBRDM as a commercial fertilizer. This investigation maintained N level constant among the fertilizer treatments in divergence to earlier application of dissimilar levels of N by our group (Roy et al., 2016). Additionally, comparative analysis of methane emissions from agricultural soils was also undertaken.

2. Materials and methods

2.1. Recycling abattoir waste as fertilizer

To prepare BBRDM, fresh blood and rumen digesta were collected immediately following annihilation of buffaloes at slaughterhouses of Magrahat II block, South 24 Parganas district, West Bengal state, India. Three parts of bovine blood and one part of rumen digesta were mixed properly as described by Roy et al. (2013) and dried at 90–110 °C for 2–3 h using the newly designed helical-ribbon mixer dryer which was installed at Magrahat village for large-scale production of the fertilizer. The recycling unit comprises of three parts: (a) cylindrical drying vessel assembled on a movable cart for convenient movement and handling, (b) helical ribbon-shaped mixing spindle with sharp edges for uniform mixing and equal distribution of heat and (c) a burner as a heat source consuming diesel or liquefied petroleum gas (LPG) that easily slid below the feed vessel (Bhowmik et al., 2021a). A patent has been obtained in India by Bhowmik et al. (2021b) (patent number 370 569) on this equipment. About 60 l bovine blood and 20 kg rumen digesta were fed to produce 14 kg of BBRDM in one batch with LPG as fuel source. Three batches were produced per day. BBRDM was stored at room temperature during the cultivation period. The shelf life of BBRDM was about 12 weeks and showed best results when applied within two weeks of manufacturing (Roy, 2018). The amendment was previously characterized by Bhunia et al. (2021a) physico-chemically as well as spectrally for better understanding of its fertilizer characteristics. The N/P/K ratio of the BBRDM was approximately 8:1:2 while C/N ratio was around 4.86 and thus categorized as Class-I organic fertilizer.

2.2. Experimental set-up and fertilizer application

2.2.1. Pot cultivation

Pot as well as field-scale experiments for two seasons were performed to study the effect of BBRDM on yield and quality of mid-season cabbage (*Brassica oleracea* L. cv. Hari Rani F1 hybrid) belonging to the family Brassicaceae. The type of amendments and application doses were considered as main experimental factors during both the studies. Pot cultivation of cabbage was conducted under a shed at the rooftop of the School of Environmental Studies of Jadavpur

University, India (latitude 22° 33′ 42″N, longitude 88° 24′ 46″E, altitude 9.9 m) during August to November, 2018. Total eighteen pots (18 cm diameter × 22 cm depth) were filled with (a) soil as control **(S)**, (b) soi l + N/P/K = 10 :26:26+urea (as chemical fertilizer) generally used for cabbage production in South 24 Parganas district **(CF)**, (c) soil + vermicompost containing N/P/K = 4 :1:2 **(VC)**, (d) soil + 6 g BBRDM kg^{-1} of soil (80 kg N ha^{-1}, low dose) **(BL)**, (e) soil + 9 g BBRDM kg^{-1} of soil (120 kg N ha^{-1}, recommended dose of BBRDM which is similar to CF and VC applications) **(BR)** and (f) soil + 13 g BBRDM kg^{-1} of soil (180 kg N ha^{-1}, high dose) **(BH)**. Soil was collected from top 30 cm of an agricultural field located in Magrahat village of South 24 Parganas district West Bengal state of India which was earlier characterized by Bhunia et al. (2021a). Soil color was light brownish gray (10YR 6/2) according to the standard Munsell Soil Color Charts having pH between 6.0 and 7.0. Water content was 17% (w/w) while the water holding capacity was 70 mL L^{-1}. Particle size distribution was as follows: sand 44%, silt 39% and clay 17%. Soil was loamy in type. N/P/K and SOC content of the soil were 1086.24 ± 39.16 mg kg^{-1}, 143.65 ± 2.83 mg kg^{-1}, 38.46 ± 2.08 mg kg^{-1} and 07.32 ± 0.61 mg g^{-1} respectively. Fertilization doses were calculated following 120:80:80 kg NPK ha^{-1} which is recommended for hybrid cabbage production in the Indo-Gangetic plains (Manjunath et al., 2018). During this investigation, half of the fertilizer was applied at the time of soil preparation as basal dose, while the remaining amount was supplied through side-dressing in two equal splits after 21 and 40 days of transplanting as recommended by Tiwari et al. (2003). Initially, application dose of CF treatment (N/P/K = 10:26:26) was determined considering the amount of fertilizer's P as well as K content; then the necessary amount of N was supplied through urea fertilization (N/P/K = 46:0:0) to maintain nitrogen levels constant among the treatments. Following a completely randomized block design method, each of the six treatments was replicated three times in this experiment. After transplantation, pots were watered thrice a week. During the cultivation, common pest attacks by aphids, bollworms, leaf miners, thrips, whiteflies, spider mites and nematodes as well as disease infestations such as clubroot, blight, leaf spots, white mold and mildews of cabbage were not significantly observed. Hence, pesticides were not applied. Plant growth parameters were assessed every two weeks to measure the physiognomic changes. The rooftop temperature varied from 38 °C to 16 °C during the entire cultivation period.

2.2.2. Multi-year field experiment

The field cultivation was carried out in Magrahat village of South 24 Parganas district (22° 13′ 48″N, 88° 22′ 12″E) at an altitude of 2.71 m above mean sea level over two consecutive years, from August to November of 2019 as season 1 and in 2020 as season 2. The soil had not been treated with any organic amendment for at least 10 years. A randomized block design method was adopted for this experiment. The cultivation plot was divided into twelve sub-plots: six of which were fertilized with well-prepared BBRDM and another six were treated with N/P/K = 10 :26:26+urea as control according to Roy et al. (2016) (Supplementary Figure S.1). Each of the sub-plots covered 15 m^2 soil surfaces. Fertilization was done manually on dry matter basis as described in Section 2.2.1 for CF and BR treatment to supply 120 kg N ha^{-1} of soil. Seedlings of approximately the same height (21 days old) were transplanted following a crop spacing of 45 cm × 60 cm (Tiwari et al., 2003). There were 48 plants in each sub-plot. Crop was irrigated adequately with an interval of 15 days. Neem oil was used once a month as bio-control agent against common pest attacks. Hand-weeding technique was followed to manage weed infestation. No chemical herbicides, insecticides or fungicides were applied during the entire cultivation period. After 8 weeks, cabbage plants were harvested and yield parameters were studied. The experimental site received an average 222 mm and 196 mm precipitation during monsoon/autumn of 2019 and 2020 respectively, while the mean temperatures ranged between 36 °C to 16 °C in season 1, and 38 °C to 14 °C in season 2. Supplementary Figure S.2 documented the climatic variations during the cultivation period. After harvesting the cabbage heads, soil samples were taken from chemical (N/P/K = 10:26:26+urea) and BBRDM fertilized sub-plots at 5–10 cm depth using a soil auger and pooled together to make two composite samples as described by Sengupta and Dick (2015), one representing BBRDM-fertilized soil **(B)** while the other denoting chemically cultivated soil **(C)**. Similar sampling methods and soil analyses were repeated in season 2, and obtained data were combined for statistical analysis (see Section 2.7).

2.2.3. Residual fertility study

Residual fertilizer effect was studied on local spinach variety (*Spinacia oleracea* L. belonging to the family Amaranthaceae) cultivated on the same soil without additional fertilization after removing cabbage plants. After 30 days of cultivation, yield characteristics were recorded as mentioned in Section 3.1. During this experiment on spinach cultivation, approximately same numbers of seeds were sown over the soil surface of the previously fertilized pots and plots for the two seasons. Soil was irrigated when required. No serious incidences of diseases or pest attacks to spinach were noticed during the cultivation. Plant leaves from main and residual plots were collected for analysis of nitrate and nitrite contents following the recommendations of European Directive (EC) No. 1881/2006 and the determination was done according to ISO 6635: 1984 (Stachniuk et al., 2018). Cabbage and spinach leaves were also purchased from local vegetable market for comparison.

2.3. Soil analysis before and after cultivation

Upon arrival at the laboratory, soil samples collected from the field were divided into two parts: one part intended for soil physico-chemical analysis (dried at 30 °C and then sieved to <2 mm), while the other part stored at 4 °C after sieving through <2 mm mesh for the determination of biological characteristics. Samples for metagenomic analysis were stored at −20 °C. Soil aggregate size distribution was studied applying wet sieving technique of Elliott (1986) while Walkley and Black's method (1934) was followed to determine SOC content. Activities of soil enzymes such as β-glucosidase, urease and alkaline phosphatase were assessed according to Eivazi and Tabatabai (1988) and Tabatabai and Bremner (1972, 1969) respectively as cited in Dick et al. (1997).

Amplicon sequencing of variable V3–V4 region of the 16S rRNA gene was carried out on Illumina HiSeq platform to estimate the abundance, diversity and composition of soil bacterial communities under chemical (C), organic (B) and unfertilized (S) regimes, and their possible interactions with soil physico-chemical changes. Metagenomic DNA was isolated from field soils (sample B, C and S, see Section 2.2.2) using Nucleospin Soil Kit (TaKaRa Bio Ltd, Japan). Prior to DNA isolation, 120 mM K_2PO_4 was used to wash soil samples following Kowalchuk et al. (1997). The isolated DNA was quantified with a ND-2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, USA). Amplification of the hypervariable V3–V4 regions of the bacterial 16S rRNA gene was performed by synthesizing 16S rRNA Forward (5′-CCTACGGGNBGCASCAG-3′) and 16S rRNA Reverse (5′-GACTACNVGGGTATCTAATCC-3′) primers and developed amplicon libraries applying Nextera XT Index Kit (Illumina Inc., USA) following the standard protocol for 16 S Metagenomic Sequencing Library preparation. Amplified PCR products were visualized on 2% agarose gel at 120 V for approximately 60 min and then the targeted bands were extracted for purification. The purified amplicons were quantified by a Qubit fluorometer (Thermo Fischer Scientific, USA), sequenced (2 × 250 bp) on an Illumina HiSeq platform (Illumina, San Diego, USA) and analyzed using Quantitative Insights Into Microbial Ecology (QIIME) bioinformatic pipeline (Caporaso et al., 2010). Raw sequences are available at the NCBI Sequence Read Archive (SRA) database with accession number PRJNA593705. Additionally, Principal Component Analysis (PCA) was carried out using R package to study possible correlation between the relative abundance of bacterial phyla and soil properties under different field treatments.

2.4. N dynamics during field experiment

Available soil N (NH_4^+-N) was measured according to alkaline permanganate method of Subbiah and Asija (1956) at the start of the cultivation (day 1) and every two weeks during the cultivation period. This method does not quantify soil nitrate and is suitable for Indian soils in general (Roy et al., 2016). P and K dynamics were not studied as fertilization was done maintaining equal amount of N instead of P and K.

2.5. Benefit–cost analysis of cabbage–spinach rotation

Analysis was performed to study the economic feasibility of using recycled slaughterhouse waste as fertilizer in commercial agriculture. The seasonal cost included land preparation, seedling purchase and transplantation, fertilization, pest and weed control, irrigation, harvesting and rental value of the land. Supplementary Table S.1 summarizes prices of various inputs and labor cost. BBRDM was sold by the slaughterhouse owner at INR 26 kg^{-1} considering the labor and production process involved (Bhowmik et al., 2021a) as his additional income. A wage rate of INR 350 per head per day was considered as labor cost during the field cultivation. The income obtained from yield per hectare was estimated considering average market price as INR 30 kg^{-1} for cabbage and INR 20 kg^{-1} for residual spinach. The benefit–cost ratio in terms of net return from cabbage–spinach rotation for both seasons over a hectare of soil was then calculated according to Tiwari et al. (2003).

2.6. Soil methane emissions

Forty milliliter borosilicate screw cap vials were filled up to 45% with field soils (collected as samples B, C and S, see Section 2.2.2) as well as waste disposal site (WDS) and kept at 30 °C under dark condition for minimum 3 days (Chan and Parkin, 2001). Methane concentrations in 5 ml aerobic headspace were measured in a Systronics GC-8205 (India) gas chromatograph coupled with flame ionization detector (GC-FID). The column temperature was set at 50 °C, detector temperature at 140 °C and hydrogen was the carrier gas having 30 ml/min flow rate (Bhunia et al., 2021a). The air–soil methane flux was calculated according to Khoiyangbam et al. (2004) with slight modifications.

2.7. Statistical analyses

All the experiments except metagenomic study were carried out in triplicates, obtained data were combined for analysis and statistical significance was determined using SPSS software for Windows version 16.0 (SPSS Inc., Chicago IL, USA). Tukey's *post hoc* analysis was performed for pairwise comparison among different pot treatments, while Student's *t*-test compared field applications. Differences were significant at 0.5% cut-off level.

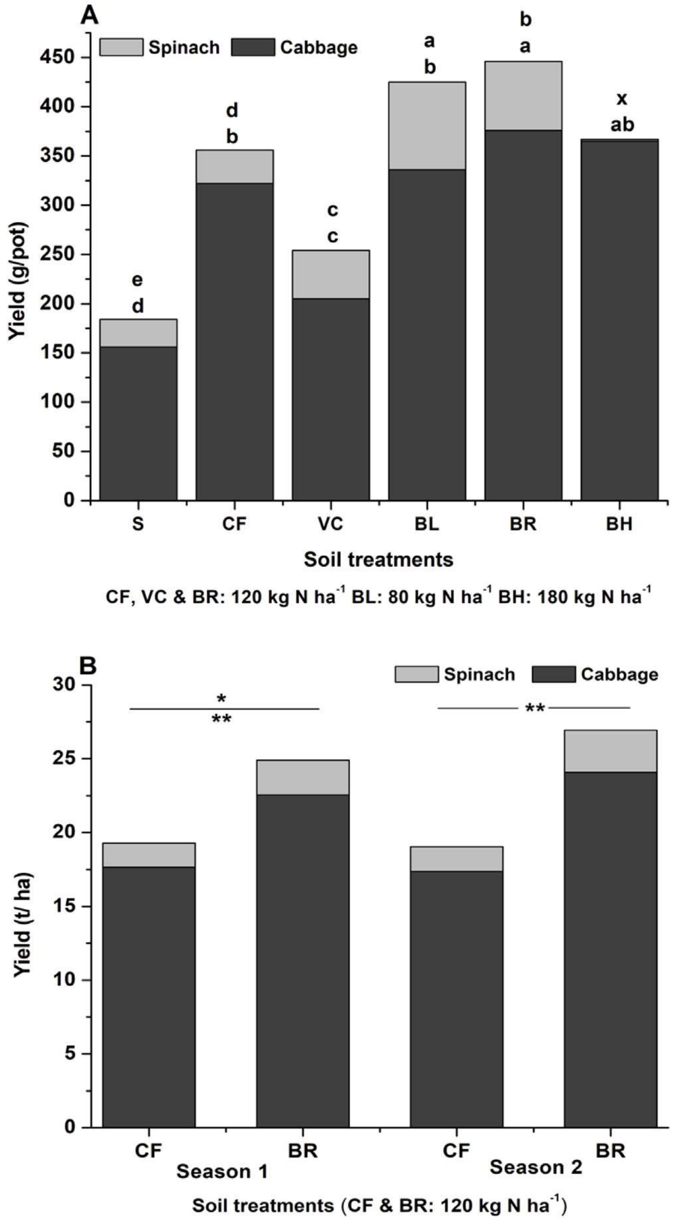

Fig. 1. Cabbage–spinach yield from (A) pot and (B) field-scale cultivation under different soil treatments- **S:** soil, **CF:** chemical fertilizer, **VC:** vermicompost, **BL:** low dose of BBRDM, **BR:** recommended dose of BBRDM and **BH:** high dose of BBRDM. Superscripts indicate significant differences among pot treatments at 0.05 level, and ** represents $p < 0.01$ and * denotes $p < 0.05$ for field observations. Details provided in Section 2.2. **X:** plants died.

3. Results

3.1. Cabbage yield and fertilizer response

Application of BBRDM at a rate of 9 g kg^{-1} of soil during pot cultivation of cabbage demonstrated higher crop yield than chemical as well as vermicompost fertilizations. In contrast, plant growth was restricted when pots were fertilized with 13 g BBRDM kg^{-1} of soil as shown in Fig. 1A. Interestingly, BL and CF treatments had similar yield effect on cabbage yield at $p < 0.05$. Also, cabbage yield was highest by applying the recommended dose of BBRDM during the field study. Yields obtained from BBRDM treated plots were higher by 27% (season 1) and 38% (season 2) in comparison with N/P/K = 10:26:26 + urea fertilized soils. Fig. 1B revealed significant differences ($p < 0.01$) in the yield between different fertilizer applications for both seasons. Our investigation also evidenced lesser disease infestation, compact head formation and well-developed cabbage root system upon addition of recommended dose of BBRDM to the field soil (Supplementary

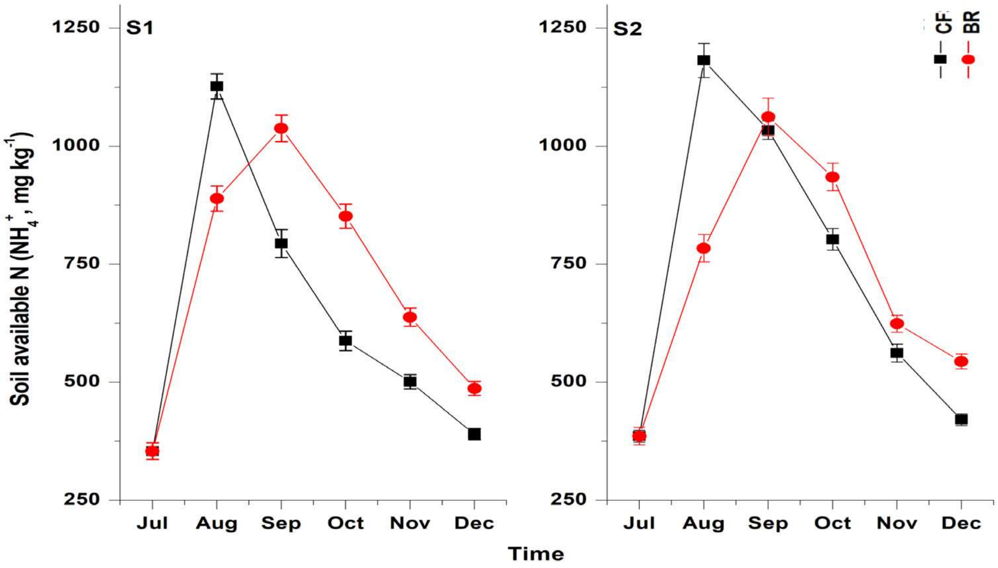

Fig. 2. N dynamics during cabbage–spinach rotation in field soil. Available soil N was measured according to Subbiah and Asija's (1956) alkaline permanganate method. Details provided in Section 2.4. Error bars represent one standard deviation from the mean ($n = 3$). **CF:** chemical fertilizer and **BR:** recommended dose of BBRDM; **S$_1$:** season 1 and **S$_2$:** season 2.

Figure S.3) in comparison to chemical fertilization. The concentration of available soil N was higher in BBRDM fertilized plots, although equal amount of N was supplied to both the field treatments. This increase as illustrated in Fig. 2 may be attributed to the delayed mineralization of the organic N present in BBRDM. Highest NH_4^+ peak was observed for CF on the 4th week and for BR on the 8th week. SOC content also enhanced over time in BBRDM treated plots.

3.2. Residual fertilizer effects on spinach cultivation

The residual effects of BBRDM were also effective for plant growth. Highest residual yield was recorded in BBRDM-fertilized soils at low and recommended doses during both pot and field experiments (Supplementary Figure S.4). On the other hand, plants died at higher application rate of BBRDM in pots after 15 days of seed sowing. Vermicompost amended soils also attained higher residual values compared to chemical fertilization during the pot cultivation of spinach. Fig. 1 exhibited significant differences at $p < 0.05$ between the yields obtained through the two soil applications.

3.3. Microbial diversity under different soil treatments

Gel electrophoresis of the PCR-amplified products showed prominent bands for sample B, C and S as displayed in Supplementary Figure S.5 which appeared to co-migrate in the gel against lane L. In order to estimate abundance, composition and diversity of microbial communities under different field treatments, reads obtained through amplicon-based 16S V3–V4 metagenomics were clustered into operational taxonomic units (OTUs) at 3% dissimilarity threshold. Fertilization effect on microbial diversity is presented in Fig. 3. We found phyla *Proteobacteria* (23.85% in B = BBRDM fertilized soils, 21.59% in C = chemically cultivated soils and 20.86% in S = unfertilized soils), *Chloroflexi* (15.58% in B, 16.94% in C and 18.65% in S), *Actinobacteria* (11.75% in B, 13.77% in C and 15.21% in S), *Firmicutes* (11.49% in B, 8.89% in C and 8.16% in S), *Acidobacteria* (8.51% in B, 8.81% in C and 9.56% in S), *Planctomycetes* (8.81% in B, 7.59% in C and 6.47% in S), *Bacteroidetes* (5.57% in B, 3.41% in C and 3.16% in S), *Verrucomicrobia* (4.72% in B, 4.41% in C and 2.74% in S) and *Gemmatimonadetes* (2.49% in B, 2.44% in C and 2.35% in S) to be dominant among the field treatments. Interestingly, soils subjected to BBRDM treatment attained higher abundance of copiotrophs such as *Proteobacteria*, *Firmicutes*, *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia* and *Gemmatimonadetes* while the dominance of oligotrophic *Chloroflexi*, *Actinobacteria* and *Acidobacteria* were more pronounced either in soils treated with chemical or in unfertilized regime as shown in Fig. 3. Supplementary Figure S.6 depicts Shannon Diversity Index which accounts for richness and evenness and Chao1 Diversity Index which measures species richness only under organic (B), chemical (C) and unfertilized (S) regimes. Compared to BBRDM application, the Chao1 index was higher in chemical treatment which indicated lower richness of bacteria in BBRDM soils. On the other hand, species evenness was slightly higher in BBRDM fertilization as this fertilization regime generated relatively greater Shannon Index value. Principal slaughterhouse pathogens reported by Franke-Whittle and Insam (2013) and Bhunia et al. (2022) were absent in soils fertilized with well-prepared BBRDM. This study also revealed possible correlations between microbial abundance and soil properties under different fertilization conditions as depicted in Fig. 4.

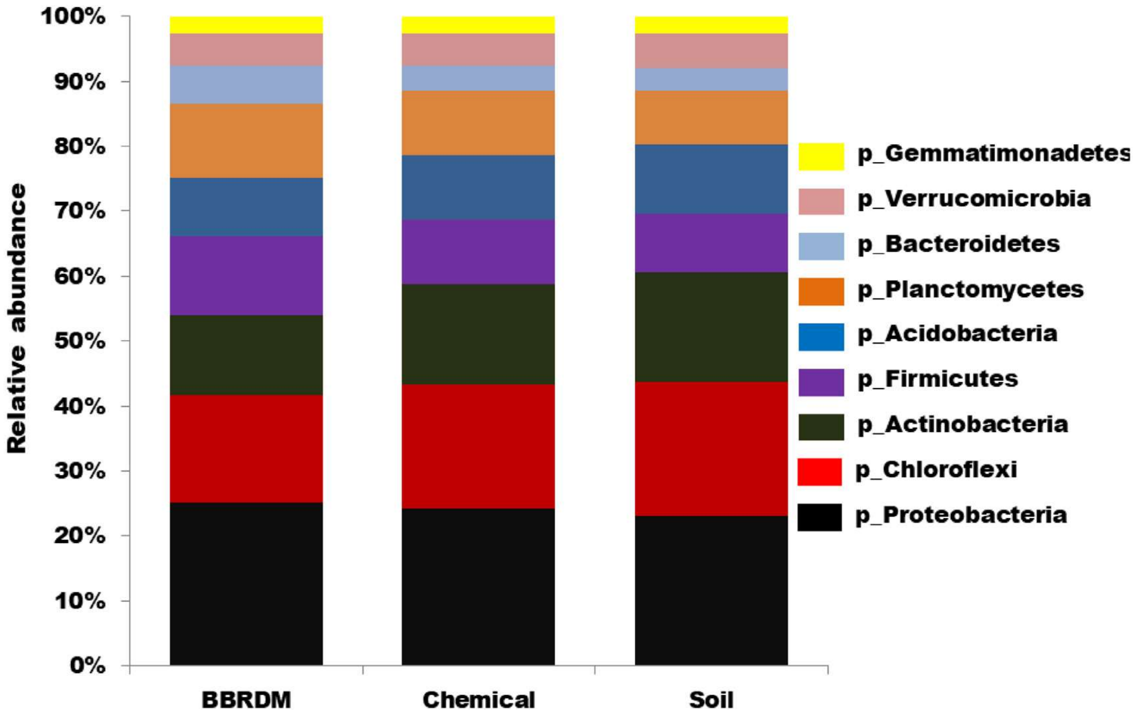

Fig. 3. Relative abundances of dominant bacterial phyla *Proteobacteria*, *Chloroflexi, Actinobacteria*, *Firmicutes, Acidobacteria*, *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia and Gemmatimonadetes* identified from multi-year cabbage–spinach rotation through amplicon-based 16S V3-V4 gene sequencing under organic (B), chemical (c) and unfertilized regimes (S). Sequencing was performed on Illumina HiSeq platform using 2 × 250 bp chemistry and analyzed by the Quantitative Insights into Microbial Ecology (QIIME) bioinformatics pipeline. Details provided in Section 2.3. Color code (read bottom-to-top) indicates the abundance of predominant phyla.

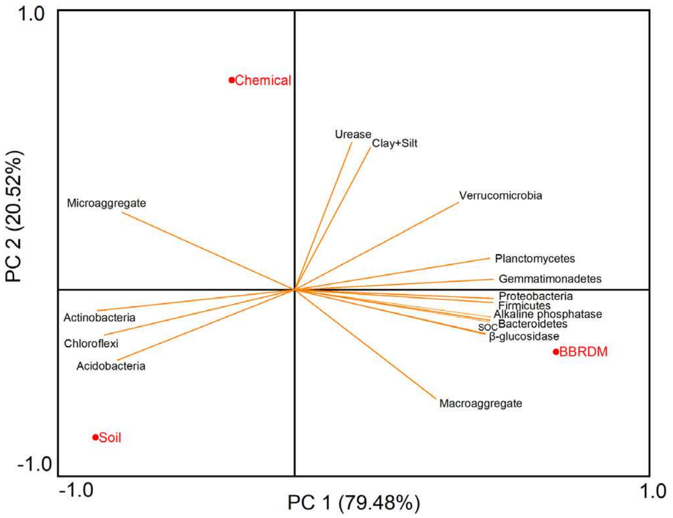

Fig. 4. Biplot of the multivariate principal component analysis (PCA) performed on soil physico-chemical characteristics and their possible correlations with bacterial community composition under different mode of fertilization. Arrows represent the correlation coefficient.

3.4. Soil characteristics before and after fertilization and possible correlation with microbial abundance

Well-judged fertilizer application and proper supply of N improved structural, physico-chemical and biological properties of the soil as evident from Table 1. BBRDM fertilization attained higher SOC concentration, enzymatic activity and better aggregate size distribution compared to the application of N/P/K = 10:26:26 + urea during the multi-year field experiment. We evidenced substantial increase, around 17% in season 1 and 19% in season 2 in macroaggregate formation when soils were treated with the recommended dose of BBRDM, while CF treatment significantly enhanced

Table 1
Fertilizer effects on soil structure and its physico-chemical and biological health.

			Treatments		
			Before cultivation	After cultivation	
	Soil parameters		Soil	Soil + CF	Soil + BBRDM
Aggregate size distribution (%)	Macroaggregate	S_1	36.15 ± 3.02	29.36 ± 4.65*	42.39 ± 13.23*
		S_2	37.93 ± 6.42	32.64 ± 4.00*	45.23 ± 7.30*
	Microaggregate	S_1	53.75 ± 4.78	58.10 ± 2.03*	46.05 ± 10.56**
		S_2	53.02 ± 6.58	54.21 ± 5.32^{ns}	43.35 ± 4.17**
	Clay+Silt	S_1	10.11 ± 0.41	12.54 ± 2.68^{ns}	11.56 ± 5.01^{ns}
		S_2	09.05 ± 2.09	13.15 ± 4.83^{ns}	11.42 ± 2.56^{ns}
Soil binding potential (g kg^{-1})	SOC content	S_1	7.40 ± 0.35	8.24 ± 0.32^{ns}	17.14 ± 0.22**
		S_2	6.09 ± 0.24	8.49 ± 0.07^{ns}	18.25 ± 0.45**
Enzymatic response (mg kg^{-1} h^{-1})	Alkaline phosphatase	S_1	143.06 ± 7.76	156.47 ± 9.88^{ns}	211.23 ± 15.50**
		S_2	148.37 ± 12.47	146.19 ± 8.99^{ns}	236.38 ± 21.48**
	Urease	S_1	91.42 ± 3.44	161.64 ± 2.01**	124.88 ± 17.69^{ns}
		S_2	89.48 ± 4.55	143.17 ± 4.30**	132.45 ± 5.27**
	β-glucosidase	S_1	62.38 ± 9.43	68.33 ± 5.08**	87.96 ± 10.22**
		S_2	59.65 ± 4.14	67.38 ± 9.82^{ns}	94.39 ± 13.40*

Differences estimated with Student's t-test. Values represented as mean of replicates per treatment ($n = 3$) with standard deviations. **ns**: not significant. Macroaggregates having >250 μm diameter, microaggregate ranges between 50 to 250 μm and those presenting diameters <50 μm were classified as clay+silt proportion. Alkaline phosphatase and β-glucosidase activity expressed as mg p-nitrophenol kg^{-1}soil h^{-1}, while urease activity was measured as mg NH_4 –N kg^{-1}soil h^{-1}. **SOC:** soil organic carbon; **CF:** chemical fertilizer (N/P/K = 10:26:26 + urea) and **BBRDM:** recommended dose of "bovine-blood–rumen-digesta-mixture"; $\mathbf{S_1}$: season 1 and $\mathbf{S_2}$: season 2.

$^{*}p < 0.05$.

$^{**}p < 0.01$.

the proportion of microaggregates. On the other hand, clay+silt distribution having diameters <50 μm did not differ significantly at $p < 0.05$ between the treatments. Our study also revealed that the unfertilized soils held relatively lower concentration of SOC, while soil binding potential in terms of SOC concentration was highest in BBRDM fertilization (Table 1). SOC had a strong positive correlation with proportion of macroaggregate formation (PCA = 0.84) under BBRDM treatment. In contrast, the use of NPK fertilizer yielded a strong negative correlation between the SOC and microaggregate formation (PCA = −0.95) as shown in Supplementary Figure S.7. Besides, the activities of alkaline phosphatase and β-glucosidase were enhanced upon application of BBRDM in field that reflected higher organic matter turnover and better soil profile under organic farming system. On the other hand, soil urease activity was elevated in CF treatment as shown in Table 1. The increase was around 18% and 68% relative to BBRDM and control treatment possibly due to direct urea fertilization to the soil. Interestingly, activity of β-glucosidase showed a strong positive correlation with SOC content (PCA = 1.0) under BBRDM fertilization (Fig. 4; Supplementary Figure S.7), while alkaline phosphatase and urease enzyme were not correlated with SOC because SOC was considered as major biological binding agent instead of total N (TN) or P (TP). According to multivariate PCA analysis, the copiotrophic abundance was positively correlated with SOC and macroaggregate formation under BBRDM treatment, whereas microaggregates having relatively lower SOC permitted faster proliferation of oligotrophs (showed strong negative correlation with SOC) in NPK-treated or unfertilized soils (Fig. 4). Thus, SOC strongly affected microbial abundance in cabbage–spinach rotation given both fertilization regimes were supplied with same amount of N.

3.5. Air–soil methane flux from cultivation field

Measurement of air–soil methane flux from agricultural soils is crucial to assess methane emission potential of soil treatments and their contribution to global climate change. The mean air–soil methane flux was recorded as 0.002 μg g^{-1} h^{-1} in unfertilized soil, 0.005 in chemically-treated and 0.008 in BBRDM-cultivated soils in 5 ml headspace, while the abattoir waste dumping sites generated 14.30 μg g^{-1} methane per hour which was around 1787 times greater than that emitted from BBRDM-fertilized plots as shown in Supplementary Figure S.8. Therefore, waste to fertilizer conversion appeared to be a sounder proposition than the waste landfilling/open dumping. There was no difference in methane emission statistically between CF and BBRDM treatments at $p < 0.05$.

3.6. Economics of cabbage–spinach rotation

Benefit–cost outcome of cabbage–spinach rotation was calculated from one hectare soil under chemical and organic treatments. We obtained highest net return from the BBRDM fertilization followed by chemical treatment during multi-year cabbage field experiment. However, the cultivation cost was higher with BBRDM due to greater input of the fertilizer on dry matter basis. In case of residual cultivation profit, we obtained sound margin from BBRDM plots, while chemically grown spinach did not meet our expectations. Supplementary Table S.1 summarizes prices of various inputs, labor cost

Table 2
Nitrate/nitrite content (mg kg^{-1} fresh weight) in vegetables after final harvest under different field treatments.

Cultivar		NO_3^-			NO_2^-		
		Organic	Chemical	Market	Organic	Chemical	Market
Cabbage	S_1	586.04 ± 27.51^a	742.66 ± 31.08^b	1019.41 ± 38.04^c	3.40 ± 0.13^a	5.17 ± 0.34^b	5.56 ± 0.66^b
	S_2	617.55 ± 18.63^a	694.19 ± 16.54^a	1228.72 ± 46.19^b	4.61 ± 0.50^a	4.83 ± 0.19^a	5.72 ± 0.51^b
Spinach	S_1	162.66 ± 12.43^a	119.84 ± 22.31^a	452.06 ± 24.8^b	0.08 ± 0.06^b	0.03 ± 0.01^a	0.23 ± 0.07^c
	S_2	156.51 ± 06.19^b	104.56 ± 18.06^a	477.23 ± 27.55^c	0.09 ± 0.03^{ab}	0.06 ± 0.04^a	0.17 ± 0.08^b

Pairwise comparison was performed according to Tukey's *post hoc* analysis. Values represented as mean of replicates per treatment ($n = 6$) with standard deviations. Superscripts denote significant differences between different treatments at 0.05 level. **organic:** recommended dose of BBRDM, **chemical:** N/P/K = 10:26:26 + urea and **market:** samples obtained from local vegetable market; $\mathbf{S_1}$: season 1 and $\mathbf{S_2}$: season 2.

and net return as mentioned earlier in Section 2.5. We obtained on average 3.75 and 2.58 benefit–cost ratio for BBRDM and N/P/K = 10:26:26 + urea, respectively which was greater than value 1 that indicated economic profitability of both the regimes, although BBRDM was advantageous over the use of conventional fertilizer in business sense.

3.7. Comparative analysis of nitrate/nitrite content in cultivated vegetables

During this investigation, we purchased cabbage and spinach leaves from local vegetable market for making a fair comparison with our study outcome in terms of nitrate/nitrite concentration in the final produce. Vegetables collected from local market displayed highest nitrate/nitrite concentration as shown in Table 2, while BBRDM-cultivated cabbage contained lower nitrate/nitrite than the produce obtained from N/P/K = 10:26:26 + urea even after supplying equal amount of N to both the regimes. Results thus indicated indiscriminate use of chemical fertilizers by farmers of the studied region. On the other hand, nitrate/nitrite concentration was higher in organic spinach due to greater availability of residual N in soil. Values in Table 2 represent statistical differences between the soil treatments at $p < 0.05$.

4. Discussion

Application of recycled slaughterhouse waste as an organic fertilizer increased field cabbage production compared to traditional N/P/K = 10:26:26 + urea fertilization. Additionally, better fruit quality, enhanced cohesion of soil aggregates, massive accumulation of SOC and promotion of growth-supporting copiotrophic microbial activity were also evident. Recent investigation of Yetilmezsoy et al. (2022) demonstrated that seasonal vegetables attained 70% higher productivity when cultivated with slaughterhouse derived soil amendment. Sankar et al. (2021) also found increased okra production upon the addition of abattoir-derived organic briquettes to soils. Earlier, Roy et al. (2016) of our research group obtained higher yield of tomato when the crop was fertilized with dried abattoir waste in comparison to chemical treatment. Ragályi and Kádár (2012) applied similar abattoir-derived fertilizer for maize, mustard and triticale and attained high productivity. The strong residual effect on spinach growth is confirmatory of our earlier study (Bhunia et al., 2021a) where higher residual yield of amaranth was obtained during bell pepper cultivation in BBRDM-fertilized soils in comparison to chemically-fertilized soils. *Brassicaceae* plants are known to contain bioactive substances and are widely used for soil biofumigation, thus preventing wilt disease. So, spinach was cultivated in residual soil after harvesting cabbage heads (Mowlick et al., 2013). Sieling et al. (2014) obtained satisfactory residual grain yield in the subsequent year following pig slurry application while McAndrews et al. (2006) demonstrated positive residual impact of cow manure on soybean growth and yield. However, spinach did not survive high application rate of BBRDM (see Fig. 1A) due to the labile carbon fraction in animal waste as explained by Bonanomi et al. (2020). According to Yuichi et al. (1992), side-dressing practice increased field N use efficacy. Indian farmers mostly use cow dung as basal manure under conventional farming practice and then apply urea either with N/P/K = 10:26:26 or with diammonium phosphate (DAP, N/P/K = 18:46:0) to paddy fields through top dressing to maximize crop yield. Sexton et al. (2006) demonstrated that top dressing with urea addition allowed faster N absorption, although farmers generally follow side-dressing or ring-dressing technique for vegetable production to expedite soil nutrient availability and to avoid phytotoxicity events (Ozdemir et al., 2019). Previously, Zhang et al. (2016a) also confirmed that side-dressing fertilization prevented N runoff and may be advantageous in organic farming.

Soil health and food security are the principal components of Organic Agriculture 3.0 that aim to transform organic farming from its current form to mainstream cultivation incorporating novel technologies (Bhunia et al., 2021b). It is imperative to manage agro-ecosystem health properly to meet the growing demand for food. As shown in Table 2, nitrate/nitrite accumulation was lower in cultivated produce in comparison with vegetables purchased from the local market. Even after supplying same amount of N to both CF and BR treatments, BBRDM-treated vegetables accumulated relatively lower nitrate/nitrite concentration probably due to slow N release in organic-fertilized soil (see Fig. 2) which is corroborative of the results of Mogren et al. (2008) and Roy et al. (2016). Similar experimental outcome was reported by Liu et al. (2014) and Hallmann et al. (2017) who confirmed conventionally grown vegetables contained higher concentration of nitrate/nitrite than the organically grown ones, where N input remained constant in the fertilization regimes. Earlier, Roy et al. (2015) and Kyriacou et al. (2019) established that greater soil N availability was the probable reason for highest nitrate/ nitrite concentration in fruit, although the levels may be considerably influenced by the variety of crop, cultivation time and mode of fertilization as described by Uddin et al. (2021). We supplied animal-derived nitrogen fertilizer to soils, comparative assessment of nitrate/nitrite in vegetables under different fertilization regimes is therefore more significant than biochemical profiling of the vegetables. Nitrates get converted to nitrite in the body leading to methemoglobinemia especially in young infants as well as increase the percentage of free oxide radicals that predispose cells to irreversible damage. Additionally, higher nitrate/nitrite consumption may induce mutagenicity, teratogenicity, birth defects, recurrent diarrhoea, recurrent stomatitis, histopathological changes in cardiac muscles, alveoli of lungs and adrenal glands and cause immune system deterioration as reported by Gupta et al. (2017). Although adverse health effects of higher nitrate and nitrite consumption are acknowledged, recent investigation of Bondonno et al. (2021) affirmed that dietary intake of nitrate-containing food may lower the risk of cardiovascular disease. Therefore, judicious fertilization and proper supply of N is recommended for green agricultural development. However, it is very difficult to assess edaphic factors of farmer's cultivation contributing to nitrate/nitrite levels as the supply of vegetables comes from different unknown sources. We assumed that factors related to structure and composition of the soil would be same because majority of the vegetable supplies come from the district where we have conducted our field experiment. During this experiment, soil sampling depth was maintained at 5–10 cm as the abundance and biomass of most soil organisms is highest in the top 10 cm of soil and declines with depth in parallel with organic matter content and prey availability (Frey, 2015). Approximately 65% of the total microbial biomass is found on the top of the soil surface. Below that depth, microbial densities typically decline by one to three orders of magnitude (Fierer et al., 2003).

SOM turnover reduced in arable soils due to intensive agricultural practices (Ali et al., 2017). Organic fertilization can be an appropriate approach to improve soil fertility and increase SOM content. According to Liang et al. (2018), animal-derived organic fertilizers replenished more SOM than lost. The C/N ratio of BBRDM was 4.68 which is a Class I ($<2.5\%$ N) organic fertilizer according to Gentile et al. (2011). Furthermore, this fertilizer supplied boron (B), zinc (Zn), copper (Cu) and iron (Fe) thus enhancing crop quality as reported in our previous study by Bhunia et al. (2021a). These micronutrients are limiting elements for plant growth because their deficiencies in the plant cause leaf chlorosis. Plant available forms of N are mainly inorganic including nitrate (NO_3^-) and ammonium (NH_4^+) which are produced through OM decomposition by soil microorganisms. Approximately 75% of the total absorbed N is required for chloroplast formation in higher plants, N promotes cell elongation and stimulates plant growth, P supports energy transfer, photosynthesis and translocation while K provides disease resistance to plants (Roy et al., 2013). C:N ratio between 1 and 15 of an organic substrate stimulates rapid N release into the soil layer for immediate crop utilization (Brust, 2019), whereas microbial N immobilization occurs in organic substrates having C:N ratio greater than 35. Thus C:N ratio has a strong impact on soil microorganisms and nutrient availability to plants. On the other hand, Zn and Fe deficiency significantly affect protein and chlorophyll synthesis in higher plants while B enhances sugar transportation and induces early flowering (Bhunia et al., 2021a). Moreover, we evidenced higher copiotrophic abundance in BBRDM-fertilized soil that expedited OM decomposition and supported proper nutrient circulation making most nutrients more available in the rhizosphere (Bhunia et al., 2021b). Therefore, BBRDM was found to be more effective for plant growth in comparison with chemical fertilizer as well as vermicompost. Ozdemir et al. (2021) demonstrated higher levels of organic matter, N, P, Fe, Mn, and Zn in poultry abattoir waste (PAS) compared to the experimental soil. Application of PAS as a fertilizer played a vital role for plant nutrient uptake, particularly in low-organic containing and alkaline soils encountered in chickpea cultivations in rain-fed farming systems. Additionally, OM protects plants nutrients and prevents them from leaching to deeper soil layers (Bhunia et al., 2022). Recent investigation of Turp et al. (2021) also established that biowastes applied to land improved structural properties, stimulated beneficial microbial communities and ultimately enhanced the availability of nutrients for cultivated crops, thereby increasing plant quality and yield. Our study demonstrated that macroaggregate formation was higher in BBRDM-fertilized plots, while CF treatment significantly enhanced the proportion of microaggregates in field having lower SOC (Table 1) which is corroborative of the previous investigation of Guo et al. (2018), Lin et al. (2019) and Bhunia et al. (2021b) who ascertained that long-term animal manure application increased SOC in macroaggregates. Increase of soil aggregation may be due to higher concentration of biological binding agent in soil, which in turn, influenced copiotrophic community to proliferate faster (see Fig. 3). Zhang et al. (2012, 2014) established a positive correlation between the aggregate stability and associated binding agents (SOC) as we found during our investigation (Fig. 4).

Soil microorganisms play pivotal role in organic matter breakdown and are dependent on their enzyme secretion potential (Sayara et al., 2020). According to Bhunia et al. (2021a), different fertilizations represent different microbial populations in an agro-ecosystem because indigenous microbial communities are highly sensitive to soil physico-chemical changes. In support of this view, Trivedi et al. (2015) demonstrated alteration of soil microbial community due to varied substrate affinity and heterogeneity of ecological niches in aggregates of various classes. For example, macroaggregates with a high SOC content supported copiotrophs like *Proteobacteria*, *Firmicutes*, *Planctomycetes*, *Bacteroidetes* and *Verrucomicrobia* (Davinic et al., 2012), while microaggregates having lower SOC preferred *k*-strategist oligotrophs including *Chloroflexi*, *Actinobacteria*, *Acidobacteria* etc. (Lin et al., 2019). The present investigation corroborated the findings of Davinic et al. (2012) and Lin et al. (2019) although oligotrophic dominance in unfertilized soils was also recorded. Similar to our observation, Ji et al. (2018) reported inverse correlation of the abundance of *Actinobacteria* and *Acidobacteria* with organic substitution ratio, while two major phyla *Proteobacteria* and *Bacteroidetes* were positively correlated with the ratio as displayed in Fig. 4. On the other hand, Wang et al. (2018) stated that phyla *Acidobacteria* and *Chloroflexi* contained many acidogenic bacteria and their presence indicated intense soil acidification due to long-term application of inorganic NPK. In this present study, enhanced copiotrophic population in response to BBRDM fertilization contributed to alleviation of soil acidification by neutralizing soil acidity via supply of alkaline matter (Fig. 3). It was reported that the members of *Planctomycetes* and *Verrucomicrobia* utilized various carbon compounds such as cellulose, xylan, pectin and sugars and promoted carbon biogeochemical cycle (Gu et al., 2019). Furthermore, Khadem et al. (2010) proved that the phyla *Verrucomicrobia* was involved in biological soil N fixation. Corroborative to the investigations of Lupwayi et al. (2019) and Urra et al. (2020), our study demonstrated higher β-glucosidase and alkaline phosphatase activity when soils were fertilized with well-prepared BBRDM. Urease activity was prominent in CF treatment due to direct urea input (Table 1) which was also described in the study of Sun et al. (2019). Frankenberger and Dick (1983) reported inverse correlation of urease activity with slow N release from the fertilizer. However, we found a strong positive correlation between β-glucosidase activity and SOC content (Fig. 4) which was similar to the findings of Zhang et al. (2015) who correlated the activity of β-glucosidase with SOC/TN in a positive way.

Enhanced greenhouse gas (GHG) emission due to variations in agricultural land use, improper management of crop residue and uncontrolled N fertilization were recognized as important issues in Asian agriculture and is vital to global climate change (Verge et al., 2007). India and China are the largest agricultural producers in Asia and these countries emitted approximately 20% of the global GHGs (Leggett et al., 2008). In India, majority of the agricultural GHG emission occurred at primary stage of food production (Pathak et al., 2010). According to Forster et al. (2007), among the major GHGs, methane alone contributed 18% to the global climate change. We observed a vast reduction in air–soil methane flux after sufficient heat treatment of abattoir waste. Majumdar et al. (2006) reported lack of moisture in organic waste decreased its CH_4 emission potential significantly. Earlier, Salminen et al. (2003) stated that due to fast metabolism of long-chain fatty acids, animal waste showed higher methane yields which might be lowered after proper waste treatment. However, according to Student's t-test there was no significant difference in methane emission between the CF and BBRDM treatment at $p < 0.05$ (see Supplementary Figure S.8). In support of our result, Zhang et al. (2016b) found decreased GHG index through prudent organic fertilizer application. According to Le Mer and Roger (2001) and Amin et al. (2013), increasing SOC stocks through sequestering C in soils may reduce GHG emissions. In addition, Cai et al. (2007) established that soil CH_4 emissions could be significantly influenced by the N supply; therefore, sensible fertilizer application and proper N management is essential to decrease net global warming potential as suggested by Zhang et al. (2016b).

Manure production can incur substantial cost to organic producers. According to Archer et al. (2007), the cost may vary widely depending upon the fertilizer production process and transportation distances. Our targeted beneficiaries were the local farmers; therefore, we demonstrated economic benefit of BBRDM application (price INR 26 kg^{-1}) compared to other organic fertilizers available in the commercial market. In the local market of South 24 Parganas district where the study was conducted, the selling price of cow dung was INR 30 kg^{-1} which was the cheapest option and horn dust was the most expensive (INR 150 kg^{-1}). Neem cake, bone meal and vermicompost manure were usually sold at INR 80 kg^{-1} (Bhowmik et al., 2021a) while we purchased urea and $N/P/K = 10{:}26{:}26$ at rate of INR 32 kg^{-1}. Compared to chemical fertilization, we obtained higher net return from BBRDM fertilization (see Supplementary Table S.1). Such higher return and low fertilization cost should be attractive to farmers' economy as evidenced through our investigation. On the other hand, BBRDM production concomitant with meat trade was further profitable for rural abattoir owners. Recent investigation of Donner et al. (2021) and Bhowmik et al. (2021a) asserted that strategies like waste to fertilizer conversion could shift the economy from linear to circular encouraging hygienic waste disposal. Moreover, Bhunia et al. (2021b) demonstrated how nutrients were recirculated together in each step of a circular bio-nutrient economy model developed through waste to fertilizer conversion. Under the framework of circular bio-nutrient economy commercial application of BBRDM is feasible as shown by the first time through this study. Moreover, our investigation demonstrated that animal-derived organic fertilizers should be the mainstay for sustainable green agriculture in the future considering the promotion of soil health, lower GHG footprint and economic profitability. This is the first report vouching the application of treated slaughterhouse wastes as organic fertilizer through demonstration of multiple positive effects.

5. Conclusions

Through this investigation it has been ascertained that careful application of recycled slaughterhouse waste promoted crop yield, enhanced soil fertility, offered quality food product and maintained farmers' profitability. This approach may reduce dependency on inorganic NPK and can limit nutrient loss through improper waste disposal that recently has become a subject of concern especially in South Asian countries. More importantly, through application of BBRDM farmers could achieve double benefit due to its strong positive effect on residual yield. Concomitantly, this practice contributed to significant reduction in GHG footprint from dumping sites. Adoption of this strategy not only facilitated sustainable agricultural production through waste to fertilizer conversion, but also benefited respective stakeholders apart from generating local employment opportunities as well as maintaining clean and healthy environment around the rural slaughterhouses. This methodology may be adopted in other developing countries having scattered, unorganized rural slaughterhouses for transition towards circular bio-nutrient economy.

CRediT authorship contribution statement

Shantanu Bhunia: Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **Ankita Bhowmik:** Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **Arnab Pramanik:** Methodology, Software, Validation, Formal analysis, Investigation. **Rambilash Mallick:** Writing – review & editing, Visualization, Supervision, Funding acquisition. **Joydeep Mukherjee:** Conceptualization, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Indian patent (370,569 of 2021) obtained on the equipment applied to produce the organic fertilizer described in this communication.

Data availability

All data have been shared

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102967.

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Review

Agronomic Efficiency of Animal-Derived Organic Fertilizers and Their Effects on Biology and Fertility of Soil: A Review

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Abstract: Healthy soils are essential for progressive agronomic activities. Organic fertilization positively affects agro-ecosystems by stimulating plant growth, enhancing crop productivity and fruit quality and improving soil fertility. Soil health and food security are the key elements of Organic Agriculture 3.0. Landfilling and/or open-dumping of animal wastes produced from slaughtering cause environmental pollution by releasing toxic substances, leachate and greenhouse gases. Direct application of animal carcasses to agricultural fields can adversely affect soil microbiota. Effective waste management technologies such as thermal drying, composting, vermicomposting and anaerobic digestion transform animal wastes, making them suitable for soil application by supplying soil high in organic carbon and total nitrogen. Recent agronomic practices applied recycled animal wastes as organic fertilizer in crop production. However, plants may not survive at a high fertilization rate due to the presence of labile carbon fraction in animal wastes. Therefore, dose calculation and determination of fertilizer application frequency are crucial for agronomists. Long-term animal waste-derived organic supplementation promotes copiotrophic microbial abundance due to enhanced substrate affinity, provides micronutrients to soils and protects crops from soil-borne pathogens owing to formation of plant-beneficial microbial consortia. Animal waste-derived organically fertilized soils possess higher urease and acid phosphatase activities. Furthermore, waste to fertilizer conversion is a low-energy requiring process that promotes circular bio-economy. Thus, considering the promotion of soil fertility, microbial abundance, disease protection and economic considerations application of animal-waste-derived organic fertilizer should be the mainstay for sustainable agriculture.

Keywords: animal waste recycling; organic fertilization; agronomic efficiency; soil health; bioeconomy; environmental sustainability

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1. Introduction

Due to increase in the world's population and changes in their dietary habits, the global demand for food is expected to be doubled within the next few decades [1]. Particularly in India and China, the adoption of a more westernized diet can contribute about 50–70% of the total growing need as both countries together represent 37% of the world population [2]. According to Food and Agriculture Organization (FAO) of the United Nations, the world population may reach 9 billion by 2050 [3], which will create huge pressure on our growers to further accelerate agricultural production. This may be achieved either by improving the farming systems or by increasing agricultural land use [4]. To feed the constantly growing population, Sjauw-Koen-Fa [5] estimated 9% expansion of arable land, 14% increase in cropping intensity and 77% more yields, while Pretty and Bharucha [6] suggested sustainable intensification of agro-ecosystems rather than the enhancement of cultivable land. The Green Revolution in the late 1960s aimed to alleviate extreme poverty, malnutrition and hunger of millions. This movement converted farming to an industrial system, which incorporated the application of modern machinery, the use of synthetic agrochemicals and genetically modified organisms to the agro-ecosystems [1]. Unfortunately, soil salinization caused by mineral weathering and human interventions and shortage of water has made extensive areas of the total cultivable lands unproductive [7].

Chemical fertilizers sustain short-term productivity of agro-ecosystems, while their indiscriminate use reduces soil fertility, adversely affects enzymatic activity and jeopardizes copiotrophic community [8]. Copiotrophs (i.e., fast growing, *r*-strategist), are a group of microorganisms that thrive in an environment rich in organic matter, particularly carbon. They are nutritionally opposite to oligotrophs (i.e., slow growing, *k*-strategist) that live in much lower C concentrations [9]. Surviving in a nutritionally deprived/rich environment must involve expression of different sets of genes among the copiotrophs and oligotrophs. Koch [10] stated the possible reasons for oligotrophs to succumb during challenges of too high nutrition are: (a) Sudden availability of too many transportable non-metabolic substances, (b) cell death due to osmotic swelling, (c) inappropriate SOS response that means blockage of DNA synthesis and (d) generation of inactive or variable but not cultivable cells. Conventional farming practices and associated synthetic fertilizations not only pollute ground water sources but also put an unbearable burden on our farmers [11]. The majority of these inorganic substances are persistent [12], not readily degraded by natural microorganisms, which can reduce soil viability and negatively affect the quality of produce [13]. Organic agriculture is thus going to be an effective alternative worldwide. Such practices encourage quality food production excluding the use of pesticides and synthetic fertilizers, and targets equilibrium in soil dynamics. The addition of animal-derived amendments to soils promotes plant growth, crop yield and fruit quality due to balanced supply of C, N and P as well as supplying micronutrients such as zinc (Zn), manganese (Mn), boron (B), copper (Cu) and iron (Fe) for crop improvement [14,15]. Organic farming makes the soil friable and fertile: It enhances cohesion of aggregates, accumulates massive organic matter (SOM) in soil and influences microbial communities and their co-occurrences [16]. Moreover, this can improve N use efficiency of crops significantly and reduce NH_3 loss through volatilization [17]. Increased SOM supports vigorous uptake of nutrients, provides food for indigenous microorganisms and contributes greater C sequestration in organic agro-ecosystems, as well as having a strong effect on soil aggregation [18,19]. Aggregation size, classes and stability also affect microbial communities of soil and their composition and diversity. For example, alpha- *Proteobacteria* was dominant in macro-aggregates with high SOM. Organic matter is divided into stable and labile fractions. Stable C fractions are highly resistant to microbial decomposition, while labile C proportions have a rapid turnover rate and are directly related to the plant nutrient supply [20]. Labile C fractions are *O*-substituted alkyl, carbonyl and methoxyl C generally abundant in animal waste that are readily available to copiotrophs as energy source [21]. Recently, Bhunia et al. [15] found copiotrophs to be abundant in soils fertilized with recycled slaughterhouse waste, although it could vary with the type of organic substances amended [16]. Simultaneously, organically treated soils attained higher urease, dehydrogenase and acid phosphatase activities, which have a key role in nutrient recycling and decomposition of soil organic matter [22]. Chae et al. [23] considered β-glucosidase as a biological indicator of soil ecosystem health. In addition, organic fertilizers protect crops from *Pythium*, *Fusarium*, *Verticillium*, *Phytopthora* and *Rhizoctonia* like soil-borne pathogens [24], while excessive chemical fertilization posed a greater risk of pest outbreak [13]. Currently, the worldwide crop production is reduced by 36% due to emerging diseases of plants and regular pest attacks [25]. The application of organic manures provides a source of food substrates of varying quality that invoked competitions among microbial communities changing the structure and function of resident soil microbiome [26]. Few studies suggested that the production of volatile and non-volatile toxic compounds released during the decomposition of supplied organic amendment can also be the attributor of plant disease suppression [27,28]. Sturz and Christie [29] documented parasitism, competition, antibiosis, and systematic induced resistance (SIR) as possible mechanisms for allelopathic exclusion of soil-borne phytopathogens. Organic cultivations may gain fast acceptance globally if adverse effects of inorganic substances are highlighted with fervor.

Over the last decade, livestock production in India has also expanded. Following the slaughtering of animals, meat sectors generate organic wastes in vast quantity, which comprises 45% of the animal body weight [30]. Due to lack of government policies and proper awareness, these are either incinerated or landfilled in developing countries like India. Landfilling and/or open disposal of livestock mortalities can pose a serious threat to environment as they are the latent reservoir of Avian influenza, *Salmonella*, *Bacillus*, *Brucella*, *Clostridium*, *Campylobacter* and bovine spongiform encephalopathy (BSE) [31]. In addition, dumping sites release various toxic compounds, leachate and CH_4 and CO_2 like major greenhouse gases (GHGs) together with annoyances from bad odors to their surroundings [32]. The unscientific management of such wastes carries calculable risks to human health. For example, zoonoses caused by the infectious livestock pathogens may increase the morbidity of farm workers [33]. Indeed, animal wastes are a rich source of organic nutrients and will propagate environmental pollution if they are not utilized responsibly. Agronomic practices with untreated animal waste can introduce organic pollutants in the agro-environment and increase the number of antibiotic-resistant bacteria in soil [1]. The propagation of antibiotic resistant genes in bacteria is driven by horizontal gene transfer (HGT), and rhizosphere soil has been considered as a major hotspot for HGT [34]. Interestingly, the accumulation of heavy metals in agricultural field due to repetitive overuse of animal-derived amendments and/or raw organic waste may enhance antibiotic resistance in indigenous bacterial population [35]. The evolutionary theory of co-selection, which drives cross- (where same gene provides resistance to both antibiotic and heavy metals) and co-resistance (when resistance is offered by different genes of the same genetic loci) is found most relevant with the occurrence and propagation of antibiotic resistant genes in agricultural soil [1]. Due to emergence of antibiotic-resistant genes, an estimated 10 million human deaths will occur per year by 2050 [36]. Moreover, even plants die due to overuse of raw organic amendments in agriculture [15,21]. To minimize such adverse effects and to ensure biosecurity, waste recycling is necessary before reusing them in agriculture, which may also support the concept of circular bio-economy. Generally, animal wastes are treated through composting, vermicomposting, anaerobic digestion and drying methods. Sometimes, rendered meals are also used in organic farming and aquaculture [37]. Special emphasis has been given to these conversion technologies and their effects on pathogen and heavy metal removal from waste as these are the major challenges in waste to fertilizer conversion.

The emerging problems in developing countries like India appear clear: (a) Production of safe, healthy and affordable food for the constantly growing population, (b) recycling and reuse of organic waste in agriculture as fertilizer, (c) reduction in GHG emissions and environmental pollution, (d) protecting soil health and landscape diversity from synthetic applications and (e) development of a bio-based economy to achieve overall sustainability. To meet the future climatic and socio-economic challenges, agriculture needs to be organic and more productive. Adoption of Organic Agriculture 3.0 that aims to shift organic cultivation from its current domain to mainstream, may solve the problems linked with food safety and environmental health and provide an opportunity for the organic sector development [38]. This version is an advancement over the previous Organic Agriculture 2.0 and 1.0, which included socio-economic empowerment of rural areas, agro-ecological intensification and development in food production incorporating novel ethics and habits [39].

Our recent work, Bhunia et al. [15], demonstrated recycling of rural slaughterhouse waste through tray-drying and showed its agronomic efficiency during successive cultivation of bell pepper and amaranth, where N fertilization at a rate of 80 kg ha^{-1} produced higher yield and better fruit characteristics. Earlier, Roy et al. [40,41] applied sun-dried mixture of bovine blood and rumen digesta as major N source for the cultivation of solanaceous vegetables in India. On the other hand, high-temperature pyrolysis converted bone-based animal waste to an alternative of rock phosphate for fertilizer production [42]. During pot cultivation of maize, Frazão et al. [43] used granular poultry litter as an effective P substitute. Furthermore, Nunes et al. [44] grew soybean and corn with composted abattoir waste, while Arancon et al. [45] assessed positive effects of animal manure vermicompost. Feasibility of these organic fertilizers varied considerably with the feedstock type and adopted treatment technology [46]. Indeed, crop nutrient-use efficiency is highly dependent on the carbon-to-nitrogen (C/N) ratio of the substances applied during cultivations as lower C/N value indicated a high fertilizer quality and net mineralization of N in soil [47].

Therefore, this review article aims to discuss: (a) Animal waste recycling and their reuse in agriculture, (b) agronomic efficiency of the animal-derived fertilizers and (c) their potential effects on biology and fertility of soil agro-ecosystems as well as (d) to develop a bio-based economy through waste-to-fertilizer conversion.

2. Recycling Animal Waste for Fertilizer Production

2.1. Composted Amendment

Under the framework of circular bio-economy, waste recycling is currently gaining much interest instead of burial and burning of livestock mortalities. Following the European Union (EU) Directive 2002/1774/EC, such traditional practices are strictly prohibited within the EU [48]. As animal wastes contain high fat and protein, they can be used as viable feedstock in various value-added applications [49]. Indeed, composting is still preferable over the decades for recycling of animal waste in agriculture, which is relatively inexpensive and also environmentally acceptable.

Biological degradation of organic waste is carried out either in presence of oxygen or in anaerobic mode. Composting is an aerobic route of organic waste valorization that typically occurs in four consecutive phases, namely mesophilic, thermophilic, cooling and maturation. During the initial stage of composting, mesophilic activities increase compost temperature up to 68 °C that can facilitate faster proliferation of thermophiles [50]. Therefore, the second phase entails efficient eradication of pathogens to ensure biosafety. However, some opportunistic pathogens such as BSE and *Salmonella* may re-colonize the compost when temperature begins to decrease in subsequent cooling stage [51]. At maturation, the composted material becomes a friable, inodorous and humus-like nutrient rich product that can replace commercial inorganic fertilizers, improving soil properties through supplying essential crop nutrients. The main factors that could affect the quality and content of compost are waste C/N ratio, mode of composting, decomposition conditions and addition of nutrients during the process [46].

Three types of composting systems are currently employed, including windrows, static bins and in-vessel composting. Franke-Whittle and Insam [30] recommended windrow composting of animal carcasses as it reduced the pathogenic load better than the in-vessel type. According to Senesi et al. [52], the presence of humic acid fractions in organic compost makes it ecofriendly and agronomically acceptable. Animal waste composting can be considered beneficial in terms of the microbial stabilization, pathogen inactivation, moisture reduction and good fertilizer quality of the end product [53]. Ragályi and Kádár [54] applied slaughterhouse compost as fertilizer in agriculture. Poultry hatchery waste comprised of infertile eggs, dead chickens, decaying tissues and blood contaminated wastewater which contained 1% N, 2.5% P and 0.2% K when it was composted with poultry litter as reviewed by Glatz et al. [55], while Nunes et al. [44] found 18.2% organic C, 1.8% N and 2% P in cattle manure compost. Furthermore, fertilization with immature compost can increase soil salinity, facilitate N immobilization due to higher C/N ratio and suppress plant growth enhancing osmotic stress [56], whereas maturate compost with a C/N ratio lower than 20 may act as valuable soil conditioner [50]. Moreover, Bhunia et al. [31] suggested additional thermal treatments to make the compost pathogen-free.

2.2. Vermicompost Manure

Like composting, this technology is also involved in organic waste stabilization under aerobic environment. Vermicomposting has emerged as a sustainable option with two major benefits of transmuting plant available nutrients into much more soluble forms along with simultaneous reduction in total as well as bioavailable heavy metals content [57]. Simply, vermicompost is a mixture of worm cast, humus, live earthworms and their cocoons. It is a finely divided peat-like material that is rich in NPK, essential micronutrients and beneficial microbial communities including N_2-fixing, P-solubilizing bacteria and actinobacteria [58]. Today, vermicompost has become an imperative component of organic farming systems as the product has better nutrient profile and higher microbial status than traditionally available compost manures [59]. In general, vermicompost fertilization improves quality of agricultural produce and can be physical, chemical and biological attributors of the soil health.

Vermicomposting, an efficient biotechnological approach of composting, employs certain earthworm species to turn the organic waste into nutrient-rich manure, during which organic fractions of solid waste are modified by associated microbial communities secreting hydrolytic enzymes, while the earthworms used accelerate the process through substrate aeration, mixing, grinding, fragmentation and enzymatic digestion [57,60]. Out of the thousands of species of earthworms, *Eisenia fetida* was found to be the most appropriate epigeic one for vermicomposting of animal waste and biogas plant slurry [61]. After 10–15 days of primary decomposition, earthworms were incorporated at 8–10 worms kg^{-1} of waste in specified composting bed, where the earthworm activity undergoes two distinct phases: An initial active phase followed by the maturation or aging stage [62,63].

At maturation, vermicompost turns dark-brown, non-sticky and odorless with a final moisture range between 25 and 30% and may then be harvested from the top of the heap separating applied earthworms. Atiyeh et al. [64] cultivated marigolds using pig manure vermicompost. Yadav et al. [61] assessed around 2.8% N, 1% P and 0.9% K from earthworm-processed cow waste, while Garczyńska et al. [65] showed that vermicompost derived from Cameroon sheep dung had an organic C content of 34%, total N of 1.7%, P of 1% and K of 1.3%. Previously, Borges et al. [66] reported that the mixture of cow and swine manure (in 50:50 ratio) provided greater mineral composition in final vermi-produce. The use of immature vermicompost may introduce crop toxicity. On the other hand, disease-causing plant pathogens such as *Pythium*, *Rhizoctonia* and *Verticillium* are suppressed when mature vermicompost is applied at a moderate rate [67]. Moreover, these can incorporate huge amount of organic matter into agricultural soils, thereby improving soil aeration, aggregation stability, water-holding capacity and nutrient availability as well as stimulate enzyme and soil microbial activity. Kumazawa et al. [68] briefly documented beneficial roles of organic matter in sustainable agricultural production.

As vermicomposting technology does not involve any thermophilic phase, complete eradication of livestock pathogens in the final product is not guaranteed, although the process surprisingly reduced enteric virus, fecal coliforms and *Salmonella* strains in various biosolids [69]. Tognetti et al. [59] inoculated earthworms after the thermophilic stage of composting to overcome the drawback. According to Swati and Hait [57], used earthworms can reduce the mobility of metal ions converting them into lesser available forms who also found such mobile metal ions were accumulated in earthworm tissues.

2.3. Anaerobically Produced Digestate

Anaerobic digestion is a series of biological process (namely hydrolysis, acidogenesis, acetogenesis and methanogenesis) that facilitates organic matter breakdown in the absence of oxygen to produce biogas that may be an alternative source of energy to replace fossil fuels. A nutrient-rich product is also derived at the end of this process known as digestate. Anaerobic digestion can be either mesophilic (at 35 °C for 15–30 days) or thermophilic (at 55 °C for 12–14 days) and effectively removes pathogens and pollutants from the digestate produced, which may then be separated into solid and liquid fractions to fertilize agricultural soils [70–72].

A wide array of organic wastes including agrarian, municipal and sewage sludge can be the feedstock of anaerobic digestion [1]. During this process, the plant-available form of N (NH_4^+) increases satisfactorily [73]. Möller and Müller [74] reported 2.2% N, 0.4% P and 0.9% K in pig slurry digestate derived from German biogas plants, while some solid digestates contained 51–61% mineral N that suggested their best use would be as fertilizer [72]. Moreover, digestate application could reduce the risks of P runoff as labile-P fractions are significantly decreased in anaerobic digestion [75]. Few studies have addressed the fertilizer value of anaerobically produced digestate. For example, Nkoa et al. [76] found poultry-derived liquid digestate to be more suitable for high N demanding crops with a short-growing period. Loria et al. [77] used swine manure digestate as N source in corn production, while Collins et al. [78] fertilized potato plants with P-rich animal manure digestate. Furthermore, digestates from animal slurries can be an efficient source of nutrients for vegetable production even under the soilless condition as reviewed by Möller and Müller [74].

In recent years, the effects of anaerobic digestion process on pathogen inactivation and pollutants removal have also been considered for sustainable management of soil fertility. The majority of the slaughterhouse pathogens such as *Salmonella*, *Giardia* and *Cryptosporidium* were destroyed just after 30 min of thermophilic digestion [79], whereas Viau and Peccia [80] failed to eradicate such pathogens adopting the mesophilic process. Masse et al. [81] acknowledged composting as a more effective way for reducing antibiotic residues from organic waste instead of anaerobic digestion. Thus, composting was recently performed extensively along with anaerobic digestion to improve the digestate quality [31], while aerobic post-treatment of anaerobically digested poultry waste was suggested by Salminen et al. [82] to reduce its phytotoxic effects.

2.4. Pyrolysed Biochar

Biochar is a carbon-rich charcoal-like organic substance obtained from pyrolysis of biomass waste, which is usually applied as soil conditioner/amender in order to improve agro-ecosystem health and crop productivity and can also reduce the adverse effects of phytopathogens [83]. This technology involves thermochemical degradation of waste materials under an oxygen-deprived environment at elevated temperature, and can be divided into three subclasses as conventional, fast and flash pyrolysis depending on their operating conditions. According to Demirbas and Arin [84], a low process temperature and heating rate would maximize the char production, while Uzoma et al. [85] pyrolyzed cow manure at 500 °C temperature to obtain a biochar with 0.1% N, 0.8% P and 3.3% organic C content. Biochar quality parameters namely pH level, surface area, pore structures, functional groups and elemental compositions differ widely with pyrolysis substrate and temperature [86]. Zwetsloot et al. [42] showed how pyrolysis temperature affected availability and chemistry of P in abattoir bone char. In a recent study, Zhang et al. [87] established that higher-temperature pyrolysis reduced environmental risks and heavy metal toxicity in biochar derived from cow manure.

Biochar application may resolve a diversity of issues including site-specific (e.g., reduction in plant available contaminants) to global-scale problems (e.g., atmospheric C sequestration, GHG mitigation) [88]. Due to the presence of recalcitrant C fraction in biochar, such amendments become resistant to microbial attacks and stay in soil for thousands of years, even though high internal surface area and porous structure of biochar facilitate an ideal habitat for colonization, growth and reproduction of bacteria, actinobacteria and mycorrhizal fungi [89,90]. Thus, biochar addition could promote a potential sink for organic C [91]. It also augmented water and nutrient retention, plant growth, enzymatic activity and cation/anion exchange ability of soil as well as prevented surface water eutrophication and environmental deterioration associated with the extensive use of chemical fertilizers [83]. Indeed, biochar can restore phosphorus sustainability in organic agro-ecosystems. Wang et al. [92] amended soils with poultry-derived biochar rather than raw litter application to reduce the risks of phosphorus leaching, while Glaser and Lehr [93] reviewed that biochars produced from agrarian residues significantly increased P availability in agricultural soils. It was also reported that animal manure biochars contained more organic nutrients compared to the biochars prepared from plant materials [94]. Moreover, biochar usages in combination with other organic amendments keep the soil healthy by positively affecting microbial community structure and soil dehydrogenase activity. Such fertilization favored higher crop yield [95], although the agronomic efficiency of animal-derived biochars was not yet fully explored.

2.5. Dried Animal Waste

After proper heat treatment, organic wastes are generally transformed into either animal/ fish feed or nutritious organic fertilizer [49]. Drying is a simple technique of heat and mass transfer that allows low-cost recycling of animal waste in agriculture as stated by Bhunia et al. [96]. EU Directive 1990/667/EC suggested that drying of biomass waste at 133 °C for 20 min may completely eradicate infectious pathogens from the process end product [97]. Kádár [98] utilized dried slaughterhouse compost for sugar beet cultivation in Hungary, while Roy et al. [40] cook-dried the mixture of abattoir-derived bovine blood and rumen digesta (BBRDM) in different ratios (1:1, 2:1 and 3:1) to assess its fertilizer potential, reduce the extensive use of chemical fertilizers and to provide a clean environment around rural slaughterhouses. During field cultivations of tomato, Roy et al. [41] applied the same fertilizer (3:1), which contained 4.9% N, 0.6% P and 0.9% K along with a 4.8 C/N ratio. In our recent study, Bhunia et al. [15] showed agronomic potential of tray-dried slaughterhouse waste, where the mixture (in 3:1 ratio) was dried at 100–120 °C for 6–8 h using a designed tray dryer system. Drying type and process temperature had significant influence on the quality of end product as we observed during our research. We also developed a new drying equipment for on-site production of the fertilizer in India. A patent has been filed on this equipment by Bhowmik et al. [99] with application number 202031033116. On the other hand, Roy et al. [100] showed the effective eradication of *Mycobacterium*, *Salmonella*, *Clostridium*, *Bacillus*, *Brucella* and *E. coli* O157:H7 adopting the drying technology. Our previous study [15] also confirmed the absence of the above-mentioned abattoir pathogens in BBRDM-fertilized soils through 16S rRNA metagenomic study. Table 1 summarizes waste conversion methods and fertilizer quality of the final produce.

Table 1. Nutritional status of organic fertilizers derived from different animal sources.

Amendment Type	Used Feedstock	Fertilizer Value (%)			References
		N	P	K	
Composted fertilizer	Poultry hatchery waste	1	2.5	0.2	Glatz et al. [55]
	Cow	1.8	2	0.1	Nunes et al. [44]
Vermicompost manure	Cow	2.8	1	0.9	Yadav et al. [61]
	Sheep	1.7	1	1.3	Garczyńska et al. [65]
Anaerobic digestate	Poultry	16.4	2.4	1.9	Salminen et al. [82]
	Pig	2.2	0.4	0.9	Möller and Müller [74]
Pyrolysed biochar	Cow	0.1	0.8	-	Uzoma et al. [85]
Dried amendment	Buffalo	4.9	0.6	0.9	Roy et al. [40,41]

3. Dose Calculation and Yield Potential Assessment

Today, the use of fertilizers in agriculture is obvious in order to meet the growing need for food. In general, fertilization maximizes crop productivity and yields better quality of produce by supplying essential plant nutrients directly or indirectly to the soils. Bhunia et al. [15] observed early-stage mortality of bell pepper plants when cultivated with excessive supply of N through BBRDM fertilization (180 kg N ha^{-1}), while N application at low (80 kg N ha^{-1}) and moderate (120 kg N ha^{-1}) rates showed profound crop yield and better fruit quality. Previously, Roy et al. [40] experienced the same problem when cook-dried abattoir waste was added to soils in higher quantity (180 kg ha^{-1}) at the time of planting, where fertilizer N content was not considered for dose calculation. Indeed, the presence of labile C fractions in animal waste and higher accumulation of NH_4^+ -N in soil due to their overuse may arrest vegetative growth of plants and induce phytotoxicity [15,21]. Previously, Bonanomi et al. [101] claimed a significant reduction in phytotoxicity, which was associated with the progressive decrease in *O*-alkyl-C fractions. Lazcano et al. [102] found higher tomato plants death due to rigorous use of compost manure and suggested that the application dosages need to be well controlled. Furthermore, Lim et al. [67] reported the application of vermicomposted manure at a higher rate could reduce crop yield due to availability of soluble salts in vermicomposts. In the case of anaerobically produced digestate, some ambiguity also exists over its agronomic effectiveness. Gutser et al. [73] stated that crops, mainly the vegetable varieties, were unable to uptake readily available form of N in a huge amount, which led to greater leaching risk. Therefore, special emphasis should be given on fertilizer dose calculation and its application frequency determination.

According to Jackson and Smith [103], while studying the effects of fertilizer application rate and time on grain yields, noted that animal manures are a potential source of N for cereal crops. Sradnick and Feller [104] established that commercially available organic fertilizers obtained from plant and animal sources generally contained a higher amount of N than P. Moreover, Hua et al. [105] showed that animal manure application increased crop productivity enhancing N use efficiency in the soils of a 40-year soybean–maize rotation. Based on the above discussion, we also realize that agronomic efficiency of animal-derived fertilizers is highly dependent on its N availability. In contrast, various studies considered animal waste as a source of P in sustainable agriculture [42,75,106]. Judicious use of animal manures maximizes economic returns increasing crop yield per unit of fertilizer applied [107]. Agronomic efficiency (AE) is a metric that includes yield potential of an applied fertilizer and relates directly to economic return [108]. In the majority of the reports, agronomic efficiency was calculated straightforward: Yield data/rate of fertilizer application as stated by Vanlauwe et al. [109], although during our study, we have calculated agronomic efficiency adopting the formula of López-Bellido and López-Bellido [110]. Mathematically, this can be expressed as:

$$\mathrm{AE} = \frac{(\text{Yield in fertilized soil} - \text{Yield in unfertilized soil})}{\text{Quantity of fertilizer supplied}} \tag{1}$$

During the field cultivation of wheat, Koutroubas et al. [111] found no significant differences in dry matter yield between the control and soils fertilized with 16 t ha^{-1} farmyard manure (FYM), while the application of composted animal manure (composting of farm wastes along with poultry manure in 3:1 ratio) at a rate of 10 t ha^{-1} attained greater maize productivity than 4 t ha^{-1} as reported by Adediran et al. [112]. Roy et al. [41] applied 225 kg ha^{-1} dried mixture of bovine blood and rumen digesta (produced in 3:1 ratio as mentioned earlier) to obtain 33 t ha^{-1} tomato yields. Authors claimed that, during the cultivation, they had provided 68.31 kg N ha^{-1} of soil, whereas Adekiya and Agbede [113] recorded 7.6 t ha^{-1} yield of tomato supplying 30 t ha^{-1} poultry manure (PM). In another study where the effects of poultry manure, wood ash and rice bran were evaluated, Moyin-Jesu [114] found that relative to other treatments, the application of 6 t PM ha^{-1} provided better cabbage head yield. Similarly, Evanylo et al. [115] assessed the effectiveness of commercial fertilizer, poultry litter and compost-based manures in an organic vegetable cropping system who reported the highest maize growth around 16.2 t ha^{-1} in soils fertilized with 2 t ha^{-1} dried poultry litter (DPL) as shown in Table 2. In contrast, PM addition did not affect maize yield satisfactorily as reported by Busari et al. [116]. On the other hand, Ragályi and Kádár [54] preferred fertilization with composted cattle waste (CCW) at a 25–50 t ha^{-1} application rate instead of chemical use for higher triticale production in

Hungary. After three years, Nunes et al. [44] cultivated soybean and maize plants with the same fertilizer at different dosages (0, 4, 8, 12 and 16 t ha^{-1}) and found a quadratic relationship between the crop yield and fertilization rate. Furthermore, Das et al. [117] confirmed that composted cattle waste was agronomically more efficient than applied swine manure compost in rice paddy. On the other hand, fertilization with vermicompost manure also demonstrated the same trend of grain yield. For example, Arancon et al. [45] recorded 16 t ha^{-1} marketable yield of bell pepper upon the application of 10 t ha^{-1} vermicomposted cow manure (VCM). In addition, Llaven et al. [118] showed that peppers treated with sheep manure vermicompost produced better-quality fruits, although vermicompost derived from cow manure (CMV) was not satisfactory according to Joshi et al. [119]. However, the market acceptance of vermicomposts is greater than the composted products probably due to the better visual aspects, larger nutrient concentrations and higher microbial population size and activity [59]. According to Rayne and Aula [120], the degree to which manure affects agro-ecosystem potential is not only dependent on its fertilizer value, but also on the rate and timing of application, soil type and climatic conditions. Conversely, the use of anaerobically produced digestate and biochar in organic farming is not very popular. Alburquerque et al. [121] achieved 44 t ha^{-1} crop yield by adding 6 t ha^{-1} digested pig slurry (DPS) to the field soils. Bougnom et al. [122] applied solid anaerobic digestate, rather than the manure, to obtain higher yield of hay plants. Furthermore, Uzoma et al. [85] assessed the yield potential of cow manure biochar (CMB) during maize cultivation in Japan. Moreover, pyrolyzed biochar could reduce the risk of nutrient leaching [92]. According to Karim and Ramasamy [123], lower fertilization rates always tended to higher fertilizer use efficiency, thus agronomic efficiency was also higher. Vanlauwe et al. [109] established a negative exponential relationship between the AE and amount of N fertilizer supplied, while Chuan et al. [124] showed a positive quadratic correlation among yield response and AE for NPK dosages as illustrated in Figure 1. Simply, higher agronomic efficiency indicates more proficient use of nutrients mainly N by the crops, although developing countries generally practiced price-based selection of fertilizer. Table 2 represents the yield potential of various animal-derived amendments at different dosages where the fertilization rates and crop yield are calculated on a dry matter basis.

Lund and Doss [125] found a strong residual impact of dairy cattle manure on plant health and soil fertility. McAndrews et al. [126] evaluated residual effects of composted swine manure measuring growth and yield parameters of field soybean. Authors reported 0.2 to 0.5 t ha^{-1} productivity, which was higher than the control as well as urea-treated residual plots. Ragályi and Kádár [54] provided evidence of greater residual fertility in soils treated with CCW even after 3–4 years of cultivation. Recently, Bhunia et al. [15] proved that dried animal waste was residually more efficient than the soils treated with chemical fertilizers and market available vermicomposts.

Table 2. Yield potential of various animal-derived amendments at different dosages (expressed as dry matter basis).

Study Country	Fertilizer Type	Application Rate (t ha^{-1})	Cultivated Crops	Yield Response (t ha^{-1})	References
Greece	FYM	0	Wheat	3.2	Koutroubas et al. [111]
		16		3.4	
		32		4.5	
Nigeria	FYM+PM (3:1) compost	0	Maize	1.6	Adediran et al. [112]
		2.5		2.1	
		5		2.2	
		7.5		2.4	
		10		4.0	
Nigeria	PM	0	Maize	1.9	Busari et al. [116]
		5		3.7	
		10		2.9	
Hungary	CCW	0	Triticale	5.2	Ragályi and Kádár [54]
		25		5.4	
		50		4.7	
		100		6.7	
		200		6.4	
India	CMV	0	Wheat	2	Joshi et al. [119]
		5		3	
		10		3.1	
		20		3.1	
United States	DPL	0	Maize	2.4	Evanylo et al. [115]
		2		16.2	
Japan	CMB	0	Maize	1.2	Uzoma et al. [85]
		10		1.3	
		15		3.1	
		20		2.4	

FYM: Farmyard manure, PM: poultry manure, CCW: composted cattle waste, CMV: cattle manure vermicompost, DPL: dried poultry litter, CMB: cow manure biochar.

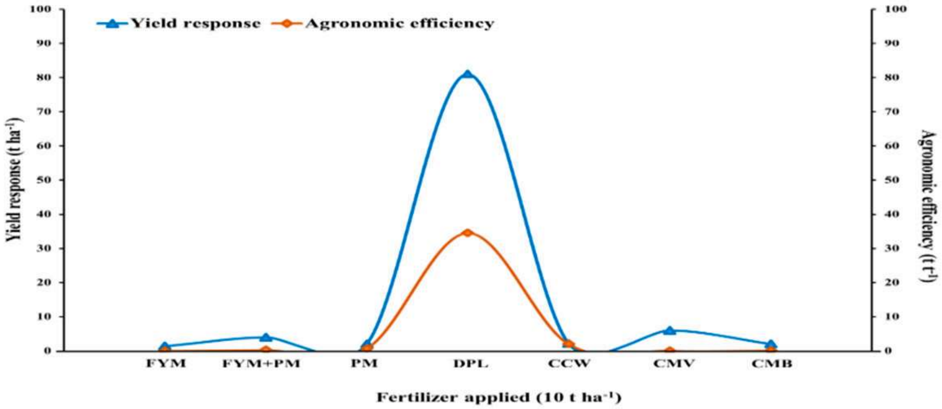

Figure 1. Positive quadratic correlation between the yield response and AE, where application rate was kept constant at 10 t ha^{-1} as dry weight basis for all the used fertilizers. This curve behaves quadratically as the highest exponent of the variable in the curve-equation was a square and the relationship found positive as increasing one variable increases the other one. Details provided in Table 2. FYM: Farmyard manure, PM: Poultry manure, DPL: Dried poultry litter, CCW: Composted cattle waste, CMV: Cattle manure vermicompost, CMB: Cow manure biochar.

4. Effects on Agro-Ecosystem Health

4.1. Aggregate Formation

Organic fertilization through the use of recycled animal waste provides sufficient strength for building up soil fertility especially in regions where soils are nutritionally poor [127]. Healthy soils are by default stable, resilient to stress factors and largely diverse with numerous taxa that form a complex food web through high levels of nutrient recycling [128]. The formation of stable aggregates may sustain crop productivity improving soil structure that provides pathways for the transportation of water, elements and gases as well as facilitates an ideal environment for microbial growth. Interestingly, aggregation stability is mainly dependent on the SOM content and dynamics [129]. Few studies reported that long-term inorganic fertilization promoted cohesion of aggregates [130–132], while no changes or even decrease was observed by Bandyopadhyay et al. [133], Xin et al. [134] and Zhou et al. [135] in comparison to unfertilized plots. In contrast, the addition of organic amendments enhanced SOM bound soil particles together into aggregates. Zhang et al. [136,137] showed a positive correlation between the aggregation stability and associated binding agents. Furthermore, it was reported that organic application decreased the proportion of micro-aggregates (<250 μm) with the mean geometric diameter and accumulated more SOM in macro-aggregates (>250 μm), as shown in Table 3. According to Lin et al. [16], micro-aggregates with recalcitrant SOM had less favorable habitat conditions due to greater cooperation and competition among the microbial groups, and found that the classes *Gaiellales* and *Pezizales* were abundant in micro-aggregates. Indeed, micro-aggregates with lower SOM chose *Actinobacteria* adopting the *k*-selection strategy, while *Proteobacteria* was dominant in macro-aggregates with high labile SOM [138]. In addition, Ma et al. [139] demonstrated that a deficiency in labile SOM enhanced both competition and cooperation among soil microbes. However, the degree of aggregation was highly influenced by the type of organic fertilizers amended and the presence of indigenous microbial communities in arable soil. Guo et al. [140] incorporated straw manure to improve structural stability of the soil, while cattle manure appeared to increase macro-aggregate proportion [141]. Furthermore, Lin et al. [16] showed more effective soil aggregation, around 30.6% larger aggregate formation in soils treated with pig manure instead of plant residues or inorganic NPK, whereas Babalola et al. [142] reported a 15.7% increase in aggregation stability after the addition of green manure compost. In addition, poultry litter usages reduced the formation of micro-aggregates by 34% compared to chemical fertilizer treatment and stimulated glomalin production [143]. On the other hand, Li-Xian et al. [144] mentioned that animal manure application with high salt content degraded soil structure to some extent.

4.2. SOM Turnover

Organic matter is a key component of arable soil, which is essential for long-term productivity of an agro-ecosystem as it contains N, P, C and other nutrients indispensable for growing plants and an energy reservoir for soil heterotrophic fauna [19] and has a priming effect on global carbon cycle as well [145]. Increasing SOM level interestingly decreases bulk density, which augments water retention, air exchange capacity and root proliferation [146]. It is well documented that the extensive use of chemical fertilizers deteriorated soil health mainly reducing the SOM content and associated microbial communities. According to Ali et al. [147], the SOM content in arable soils can be lower due to intensive cultivation throughout the year. In order to increase the SOM level, organic farming is preferred as suggested by Liang et al. [22], Maillard and Angers [148] and Wang et al. [149] because organic agriculture could replenish SOM more than that lost. The SOM cycling, formation and decomposition, is mainly mediated by the structure, composition and activity of indigenous microbial communities [150,151]. Later, Tian et al. [152] stated that fertilization deliberately influenced SOM content and quality affecting the composition of microbial communities. However, Kong et al. [153] reported that the turnover rate could also be influenced by the factors like irrigation, crop-rotation, soil environment and climate change.

A large number of field experiments have revealed that the long-term manure application alone or in combination enhanced SOM content and its fractions, while Gregorich et al. [154] did not find any significant alteration in the turnover rate of SOM under continuous chemical supplementation. Additionally, Whalen et al. [155] reported that the soil organic carbon (SOC) and total nitrogen (TN) concentration were increased up to 2.02 and 0.24 t ha^{-1} yr^{-1}, respectively, when composted cattle manure was added to farmland soils. Similarly, Brown and Cotton [156] found a three-fold higher SOC level in compost-amended soil, while Majumder et al. [157] observed NPK + FYM fertilized plots had similar labile C pool in comparison to the control. Bouajila and Sanaa [158] showed that matured compost application responded better to SOM fractions than the fresh or immature one due to the presence of higher stable C. Dass et al. [159], Jayakumar et al. [160] and Zhao et al. [161] experienced the same effects with animal manure vermicompost. Previously, Compton and Boone [162] showed how soil N dynamics was affected by the quality and quantity of SOC. According to Lal [163], fertilization affects SOC pools mainly increasing the humification rate. During the organic cultivation of tomato by land application of dried buffalo waste, Roy et al. [41] evidenced a temporary increase in plant-available soil N that may be due to lack of N immobilization, while in applying animal-derived biochar in organically fertilized soils, Plaza et al. [164] documented a drastic reduction in soil C loss through organo-mineral complex formation. Furthermore, Lin et al. [16] obtained highest TN and SOM value in NPK + pig manure soil. Authors amended plant residue with virgin NPK that did not affect SOM level significantly in comparison to control treatment. However, readily available SOC and high TN content allowed faster proliferation of copiotrophs as reported by Zhan et al. [165], which are involved in decomposition of organic matter to supply essential plant nutrients. We can consider them as potential indicators of healthy soil.

4.3. Microbial Abundance and Community Composition

Soil microbes are an integral part of agro-ecosystem health, can be classified as bacteria, actinobacteria, cyanobacteria, fungus, mycorrhizae, protozoa, algae, and each of them has a specific function in maintaining soil quality. According to Moeskops et al. [166], these serve residue decomposition, nutrient cycling, N fixation, C sequestration and stable aggregate formation as well as having a major role in soil-borne disease suppression. Moreover, the ability of organisms to degrade SOM depends on their enzyme secretion potential [129]. An active microflora is therefore crucial for sustainable crop production. Xu et al. [167] considered rhizosphere microbes as early warning indicators of soil health as they respond quickly to environmental changes. Indeed, the abundance, structure and activities of such indigenous microbial communities could be greatly influenced by the composition of plant species, agricultural practices and various abiotic factors as stated previously by Yu et al. [168]. Their responses towards diverse fertilization regimes have been well studied by so many authors over the past several years. Geisseler and Scow [169] found a 15.1% increase in microbial biomass production and diversity upon the application of mineral fertilizers compared to non-fertilized plots, but later on Wang et al. [170] observed a dramatic reduction in bacterial richness under the same fertilization regime. In contrast, Roy et al. [40] obtained higher numbers (in terms of cfu mL^{-1}) of total bacteria, N fixing *Azotobacter*, P-solubilizing bacteria, cyanobacteria and fungi in soils fertilized dried abattoir waste, whereas rice husk biochar only increased the abundance of genera *Thiobacillus*, *Pseudomonas* and *Flavobacterium* that contributed to P availability in soil [171]. In addition, Gopal et al. [172] confirmed that the populations of *Azotobacter*, *Azospirillum*, *Nitrobacter*, ammonifying bacteria and P-solubilizers were superior in *Eudrilus* sp. composted cow manure. The repetitive overuse of ammoniacal fertilizers significantly reduced the pH level in soil, which is closely associated with decreased microbial diversity and changes in indigenous community composition, while the addition of animal-derived amendments to soils prevented the acidification problem and related effects on soil microbiota as stated by Sun et al. [173]. However, it is very difficult to understand the complex responses of microbial communities towards organic and conventional farming as both the fertilization regimes have different bacterial and fungal populations (Table 3).

During their studies, Chaudhry et al. [174], Wang et al. [170] and Li et al. [175] demonstrated copiotrophic abundance in soils, which was managed organically, while Li et al. [176] observed a slow recovery of oligotrophs when SOC and TN levels were decreased in arable field with the progression of time. Oligotrophs and copiotrophs are physiological traits and can be distinguished by their growth kinetics and substrate affinity for metabolism. Chen et al. [177] obtained a higher Michaelis–Menten constant for copiotrophs that usually stayed in environments with high nutrient levels and preferentially consumed the labile C pools, while in contrast, oligotrophs exploit a soil that is nutritionally poor with low energy flow but, have higher biomass yield for each unit of substrate consumed [9]. Thus, oligotrophs are less reactive to abrupt resource availability and relatively slow-growing. Studies suggested that the main driving factor behind such shifts in community compositions may be the type of organic C incorporated and not the application of P and N [178–180]. The inability of copiotrophs to grow under nutrient-deprived condition includes possessing a relatively lower affinity for the substrate combined with a lack of adequate regulatory mechanisms for starvation as reported by Koch [10]. By applying the copiotroph-oligotroph concept to soil microorganisms, we can make specific predictions about the ecological attributions of various taxa and understanding of structure and function of resident bacterial communities in better way. Furthermore, Ding et al. [181] specified the roles of pH in shaping bacterial community structure who found *Proteobacteria*, *Acidobacteria* and *Actinobacteria* to be pre-dominant in the combined application of NPK + organic manure. Jones et al. [182] recorded the highest Acidobacterial abundance in chemically fertilized soils with a lower pH level. However, Shanks et al. [183] documented *Bacteroidetes* as the most abundant phyla in soils amended with composted cattle manure, whereas Li et al. [176] did not find any significant change in relative abundance when they compared the compost with control treatment. Moreover, the genus *Thermogemmatispora* (phylum Chloroflexi) was reported as key stone taxa in pig manured soils by Lin et al. [16] who noticed a decrease in its relative abundance under the NPK + pig manure treatment. On the other hand, fertilization with vermicompost manure at 3.75 t ha^{-1} rate diminished the richness of oligotrophic *Actinobacteria*, *Acidobacteria* and *Gemmatimonadetes* [184]. In a recent study, Bhunia et al. [15] obtained copiotrophic *Proteobacteria*, *Planctomycetes*, *Bacteroidetes*, *Chloroflexi* and *Firmicutes* as dominant in soils from bell pepper rhizosphere following the application of recycled slaughterhouse waste, while their richness diminished when treated with N/P/K = 10:26:26 + urea. Likewise, Wu et al. [185] observed that *Nitrosospira* abundance in organically fertilized soils that belonged to the ß-subclass of *Proteobacteria* improved fertilizer N use efficiency during the cultivation of grapes. Compared to bacteria, fungus poses more oligotrophic features as they prefer nutrient-rich environment to grow, therefore, the species of fungal phylum *Basidiomycota* including *Irpex*, *Pycnoporus*, *Trametes*, *Schizophyllum* and *Fomes* predominantly show oligotrophy in organic soils [186]. However, Wang et al. [170] demonstrated more ecologically similar groups in arable soils after the addition of organic fertilizers that indicated less interaction between the microbes.

4.4. Enzymatic Activity

Soil enzymes are also crucial for maintaining agro-ecosystem productivity. According to Das and Varma [187], ß-glucosidase, dehydrogenase, phosphatase, urease and invertase are the major enzymes that are generally found abundant in agricultural soils. The majority of these enzymes directly originate from viable microbial cells [188]. Their activities together with microbial biomass C (MBC) express biological status of the soil at a given time, and therefore enzyme levels can be used to determine the degree of alteration in soil structure. Moreover, Shi [189] established a positive correlation between the organic matter turnover and enzymatic activity in agricultural soils, whereas Lupwayi et al. [190] showed how MBC and activities of some of the enzymes (ß-glucosidase, NAGase, acid phosphomonoesterase and arylsulphatase) that mediate major biogeochemical cycles were affected by manure applications at different dosages. Additionally, soil enzymes are found highly sensitive to pH changes, while different fertilizers responded differently to the soil pH [191], although it is well documented that these enzymatic activities were enhanced when soils were fertilized with organic amendments. For instance, the activity of alkaline phosphomonoesterase in clay loamy soils was increased up to 300 mg *p*-NP kg^{-1} h^{-1} with the addition of swine manure biochar at the rate of 0.5%, which was around 150 mg *p*-nitrophenyl phosphate (*p*-NP) kg^{-1} h^{-1} in control treatment [192]. Lupwayi et al. [190] obtained 1956 pmol methylumbelliferone (MUF) g^{-1} h^{-1} ß-glucosidase activity in soils amended with composted cattle manure, whereas the NPK fertilized plots had 1534 pmol MUF g^{-1} h^{-1} activity of ß-glucosidase. Antonious et al. [193] monitored soil enzyme activity before and after animal manure application and found an increased urease and invertase activities after incorporation of vermicomposted horse manure to native soils. Furthermore, Panuccio et al. [194] showed a higher dehydrogenase activity (255 µg trifenil tetrazolium formazan or TTF g^{-1} h^{-1}) in loamy-sand soil, which was treated with 50% solid digestate produced from anaerobic digestion of animal manure and maize silage mixture, relative to control treatment (70 µg TTF g^{-1} h^{-1}). These positive results reflect higher metabolic profile of soil under organic farming systems.

Table 3. Alteration in agro-ecosystem health under different fertilization regimes.

Soil Health Parameters	Type of Fertilizer Applied		References
	Chemical	**Organic**	
Aggregate formation	Increases the proportion of micro-aggregates in soil (<250 µm)	Accumulates more SOM in macro-aggregates (>250 µm)	Lin et al. [16]
SOM turnover	No significant alteration in SOM turnover rate	More labile SOC pools in organically fertilized soils	Gregorich et al. [154]/Brown and Cotton [156]
Microbial abundance	Oligotrophic (*Actinobacteria*, *Acidobacteria* and *Gemmatimonadetes*)	Copiotrophic (*Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Planctomycetes*)	Bhunia et al. [15]
Enzymatic activity	Relatively less but greater than the control treatment	Higher	Lupwayi et al. [190]
Disease suppression	Poses a greater risk of pest outbreak	Protects crops from *Pythium*, *Fusarium*, *Verticillium*, *Phytopthora* and *Rhizoctonia* like soil-borne pathogens	Kim et al. [13]/Bailey and Lazarovits [24]

4.5. Disease Suppression

Research shows that the disease suppression ability of a crop is mainly confined to the biological properties of soil [195]. As highlighted in the literature, organic fertilizers can also be beneficial in terms of their disease suppression potential, while extensive and imbalanced supply of virgin nutrients pose a greater risk of pest outbreak decreasing the natural resistance in crops [13]. In recent years, the emergence of novel soil-borne plant pathogens and their resistant behaviors towards various phytochemicals have become a challenge to agricultural biologists. However, crops grown in organically cultivated soils exhibited lower attacks of pests and diseases as stated by Bailey and Lazarovits [24] (Table 3). Among the considered amendment types, composted fertilizers and vermicompost manures are generally applied to manage plant diseases and pests attacks without affecting the environment and human health, although their effectiveness against phytopathogens is attributed to microbial populations and community interactions present within these recycled products [196]. Yatoo et al. [197] claimed that vermicompost had better ability to resist plant diseases in comparison to commercial compost manures. Previously, Manandhar and Yami [198] found vermicompost to possess highest efficiency of disease control when they compared the effect of composted and vermicomposted fertilizers on foot rot disease of rice caused by *Fusarium moniliforme*. Arancon et al. [199] reported similar results applying vermicompost manure against spider mites attack to tomato seedlings, while wilt disease of tomato plants caused by the infection of *Fusarium oxysporum* was effectively suppressed when soil fertilized with dairy solid-based vermicompost [200]. Likewise, Szczech and Smolinska [201] assured that vermicompost application derived from animal manures prevented the abundance of pathogenic *Phytophthora nicotianae*. Pane et al. [202] recorded better inhibition in mycelial growth of *Pythium*, *Sclerotinia* and *Rhizoctonia* upon the addition of animal manure-based composts. In contrast, Bonanomi et al. [203] reported *Rhizoctonia solani* to thrive on animal-derived amendments rich in sugar-containing labile carbon fractions. In a continuation, Bonanomi et al. [21] demonstrated that organic amendments with high labile C fractions would be conducive to plant damping-off disease in short-term but, became suppressive after 100–300 days of application. Recently, Tao et al. [204] showed that the addition of bio-organic fertilizer shielded plants from pathogen infection increasing synergistic formation of biofilm at the root–microbiome interface, which may act as a plant-beneficial consortium against the soil-borne phytopathogens. An overall depiction of animal waste recycling and their reuse in farming systems to intensify agricultural productivity is reflected in Figure 2.

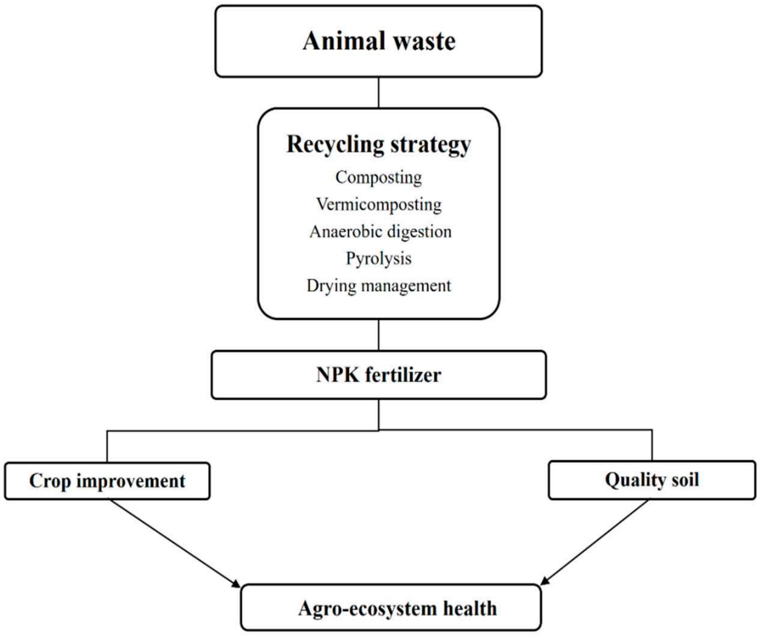

Figure 2. An approach to achieve agricultural sustainability through waste to fertilizer conversion.

5. Circulation of Nutrients Together with Economy

In this section, we will discuss how a bio-based economy is developed through waste-to-fertilizer conversion. In recent years, the per capita consumption of meat is increasing day after day leading to huge production of animal wastes daily. As we mentioned earlier, most of the time, these wastes are not properly disposed of, which adversely affects the environment and society as well as leading to economic losses. Composting, vermicomposting, anaerobic digestion, pyrolysis and drying are the major treatment alternatives that make such wastes suitable to supply NPK, without which food production would not be possible. This was discussed briefly in Section 2. As animal wastes have greater fertilizer value, as shown in Table 1, these may reduce the use of inorganic NPK sources in agriculture. Waste-to-fertilizer production not only improves the agro-ecology but also can be the backbone of our economy. It is necessary to shift our economic mindset from linear to circular to provide a permanent solution along with societal benefits under the framework of sustainable bio-economy practice. According to the European Commission [205], bio-economy includes the conversion of renewable bio-based wastes into diversified value-added products, which is, by default, circular as described by Carrez and Van Leeuwen [206], Sheridan [207] and Stegmann et al. [208]. This study highlighted a transition from the utilization of virgin nutrients to nutrient cycling, where nutrients are circulated together with economy.

This transition also includes efficient use of nutrients, generates demands for organic fertilizers and measures safe and profitable production and consumption of recycled nutrients as previously recommended by Valve et al. [209]. Figure 3 represents a pictorial overview of circular nutrient economy, which reflects economic, agricultural and environmental sustainability in each step. It starts with the waste production from different livestock farms and meat processing units, and then such wastes are recycled into organic fertilizers like compost, vermicompost, anaerobic digestate and biochar. When these amendments are applied in agriculture, SOM turnover as well as the C/N ratio of soil increases. Such fertilizers also supplies micro-nutrients that are indispensable for plant growth, thus a noticeable improvement in crop productivity was observed by Zhang et al. [14] and Bhunia et al. [15] during their studies. In fact, animal-derived organic amendments can positively affect the structure, nutrient turnover and many other properties of the soil as we briefed in Section 4. Human and livestock consumption of plant produce closed the nutrient loop, which will start again with the slaughtering of livestock animals. This novel approach can recirculate the economy transforming nutrient flows from linear to circular. Adopting this approach, local farmers, livestock owners and meat producing sectors would be benefited creating a ground for profitable business.

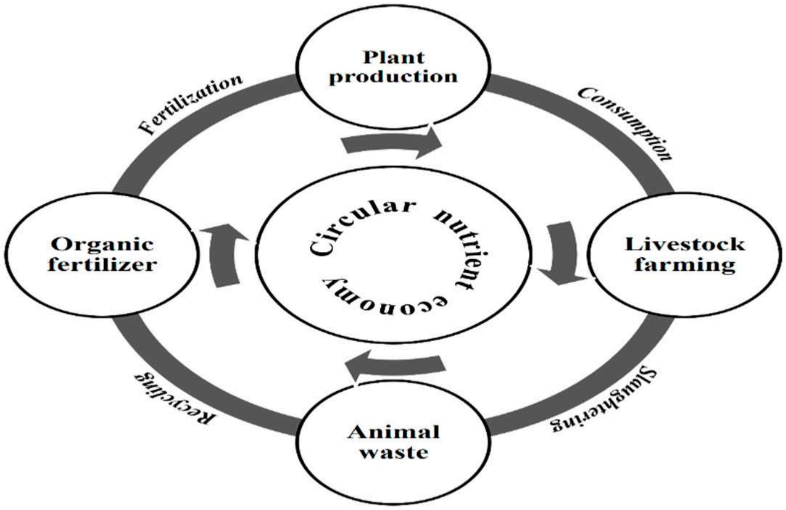

Figure 3. Circular nutrient economy and its elements.

6. Conclusions

Nutrient recycling through waste to fertilizer conversion safely disposes of livestock waste without polluting the environment. Among the existing conversion alternatives, composting, vermicomposting and thermal drying are relatively cost-effective and environmentally sound methods that can satisfactorily incorporate animal nutrients into the soil agro-ecosystem, which may be lost due to improper disposal of animal waste. No single technology can allow the complete destruction of abattoir pathogens, mainly the re-survival problem of BSE. Thus, the use of techniques in combinations is preferable, although adoption of dry heating technology may be advantageous in rural meat sectors. This review has categorized animal-derived amendments according to their source of origin and treatment technology adopted for recycling and highlighted how dose calculation and determination of fertilizer application frequency are crucial for maximizing crop production and soil fertility. A positive quadratic correlation between the yield response and agronomic efficiency was established by meta-analysis and the effects of diverse animal-derived fertilizers on soil aggregate formation, SOM turnover, microbial abundance, enzymatic activity and soil-borne disease suppression were also studied. In this study, a special emphasis has been given to the inactivation of waste pathogens that generally contaminate the rhizosphere soil if not treated properly before the land use. We furthermore conclude that, rather than the use of chemical fertilizers, the application of properly recycled animal-derived amendments at the appropriate dose will be more beneficial in terms of cost saving, agro-environmental quality and better crop productivity that should be the mainstay for sustainable agriculture. The main feature of this research is the circular nutrient economy, which exemplified how nutrients circulate together with the economy and affects sustainable development of the society. In view of animal waste valorization, future research on the circular nutrient economy should be encouraged to introduce organic fertilizers into mainstream cultivation.

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