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Integrated organic farming systems: Approach for efficient food production and environmental sustainability

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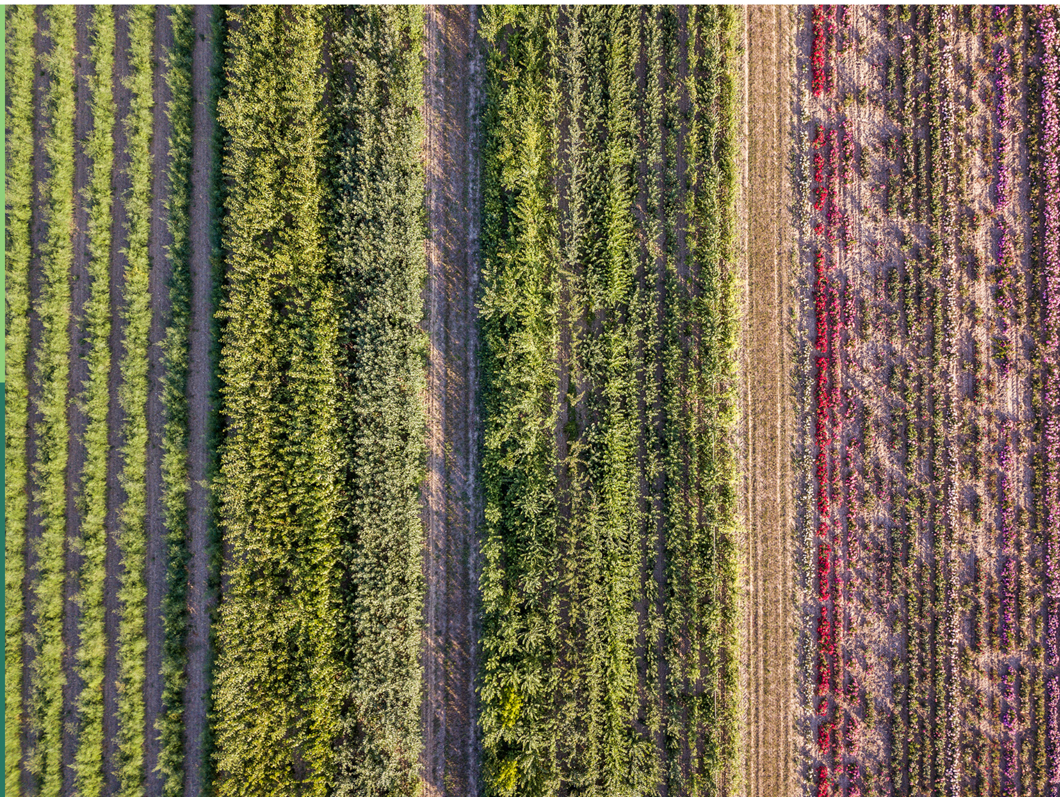

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Integrated organic farming systems: Approach for efficient food production and environmental sustainability

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REVIEW
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Sustainable Intensification of Maize in the Industrial Revolution: Potential of Nitrifying Bacteria and Archaea

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Sustainable intensification is a means that proffer a solution to the increasing demand for food without degrading agricultural land. Maize is one of the most important crops in the industrial revolution era, there is a need for its sustainable intensification. This review discusses the role of maize in the industrial revolution, progress toward sustainable production, and the potential of nitrifying bacteria and archaea to achieve sustainable intensification. The era of the industrial revolution (IR) uses biotechnology which has proven to be the most environmentally friendly choice to improve crop yield and nutrients. Scientific research and the global economy have benefited from maize and maize products which are vast. Research on plant growth-promoting microorganisms is on the increase. One of the ways they carry out their function is by assisting in the cycling of geochemical, thus making nutrients available for plant growth. Nitrifying bacteria and archaea are the engineers of the nitrification process that produce nitrogen in forms accessible to plants. They have been identified in the rhizosphere of many crops, including maize, and have been used as biofertilizers. This study's findings could help in the development of microbial inoculum, which could be used to replace synthetic fertilizer and achieve sustainable intensification of maize production during the industrial revolution.

Keywords: biotechnology, food security, bacteria, archaea, sustainable agriculture

INTRODUCTION

An agroecosystem where yields are increased without an adverse effect on the environment and a need for additional non-agricultural land is referred to as sustainable intensification (SI) (Pretty and Bharucha, 2014). The focus on agricultural intensification to increase yield for the growing population has escalated environmental degradation (Armstrong Mckay et al., 2019). Furthermore, many agriculturists have yet to adopt environmental sustainability because the problem of low yields has not been addressed (**Figure 1**). Sustainable intensification can concurrently address environmental security and food security. This is because as agricultural production would be increased, environmental degradation would be reduced simultaneously without acquiring more land for farm use (Hunt et al., 2019). The components of SI (**Figure 1**) protect the process of an ecosystem and biological diversity while achieving an increase in food production. However, to achieve this aim, the development of suitable techniques for estimating both the

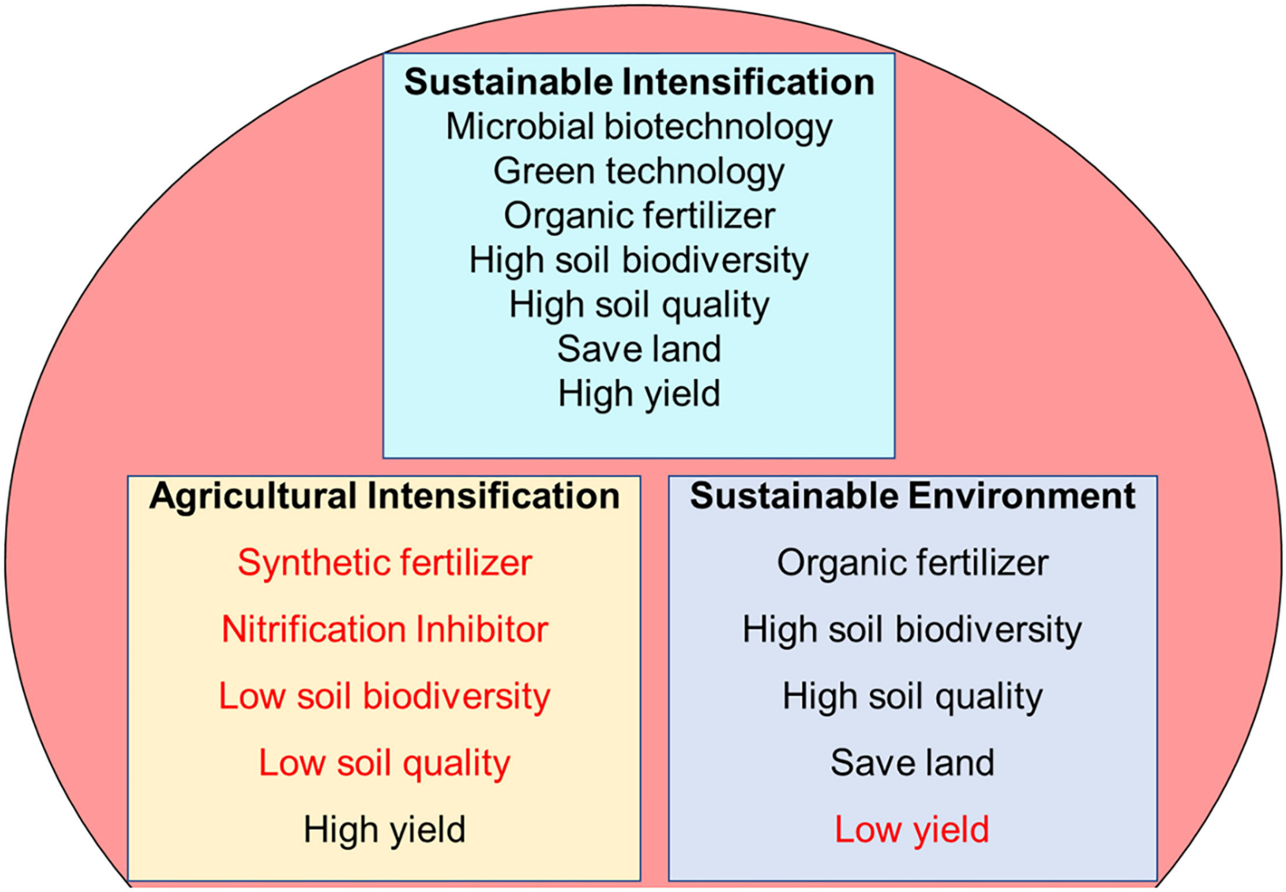

FIGURE 1 | Components of sustainable intensification, agricultural intensification, and sustainable environment.

sustainability and intensification of agriculture is needed (Hunt et al., 2019). Therefore, studying the interaction of microorganisms and the ecosystem would help maximize their services to ensure a better ecosystem.

Industrial revolution (IR) connotes industrialization that began way back in the seventeenth century (More and More, 2002). The industrial revolution brought about the expansion of farm crop yield and goods produced from them. Over the years IR has improved drastically as a result of mechanical production, electricity, electronics, telecommunication, computers, cyber-physical systems, genetic engineering, green revolution, and the internet (Prisecaru, 2016; Vu and Le, 2019). This has affected the agricultural sector also because the innovation of technology is crucial to the renovation and cultivation of food. Food security is part of the challenges the industrial revolution intends to resolve (Prisecaru, 2016). Expectations have been raised regarding using new technologies to conserve resources and improve food nutrients. People are malnourished because nutrient requirements are not being met. Therefore, there is a need for further global green revolution if the world needs to be fed. The industrial revolution could contribute to the security of food by improving crops by artificially adjusting important microbes associated with crops.

Maize is an important staple crop in the industrial revolution and is still in high demand worldwide, considering its importance as food, additives in industrial products, scientific research, and economy. The necessity to intensify its production sustainably is of paramount importance. Modifications in the nitrogen cycle have acutely disturbed the structuring and functioning of the natural ecosystem. The suitable range of nitrogen levels has been altered within the ecospheres and has posed a challenge to the issue of nitrogen maintenance (Xu et al., 2016). The increasing nitrogen level is partly caused by the input of nitrogen-based synthetic fertilizers. Consequently, to avert the challenge with the use of synthetic fertilizers, the inoculation of plant growth-promoting microbes wholly or together with manures would be critical in improving maize productivity for industrial revolution. Nitrifying bacteria with traits that promote plant growth have the potential of achieving sustainable intensification. This review discusses the role of maize in the industrial revolution, progress toward sustainable production, and the potential of nitrifying bacteria and archaea to achieve sustainable intensification.

SIGNIFICANCE OF MAIZE IN THE INDUSTRIAL REVOLUTION

Maize accounts for a significant amount of daily food in most developing regions. It is referred to as yellow gold because of its usefulness as food, animal feeds, and manufacturing processed food and non-food materials. Several studies have been carried out on maize because of its economic importance. Maize is one of the few crops that have attracted the attention of researchers in the area of genetic enhancement (Badu-Apraku and Fakorede, 2017). Aside from its importance, researchers choose to work with maize because it is suitable to cultivate and easy to collect data from Chen et al. (2015). Considering the foresight of industrial revolution, it is necessary to elaborate its role and point out how it can be cultivated in an environmentally friendly way.

Maize Products

The IR has caused an increase in agricultural products, both raw materials and industrialized. The processing of food rapidly has created sufficient time for human liberation, labor market participation and children care (Reardon et al., 2019). This has resulted in a reduced death rate and increased birth rate, causing a sharp increase in population, and placing high demand on resources. According to Dowswell et al. (2019), 20 million tons of maize is used for starch, 10 million tons are used for ethanol fuel production, 3 million for cereal and baked products, 0.7 million for cereal and hybrid seed sales. As a result of its reduced price as compared to other crops, maize has been used as feed formulae in animal rearing.

Maize is of high nutritional value and has been considered raw material for many industrial productions (Adiaha et al., 2016). This includes biomethane production, bioplastic, paper making, packaging and many additives. The agricultural sector substantially contributes to job creation and international marketing (Rekha and Singh, 2018). The effect of any technology in agriculture should be weighed against product output, profits, health, and environmental effect (Reardon et al., 2019). Over processed food has led to obesity, diabetes, and several health problems, hence the need to ensure the fortification of foods with sufficient nutrients (Reardon et al., 2019).

Economic Importance of Maize

Since the transcend of IR, global economic growth has been increasing. The production of maize ranks first in Latin America and Africa, while in Asia it is ranked third after rice and wheat (Dowswell et al., 2019). The demand and supply for maize globally for food and non-food products are usually on the increase. Yearly, 15 million metric tons (MMT) are used for animal feed, 4.25 MMT for industrial use, 1.36 MMT is used as food (Yadav et al., 2016). Considering its value for domestic, industrial and economic use (Adiaha et al., 2016), investing in the increase in maize production is an opportunity for any country. Maize is grown in 170 countries using 184 M ha of land with a production of about 1016 MMT (Food Agricultural Organization of the United Nations, 2017)). Various countries have benefited from the exportation and importation of maize.

In India maize has an annual production of 24.26 million metric tons (MMT) (Yadav et al., 2016). There was a rapid increase in the production of maize from 1950 to 1980, while 1983 marked a sharp decrease in maize production (Dowswell et al., 2019). It generates income for the government as it is used by countries as a commercialized product (Adiaha et al., 2016). Companies and individual entrepreneurs are collaborating with large-scale farmers to produce high-quality maize seeds. This helps mitigate their high demand and insufficient supply (Jonga et al., 2018). Seed quality determines crop yield and productivity. Jonga et al. (2018) advised that a quality management system should be put in place by the companies and entrepreneurs to ensure better products continuously.

Scientific Research on Maize

The role of maize in scientific research for the industrial revolution cannot be overemphasized (**Table 1**). Some upcoming scientists wonder why there is intensive research on maize when compared to other cereals. Aside from its importance as food and uses in industrial products, maize is easy to cultivate and manage, thus the results are observed easily and juxtaposed occasionally to other plants. Notable of its use in genetic studies, Jiao et al. (2017) referred to it as a model species for agricultural and genetic research. Maize plant has been used to check the quality of soil (Adiaha et al., 2016). The cob is useful in the treatment of waste. Okoya et al. (2015) reported the efficiency of maize cob in the removal of lead and chromium from waste.

Food Security

The quantity and quality of food have been threatened by unfavorable environmental conditions. To meet the needs of the high population, the quantity of food must be increased without jeopardizing the quality. In search of a solution, maize has been a choice crop by researchers (Adiaha et al., 2016; Otsuka and Muraoka, 2017). According to Abate et al. (2015), after considering factors that can be used to combat food security, maize was chosen as the best cereal to be cultivated in Ethiopia. He further explained that in terms of calorie intake, maize is the most important staple food. Otsuka and Muraoka (2017) acknowledged maize to be the most important cereal, considering its production and consumption. The development of the agricultural sector is necessary to reduce poverty and secure food. The need to secure food should be reinforced with green revolution that would drastically increase the yield of crops in a sustainable way. Therefore, maize which is easily cultivated and possess lots of nutrient has the potential to combat food insecurity globally. Maize cultivation has dropped the rate of poverty and improved the lives of local farmers, especially in developing countries Adiaha et al. (2016).

INDUSTRIAL REVOLUTION OF MAIZE

The agricultural sector, in general, has benefitted from industrial revolution using green and microbial biotechnology. Presently, green revolution has been anchored on genetically modified food and agrochemicals alongside several other inventions and technology (Llewellyn, 2018). Otsuka and Muraoka (2017)

TABLE 1 | Significant research findings related to the study of maize.

Scientific research on maize	Result	References
Agronomic assessment of a Controlled-Release Polymer-Coated Urea-Based Fertilizer in Maize	20% significant increase in maize yield compared to traditional fertilizer. Soil property was improved, and nitrogen loss was reduced	Gil-Ortiz et al., 2021
Evidence for phloem loading *via* the abaxial bundle sheath cells in maize leaves	The transfer of sucrose toward phloem was carried out by abaxial bundle sheath cell and it is subject to dorsoventral pattern	Bezrutczyk et al., 2021
How to increase maize production without extra nitrogen input	Increasing the density of plant increase the yield of maize by 5.59% Greenhouse gas reduced	Hou et al., 2020
Early isotopic evidence for maize as a staple grain in the Americas	Maize consumption started 4,000 calendar years before present	Kennett et al., 2020
Comparison between organic and inorganic fertilizer	The cost of production using organic fertilizer is one fourth cheaper than inorganic Significant increase in broadness and number of leaves in the plants with organic fertilizer	Deba et al., 2019
The function of ZmUBP15, ZmUBP16, and ZmUBP17	Help plant tolerate cadmium and salt stress They are mostly found in the plasma membrane	Kong et al., 2019
The role of cytoplasmic diversification on plant agronomic productivity and trait	A significant influence on the yield component of plants as a result of interaction between cytoplasm, nucleus, and testers	Calugar et al., 2018
Determination on how cells and tissues rely on autophagy	The evident alteration was seen in plants missing the core autophagy component ATG12 Autophagy influences eukaryotic membrane under nutrient stress	Mcloughlin et al., 2018
Effect of climate change on maize cultivation	Yield loss majorly as a result of drought stress Elevated CO_2 and heat had no effect on the crop	Webber et al., 2018
Assemble and annotation of maize genome using single molecule real-time sequencing and high-resolution optical mapping	Contig length was significantly increased and there was a deletion in the low gene density region	Jiao et al., 2017
Effect of heat and drought on rubisco activity which is associated with photosynthetic limitation	Rubisco activities was most affected at high temperature, but it was unrelated to the amount of rubisco activities The reduced rubisco affected CO_2 assimilation rate Rubisco can be used to improve plant photosynthetic performance in warm climate	Perdomo et al., 2017
Molecular basis of carpel fusion in ovary development	Certain miRNAs influence incomplete carpel fusion which code for auxin response factor and growth regulating factor	Li et al., 2017
Cadmium stress tolerance of plant using dark septate endophyte	Cadmium phytotoxicity reduced significantly while maize growth increased This was done by triggering the antioxidant system, altering cadmium and partitioning the subcellular cadmium into the cell wall.	Wang et al., 2016

stated that the green revolution has helped resolve food crisis however, some countries are yet to meet the global standard of maize yield and attributed this to low soil quality. Also, food insecurity is rising, crop yields are lower than expected when compared to farmers' input, many crop plants are susceptible to disease and the environment is being depleted. An improvement in the present green revolution is necessary, this could be achieved by scientific and biotechnological research toward agricultural production.

The focus is now on sustainably feeding the growing population. Increasing land productivity is a crucial requirement in meeting the growing demand for food in every region. Implementing technology in agriculture can cause a global transformation. Brill (1981) suggested the possibility of getting a hybrid plant with foreign genetic material that would make it possible for the plant to efficiently use atmospheric nitrogen. The possibility of using recombinant DNA techniques in microbial breeding for agriculture is still at a primitive stage, while engineering of beneficial soil microorganisms associated with the specific crop is ongoing. The inoculation of bacteria into soil has been seen to have a positive effect on plant growth (Ndeddy Aka and Babalola, 2016). The beneficial microorganisms can be cultured, grown in fermentation tanks and isolated for use. This can be taken practically to revolutionize industrial maize production.

ACHIEVING SUSTAINABLE INTENSIFICATION

In-depth knowledge of the dynamism of nitrogen would require research on the distribution, function, structure, and contribution of Bacteria and Archaea associated with its cycling process (He et al., 2012). Inoculation of microorganisms is a biotechnological environmentally safe alternative to increase crop production (Alori et al., 2017; Olanrewaju et al., 2017). The microorganism with the highest benefit could be useful for biotechnology breeding (Walters et al., 2018). Integration of microbes with organic material can also be considered Enebe and Babalola (2018). This would reduce the need for synthetic

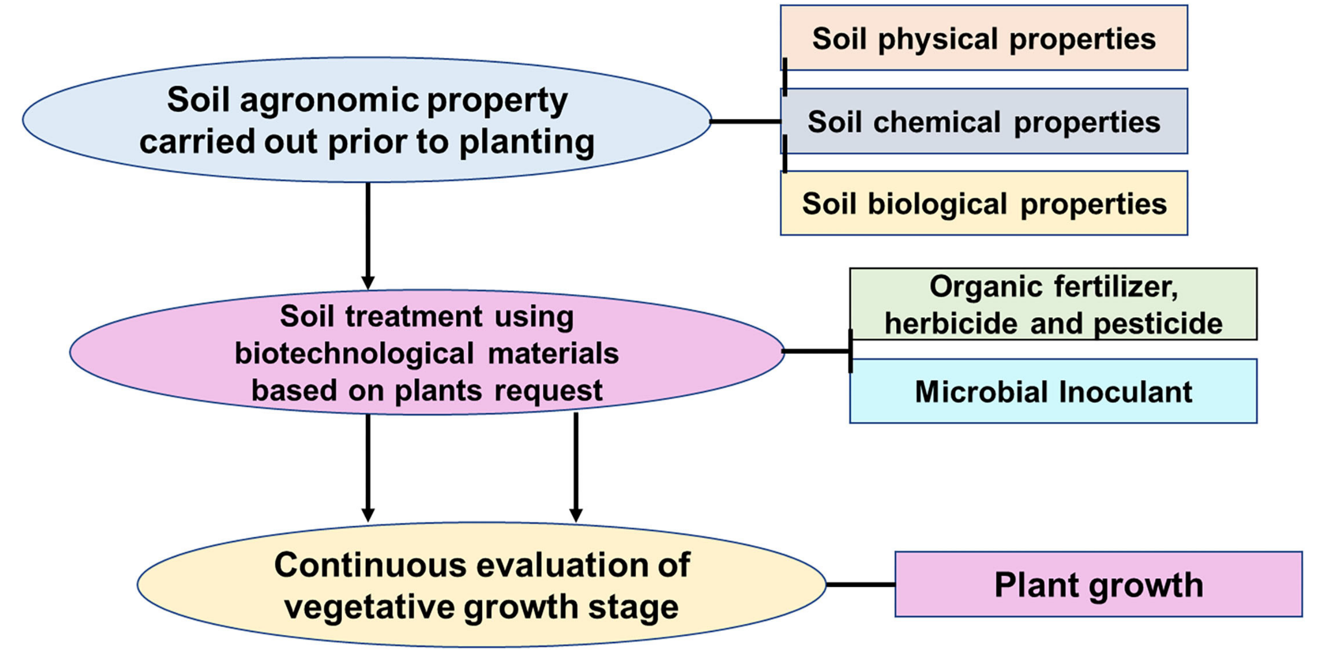

FIGURE 2 | Components that can help achieve sustainable intensification.

fertilizer and achieve SI. A new system incorporating different components that can boost maize production can be put in place (**Figure 2**).

PLANT GROWTH PROMOTING MICROORGANISMS

Unavailability of nutrients, pest infestation, and drought are some of the challenges to plant growth. Some microorganisms referred to as plant growth promoters have been observed to have traits that could help combat these challenges. One of the ways to address these challenges is to assist in the cycling of geochemical making nutrients available for plant growth (Etesami and Adl, 2020). Inoculation of microorganisms is a biotechnological alternative to increase crop productivity, increase the availability of nutrients, reduce the use of synthetic fertilizer, and achieve SI (**Table 2**). *Bacillus subtilis* was reported by Zheng et al. (2018) to be able to influence the physical, chemical and hydrological characteristics of the rhizosphere, thus improving drought tolerance of plants in the long run. They ascribed this attribute of *Bacillus subtilis* to their production of extracellular polymeric substances. Using genomic information, Wang et al. (2018) ascertained the usefulness of *Streptomyces albireticul* and *Streptomyces alboflavus* as a biocontrol agent.

NITRIFYING BACTERIA AND ARCHAEA

Surprisingly, nitrifying bacteria and archaea (**Table 3**) have not been focused on as plant growth-promoting bacteria. Considering their importance in nitrate production and oxidizing ammonia in soils and substrates, this calls for attention in scientific research. Aside from their major function of nitrification, they could have other plant growth-promoting traits. They can be classified into three distinct groups depending on the key enzymes possessed. The first group is the ammonia-oxidizing bacteria and archaea, second is the nitrite-oxidizing bacteria (**Table 3**) and the third is comammox bacteria (oxidation of ammonia to nitrate) (Stein and Klotz, 2016). Key enzymes used by these organisms are ammonia monooxygenase (AmoA), hydroxylamine oxidoreductase (HAO), and nitrite oxidoreductase (NXR) (Kuypers et al., 2018).

Based on nutrition, the nitrifying bacteria and archaea could be divided into heterotrophs and autotrophs (Liu et al., 2015). The heterotrophs depend on other organisms or dead organic matter for food while the autotrophs can synthesize their food. The autotrophs could further be divided into photoautotrophs (possess bacteriochlorophyll and use solar energy to produce food) and chemoautotrophs (using the oxidation of certain chemicals to produce food). Cellular respiration of nitrifying bacteria and archaea could either be aerobic (with oxygen) or anaerobic (without oxygen) (Muck et al., 2019). The group of organisms involved in anaerobic ammonium nitrification is known as anammox, they carry out nitrification in oxygen-depleted zones (Rich et al., 2018).

Nitrifying microbes include chemolithotrophic members, members of Betaproteobacteria, Gammaproteobacteria, and members of the Thaumarchaeota (Stein, 2019). The reactions occur under varying soil characteristics with some abiotic components contributing to it (Heil et al., 2016). Also, there are heterotrophic and methanotrophic bacteria that oxidize ammonium to nitrite efficiently (Stein and Klotz, 2016). High

TABLE 2 | Microorganism with plant growth-promoting traits that have been used on maize.

Microorganism inoculate used in maize	Type of experiment	Plant growth promoting trait	Result	References
Aspergillus niger	Field	Zinc and phosphate solubilization at a wider temperature and pH range	Inhibit production of aflatoxin Increase harvest index and yield Improves maize nutrient content	Naeem et al., 2021
Rhizophagus Irregularis, Glomus mosseae, Paraglomus occultum	Greenhouse	Increase soil fertility and enhance plant growth	Significant increase in root colonization and maize growth	Fasusi et al., 2021
Anabaena-Nostoc consortium, Anaebaena-Trichoderma biofilm	Field	Carbon Nitrogen mobilization	Higher efficiency was recorded in terms of economic, energy and environmental use Increased cob yield	Sharma et al., 2021
Azospirillum brasilense	Field	Increase chlorophyll content of plant	Increased yield and productivity	Cardozo et al., 2021
Metarhizium sp	Greenhouse	Possess entomopathogenic properties	Antagonistic effect on maize pathogen *Spodoptera frugiperdia*	Silva, 2021
Azospirilum brasilense and *Bacillus subtilis*	Greenhouse	Zinc solubilization	Modified root system which efficiently improves water and nutrient use	Moreno et al., 2021
Trichoderma harzianum	Field	Induce resistance of plant against herbivorous attack	Alter and reduce the community and abundance of pests	Contreras-Cornejo et al., 2021
Bacillus sp. and *Paenibacillus*	Field	Auxin production	Improve maize yield	De Carvalho Nascimento et al., 2021
Bacillus subtilis and *Pseudomonas koreensis*	Greenhouse	Siderophore production	Reduces infectious disease caused by *cephalosporium maydis*	Ghazy and El-Nahrawy, 2021
Burkholderia cepacia and *Acinetobacter baumannii*	Net house	Zinc solubilization	Improve the level of protein and sugar accumulation	Upadhyay et al., 2021
Claroideoglomus etunication	Greenhouse	Facilitate revegetation of contaminated soil	Enhance plant growth in lanthanum contaminated soil	Hao et al., 2021
Azotobacter chroococcum	Field	Promotes absorption of plant nutrients	Increase total nitrogen and phosphorus content in plant	Song et al., 2021
Anabaena cylindrical and *Azospirillum brasilense*	Field	Nitrogen-fixing bacteria	Higher nitrogen content of maize	Gavilanes et al., 2020
Arthrobacter arilaitensis and *Streptomyces Pseudovenezuelae*	Greenhouse	Ammonia, Indole-3-acetic acid, and Siderophore activity	Plants tolerated drought better Physiological parameters show significant increase	Chukwuneme et al., 2020
Trichoderma harzianum, Bacillus amyloliquefaciens	Greenhouse	Phosphate solubilization	Stimulate root growth which promotes the absorption of nutrients in the soil	Mpanga et al., 2019

temperature changes soil nitrifying communities as a result of an increase in the rate of chemical production (Nguyen et al., 2019). pH between 7 and 9 is best for the activity of ammonia oxidizing bacteria and nitrite-oxidizing bacteria, as higher than that disrupts their activity (Heil et al., 2016). Environmental factors determine the group of nitrifying microorganisms that would be prevalent in a habitat or substrate.

Nitrifying bacteria are widely used in aquaculture management (Ruiz et al., 2020; Ajijah et al., 2021) and waste management (Sepehri et al., 2020; Zhao et al., 2020). It is rarely used in cropping. *Nitrobacter*, on the other hand, has been used as a biofertilizer both alone (Doost et al., 2019) and in groups of micro-consortiums (Vatandoost et al., 2019). Doost et al. (2019) discovered that the protein content of Canola improved when compared to the control. Beyond aquaculture and waste management, there is still a need to expand the use of nitrifying bacteria in cropping systems. Nitrifire 5x, MicrobeLift Nite-out II, Scape bac up, Nitrobacter multi-probiotic, Nbc1, and Nbc2 are some of the commercially available application-based nitrifying bacteria. Although these products were intended for use in aquaculture, their novel application in crop management can be investigated.

Excess ammonia in the soil as a result of synthetic ammonium-based fertilizer affects the environment negatively (Lehtovirta-Morley, 2018). The presence of nitrifying bacteria in the soil reduces ammonia. This makes the soil less acidic and, as such, other beneficial microorganisms can proliferate, thus promoting soil quality. Also, nitrate, which is eventually produced from the nitrification process, elongates lateral roots (Mantelin and Touraine, 2004), mediates signaling pathways of phytohormones, expands leaves, and induces flowers in plants (Hachiya and Sakakibara, 2016). Furthermore, plants' yields and growth are increased and there is little or no dependence on synthetic fertilizer and other agrochemicals that degrade the soil.

ELECTRON TRANSPORT CHAIN

The enzymatic process of nitrification can be divided into three pathways: NH_3 oxidation pathway, NH_2OH oxidation pathway, and NO_2 oxidation pathway. The enzymatic process is carried

TABLE 3 | Well-identified nitrifying bacteria and archaea genera and their physiological group.

Domain	Phylum	Class	Order	Family	Genera	Physiological group	References
Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	*Nitrosospira*	Ammonia oxidation	Schaechter, 2009
	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	*Nirosococcus*	Ammonia oxidation	Gerardi, 2003
	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	*Nitrosomonas*	Ammonia oxidation	Koops and Stehr, 1991
	Proteobacteria	Deltaproteobacteria	Nitrospinales	Nitrospinaceae	*Nitrospina*	Nitrite oxidation	Gerardi, 2003
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Nitrobacter*	Nitrite oxidation	Brenner et al., 2005
	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	*Nitrococcus*	Nitrite oxidation	Schaechter, 2009
Archaea	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceaa	*Nitrospira*	Nitrite oxidation	Schaechter, 2009
	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	*Candidatus Nitrososphaera*	Ammonia oxidation	Tourna et al., 2011
	Thaumarchaeota	Nitrososphaeria	Nitrosopumilus	Nitrosopumilaceae	*Candidatus Nitrosopumilus*	Ammonia oxidation	Qin et al., 2017

out by an electron transport chain and the reaction is exergonic (a biochemical reaction that releases energy) (Wendeborn, 2019). Ammonia monooxygenase (AMO) turns ammonia into NH_2OH with the gain of two electrons (Daims et al., 2015). The electron is obtained from subsequent oxidation of hydroxylamine, and the energy liberated is obtained from the linked reaction of oxygen reduced to water (Wendeborn, 2019). AMO exists in an integral membrane protein and is a member of the copper membrane monooxygenase (CuMMO) family. The mechanism by which CuMMO carries out its oxidation could help in the development of monitored synthetic oxidation (Lancaster et al., 2018).

$$NH_3 + 2H^+ + O_2 + 2e^- \rightarrow NH_2OH + H_2O$$
$$NH_3 + ½O_2 \rightarrow NH_2OH$$
$$½O_2 + 2H^+ + 2e^- \rightarrow H_2O$$

Four electrons are used by hydroxylamine oxidoreductase (HAO), a multiheme enzyme used to oxidize NH_2OH to NO_2-. Two of the electrons used in oxidizing hydroxylamine return to AMO while the remaining two enter the respiratory electron transport chain, terminating the electron acceptor using O_2 (Daims et al., 2015). This reaction is also exergonic and the energy produced is higher if coupled with a reduction of water (Wendeborn, 2019). According to Lancaster et al. (2018), oxygen is not required for HAO activity, and NO is the product of NH_2OH oxidation and not NO_2. He further explained that NO is a reactive molecule, as its transformation to other forms of nitrous oxide could be a non-enzymatic reaction. This might be true, however (Wendeborn, 2019), reported some organisms that can oxidize NO_2 to NO_3.

$$NH_2OH + ½O_2 \rightarrow NO_2^- + 2e^-$$

Nitrite oxidoreductase possessed by some NOB oxidizes nitrite to nitrate with the use of electrons donated from oxygen. However, it can also be produced when nitrite donates electrons to reduce CO_2 to glucose by some photosynthetic bacteria (Wendeborn, 2019).

$$NO_2^- + ½O_{2\rightarrow} NO_3$$
$$2NO_2 - +CO_2 + H_2O \rightarrow CH_2O + 2NO_3^-$$

Comammox (complete ammonia oxidizer) was predicted by Costa et al. (2006) and discovered in *Nitrospira* by Daims et al. (2015) and Van Kessel et al. (2015). They can utilize eight electrons to oxidize NH_3 to NO_3^- (Lancaster et al., 2018). Broda (1977) predicted two chemolithotrophic organisms that can carry out anammox (Anaerobic ammonia oxidation). One of the bacteria responsible for anammox was identified as Planctomycetales in 1999 (Strous et al., 1999). Anammox microorganisms in an environment where oxygen is depleted can make use of nitrite instead of oxygen as the electron acceptor producing dinitrogen (Wendeborn, 2019). Considering the complex metabolic pathway in the nitrification process, there might be more discoveries to be made to manage the process efficiently.

AVAILABILITY OF AMMONIA IN THE SOIL AND ORGANIC WASTE

Ammonia-based substance is the substrate used by AOA and AOB. They can obtain it from ammonia-based organic waste or soil organic matter. Organic waste improves the quality of soil because it positively affects the growth of soil microorganisms. The natural process of nitrification does not provide sufficient nitrate. Therefore, to strike a balance between the modern process and the natural process, it would be good to provide a technology that would mimic the natural process. Organic fertilizers have been made from composting of organic waste and vermicomposting (Caceres et al., 2018). Plant growth-promoting microorganisms can be used along with these organic materials (Domenico, 2020). One of the biological approaches suggested for SI is to increase biological diversity in the agricultural systems (Petersen and Snapp, 2015). Nitrate has been successfully produced from ammonium contained in vegetable waste using

Nitrosomonas sp. and *Nitrobacter* sp. by Naghdi et al. (2018). Synthetic fertilizer is the cause of excessive amounts of nitrate because it speeds up the rate of nitrification. The gradual and systematic production of nitrate is considered safe for the ecosystem and a better alternative to synthetic fertilizer.

IDENTIFICATION AND ISOLATION OF NITRIFYING BACTERIA AND ARCHAEA

Microorganisms are ubiquitous, however, their composition varies in different habitats as a result of varying environmental factors. In time past isolation and identifying of microorganisms are usually carried out after culturing. Recently, metagenomics survey has enabled the easy identification of microorganisms. Known and unknown nitrifying microorganism strain has been identified from different habitats *via* metagenomics analysis (Clark et al., 2021). The establishment of the presence of nitrifying bacteria and archaea provides a guide on what type is to be isolated and cultured. Although nitrifying bacteria and archaea have been difficult to culture, however, some researchers have been successful in that regard (Könneke et al., 2005; Mellbye et al., 2017). Könneke et al. (2005), isolated nitrifying archaea using serial dilution and incubated them with a medium enriched with ammonia at 21–23°C. The use of mineral salt media with varying formulations has been used by Mellbye et al. (2017). Furthermore, Fujitani et al. (2015), explained the possibility of isolating them from nitrifying granules in a wastewater plant and cultivating them in a liquid culture rich in ammonia. Molecular characterization of the nitrifying microorganisms can also be carried out using 16S rRNA gene sequencing after serial dilution, DNA extraction and PCR amplification (Hastuti et al., 2019). Cultivating nitrifier community unique to maize plant can be carried out and used to increase their population in maize rhizosphere. This would increase the bioavailability of nitrogen in the soil, thereby replacing nitrogen-based fertilizers.

CONCLUSION AND PERSPECTIVE

Sustainable intensification proffers the solution to the conflicts of meeting the increasing demand for food and ensuring a sustainable environment. Industrial revolution merges trends in intelligent automation with artificial intelligence, this results in remarkable improvement in technology, growth in economy and unimaginable progress. Maize accounts for a significant amount of daily food in most developing regions and it is important to scientific and industrial use. Considering the need to increase maize production, microorganisms with growth-promoting properties can help achieve proper management, sustainable agriculture and sustainable environments. Agriculture has used large amounts of land globally, with major implications for reactive nitrogen from synthetic fertilizers and the use of nitrifying inhibitors to inefficiently manage the system. Nitrifying bacteria and archaea can transform ammonia locked up in soil organic matter and organic waste matter. They can be inoculated wholly or together with ammonium-based organic waste into the rhizosphere of maize. Although the biotechnological formulation and use are still in their primitive stage. Identifying and isolating nitrifying microorganism communities and structures associated with maize is a step toward achieving SI.

AUTHOR CONTRIBUTIONS

OEA reviewed and wrote the first draft of the paper, while OOB critiqued, proofread, and contributed substantially to the structure of the manuscript. Both authors contributed to the article and approved the submitted version.

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In vitro Screening of Sunflower Associated Endophytic Bacteria With Plant Growth-Promoting Traits

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Harnessing endophytic microbes as bioinoculants promises to solve agricultural problems and improve crop yield. Out of fifty endophytic bacteria of sunflowers, 20 were selected based on plant growth-promoting. These plant growth-promoting bacteria were identified as *Bacillus, Pseudomonas,* and *Stenotrophomonas*. The qualitative screening showed bacterial ability to produce hydrogen cyanide, ammonia, siderophore, indole-3-acetic acid (IAA), exopolysaccharide, and solubilize phosphate. The high quantity of siderophore produced by *B. cereus* T4S was 87.73%. No significant difference was observed in the *Bacillus* sp. CAL14 (33.83%), *S. indicatrix* BOVIS40 (32.81%), *S. maltophilia* JVB5 (32.20%), *S. maltophilia* PK60 (33.48%), *B. subtilis* VS52 (33.43%), and *P. saponiphilia* J4R (33.24%), exhibiting high phosphate-solubilizing potential. *S. indicatrix* BOVIS40, *B. thuringiensis* SFL02, *B. cereus* SFR35, *B. cereus* BLBS20, and *B. albus* TSN29 showed high potential for the screened enzymes. Varied IAA production was recorded under optimized conditions. The medium amended with yeast extract yielded high IAA production of 46.43 μg/ml by *S. indicatrix* BOVIS40. Optimum IAA production of 23.36 and 20.72 μg/ml at 5% sucrose and 3% glucose by *S. maltophilia* JVB5 and *B. cereus* T4S were recorded. At pH 7, maximum IAA production of 25.36 μg/ml was obtained by *S. indicatrix* BOVIS40. All the isolates exhibited high IAA production at temperatures 25, 30, and 37°C. The *in vitro* seed inoculation enhanced sunflower seedlings compared to the control. Therefore, exploration of copious endophytic bacteria as bioinoculants can best be promising to boost sunflower cultivation.

Keywords: bioinoculants, *Helianthus annuus*, plant growth promotion, seed inoculation, South Africa, sustainable agriculture

INTRODUCTION

The environmental problems posed by the use of agrochemicals on farmlands have necessitated the need to search for ecofriendly and sustainable approaches by harnessing endophytic bacteria as the best alternative bio-input (Basu et al., 2021; Bhutani et al., 2021). Devising suitable methodologies for the characterization of agriculturally important endophytic microbes from economic crops, however, promise to avert future food insecurity. The microbes found inhabiting the internal tissue of plants are called endophytic microbes and their mutual interdependence with the host plants contributes to plant growth and health (Mukherjee et al., 2021). Hence, the overview of the multifaceted functions of endophytic microbes can systematically bring new insights into agriculture biotechnology by maximum exploration and applications.

Endophytic microbes employed direct and indirect mechanisms to ensure sustainable plant nutrition (Zaman et al., 2021). Nitrogen fixation, IAA production, phosphate solubilization, and ACC deaminase activity contribute to soil nitrogen pool, root development, plant growth, and resilience to abiotic drought stress. The antibiosis, induced systemic resistance, hydrogen cyanide, siderophore, and enzyme production by endophytic microbes protect plants from pathogen attack, so indirectly enhance plant growth (Adeleke and Babalola, 2022). An increase in plant growth and crop yield upon inoculation with plant growth-promoting endophytic bacteria in the genera *Bacillus, Enterobacter, Klebsiella, Pantoea,* and *Rhizobium* has been documented (Nascimento et al., 2020; Mowafy et al., 2021; Preyanga et al., 2021). In addition, some endophytic bacteria isolated from oilseed crops have been reported to enhance plant growth due to their plant growth-promoting traits (Lally et al., 2017; Abdel-Latef et al., 2021). Nevertheless, scant information is available on endophytic bacteria isolated from sunflower cultivated in Southern Africa. Hence, research findings into the sunflower microbial world for maximum exploration as bioinoculants will help improve crop yield sustainably.

Aside from maize, cowpea, wheat, and sorghum, sunflowers are one of the most edible and economic crops cultivated in the North West Province of South Africa and other countries of the world (Adeleke and Babalola, 2020). Sunflower cultivation promise to ensure food security, and the supply of nutritional and healthy food for both livestock and human beings (Seiler et al., 2017). The economic value of sunflowers is enormous, such that, sunflower oil is widely distributed and available in South African markets. The seed inoculation and optimization of endophytic bacteria under different growth conditions remain fundamental to testing their effects on sunflower seed germination. Furthermore, harnessing copious endophytic microbes on a large scale can potentially boost sunflower oil production in South Africa. Hence, this research was designed to isolate, characterize and screen sunflower-associated endophytic bacteria with plant growth-promoting traits *in vitro*.

MATERIALS AND METHODS

Helianthus annuus Sampling

The roots and stems of *H. annuus* cultivar PAN 7160 CLP were sourced from commercial farmland in Lichtenburg, North West Province, South Africa in February 2020. The climatic conditions of this region were characterized by an annual rainfall of 360 mm and a temperature range of 3–21°C during winter and 22–34°C during summer. The healthy sunflower samples were carefully uprooted, labeled, placed inside sterile zip-lock bags, and transported to the Microbial Biotechnology Research Group laboratory, North-West University, South Africa at 4°C for further analysis. A total of 24 samples were randomly collected in triplicates from four points within the field for the isolation of endophytic bacteria.

Root and Stem Surface Sterilization and Isolation of Endophytic Bacteria

The sunflower roots and stems were cut into small sizes with a sterile scalpel and then washed in sterile distilled water. The samples were surface sterilized by soaking in 70% ethanol for 3 min, followed by 3% sodium hypochlorite for 3 min, 70% ethanol for 30 s, and lastly rinsed 5 times with sterile distilled water. The sterilization level of the samples was assessed by plating the last rinse on Luria Bertani (LB) media (Miller, Sigma Aldrich, USA). Five grams of plant material were weighed (Radwag weighing machine; Lasec; Poland), suspended in 1 M phosphate-buffered solution (FBS), and manually macerated in a mortar and pestle until smooth suspensions were obtained. One gram of the macerated samples was weighed and aseptically dispensed into sterile test tubes containing 9 ml sterile distilled water and mixed properly. Then, 1 ml from the mixture was aseptically pipetted and serially diluted up to 10^{-9} dilutions. From dilutions 10^{-5} and 10^{-6}, 0.1 ml of the suspension were gently dispensed into Petri plates in triplicates. The plates were pour-plated with molten sterilized Luria Bertani (LB) media and incubated at 28 ± 2°C for 24 h. Distinct bacterial colonies formed on the plates were counted and recorded. Colonies from each plate were observed and selected based on morphological characteristics. The pure bacterial cultures were obtained by repeated streaking on fresh LB agar plates. Pure bacterial colonies were preserved on LB medium amended with 30% (v/v) glycerol at −20°C.

Biochemical Characterization of Pure Bacterial Cultures

Gram staining and various biochemical tests were performed to characterize the bacterial isolates. Oxidase, urease, starch hydrolysis, citrate, and sugar fermentation tests were performed following the modified methods of Majeed et al. (2018). Other biochemical tests performed include Voges-Proskauer, hydrogen sulfide production, citrate test, nitrate utilization, methyl red test, and indole production test. All the chemical reagents used for these tests were procured from Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

Screening of Bacterial Isolates for Plant Growth-Promoting Traits

Ammonia Production Test

The ability of bacterial isolates to produce ammonia was tested according to the methods described by Alkahtani et al. (2020). Briefly, ammonia production was performed as follows: 0.1 ml of 24-h old bacterial culture (10^6 CFU/ml) was aseptically inoculated into test tubes containing 10 ml sterile peptone broth (peptone 0.2 g, 10 ml sterile distilled water) and incubated on a rotary shaker (SI-600, LAB Companion, Korea) at 120 rpm, at ambient temperature for 96 h. After incubation, a 0.5 ml Nessler's reagent was gently dispensed into each test tube, then allowed to stand for 5 min for color development. A color change from yellow to dark brown indicated a positive reaction. A tube without bacterial inoculation served as a control.

Phosphate Solubilization

The phosphate solubilization potential of the bacterial isolates was evaluated according to the methods described by Premono et al. (1996). The qualitative test was performed on modified Pikovskaya agar composed of (g/L; tricalcium phosphate ($Ca_3(PO_4)_2$) 5, glucose ($C_6H_{12}O_6$) 10, manganese sulfate ($MnSO_4{\cdot}H_2O$) 0.002, sodium chloride (NaCl) 0.2, potassium chloride (KCl) 0.2, magnesium sulfate ($MgSO_4$) 0.1, ammonium sulfate ($(NH4)_2SO_4$) 0.5, yeast extract 0.5, agar 15, at pH 7. Twenty-four-hour-old bacterial cultures were spot inoculated directly at the center of each Pikovskya's agar (PA) plate. The plates were incubated at 28°C for 5 days for the visible zone of clearance (ZOC). The ZOC (mm) around the colony on the cultured plates indicated positive results for phosphate solubilization, while the un-inoculated plate served as control. The ZOC (mm) around the colony was measured, and colony diameter measurements (mm) were summarized as low (+), medium (++), and high (+ + +). The phosphate-solubilizing index (PSI) was enumerated as:

$$PSI = \frac{\text{colony diameter (mm)} + \text{ZOC (mm)}}{\text{colony diameter (mm)}}$$

PSI was grouped as low (PSI < 2.00), intermediate ($2.00 \leq$ PSI < 4.00), or high (PSI ≥ 4.00) based on Marra et al. (2011) methods.

For the quantitative assay, the phosphate solubilizing-producing ability of the bacterial isolates was performed by inoculating 10 ml sterile Pikovskaya broth in 50 ml Falcon tubes with 0.1 ml (10^6 CFU/ml) freshly grown bacterial culture, incubated at 30°C for 5 days at 180 rpm on a rotary shaker (SI-600, LAB Companion, Korea). The supernatant was obtained after cold centrifugation of 10 ml bacterial cultures at 10,000 rpm for 5 min at 4°C. Four milliliters of the color reagent (1:1:1:2 ratio of 3 M H_2SO_4, 10% (w/v) ascorbic acid, 2.5% (w/v) ammonium molybdate, and distilled water) were added to 10% (w/v) of 5 ml trichloroacetic acid inside test tubes. The inoculated tubes were allowed to stand for 15 min at room temperature. The quantity of phosphate content was measured according to phosphomolybdate, a blue method, at an absorbance of 820 nm. The phosphate solubilization potential of endophytic bacteria in the Pikovskaya broth was determined from the phosphate (KH_2PO_4) standard curve. Medium without bacterial inoculation served as control.

Siderophore Screening

The siderophore production ability of the bacterial isolates was performed according to the methods of Khan et al. (2020) with few modifications. Each bacterial isolate was aseptically inoculated into a sterilized medium amended with CAS, i.e., chrome azurol S. Preparation of CAS solution was performed by weighing 60.5 mg CAS into 10 ml of 1 mM iron (III) solution ($FeCl_3.6H_2O$) (a). The iron (III) solution was diluted in 10 mM HCl. The solution (a) was gently mixed with 0.0729 g hexadecyltrimethylammonium (HDTMA, Merck, SA) bromide suspended in 40 ml sterile distilled water (b) on the magnetic stirrer. From the mixture of "a" and "b," solution, 100 ml was measured and added to 900 ml sterilized LB medium at pH 6.8. The sterilized medium at 121°C for 15 min was allowed to cool and pour plated into sterile Petri dishes. Each bacterial culture was spot-inoculated at the mid-point of the solidified agar plates and incubated at 28°C for 5 days. The development of yellow ring coloration around the bacterial colonies on the plates indicated positive reactions for siderophore production. Un-inoculated plates served as control.

The quantity of siderophore production was determined by inoculating LB broth solution containing CAS with 0.1 ml of 24-h old bacterial culture and incubated at 180 rpm on a rotary shaker (SI-600, LAB Companion, Korea) for 7 days. The grown bacterial culture was centrifuged at 8,000 × g for 10 min. From the filtrate, 0.5 ml was added to 0.5 ml CAS reagent, mixed, and incubated for 2 min at room temperature. The quantity of siderophore produced was measured at 630 nm using a spectrophotometer (Thermo Spectronic, Merck Chemicals, SA). The siderophore values were obtained from the regression equation of the standard curve. The experiment was carried out in triplicate.

Test for Hydrogen Cyanide

The test for HCN production by bacterial endophytes was determined according to the modified method of Igiehon et al. (2019). Nine milliliters of LB broth amended with 0.4% (w/v) glycine was aseptically dispensed into test tubes, sterilized, allowed to cool, and then inoculated with 0.1 ml (10^6 CFU/ml) fresh bacterial inoculum. Whatman filter paper No.1 was dipped in 0.5% (w/v) picric acid and subsequently in 2% (w/v) sodium carbonate. Then, the filter paper was plugged into each test tube (without touching the broth solution), then screw-cap and stop-up with parafilm and incubated at 28°C for 5 days. The test tubes were examined daily for color changes in the filter paper. A color change from yellow to brown indicated a positive result. The un-inoculated tube served as control. The experiment was carried out in triplicate.

Screening of Extracellular Enzymes

Screening for enzyme production was performed using plate assay techniques. The enzymes screened include, mannanase, cellulase, amylase, xylanase, and protease.

Mannanase

Screening of bacterial isolates for mannanase production was performed as described by Blibech et al. (2020) with few modifications. Briefly, media composition of g/L; Locust bean gum (3), dipotassium hydrogen phosphate (K_2HPO_4) 1, iron sulfate ($FeSO_4$) 0.001, ammonium chloride (NH_4Cl) 1, sodium chloride (NaCl) 0.5, calcium chloride ($CaCl_2$) 0.1, magnesium sulfate ($MgSO_4$) 0.5, and agar 13 at pH 7.2 was sterilized at 121°C for 15 min, allowed to cool. The media were poured plated and allowed to solidify. A 24-h old bacterial culture was gently inoculated in the middle of the agar plates and then incubated at 28°C for 48 h. Each cultured plate was flooded with iodine solution and observed for 15 min. The staining solution was poured off and further treated by flooding with 1 M sodium chloride (NaCl) for 15 min for the visible ZOC around the colonies. The colonies with a ZOC (mm) indicated mannanase production. The un-inoculated plate served as control. The experiment was carried out in triplicate for each bacterial isolate.

Cellulase

The qualitative screening of endophytic bacteria for cellulase production was performed using plate assay techniques according to Alkahtani et al. (2020) with little modifications. Freshly grown pure bacterial cultures were inoculated by single streaking on carboxymethyl cellulose (CMC) amended media composed of g/L; dipotassium hydrogen phosphate (K_2HPO_4) 1, sodium nitrate ($NaNO_3$) 3, iron sulfate ($FeSO_4$) 0.01, CMC 1, potassium chloride (KCl) 0.5, magnesium sulfate ($MgSO_4$) 0.5, and agar 20 at pH 7.0. The inoculated CMC plates were incubated at 28°C for 48 h and then flooded with 1% (w/v) Congo red (CR) for 10 min. The CR on the plates was gently washed off and the plates were further washed with 1 M NaCl for 15 min. The ZOC (mm) encircling the colonies indicated a positive result for cellulase production. The un-inoculated plate served as control. Negative result plates were further flooded with 5% acetic acid solution for 2 min and then washed with sterile distilled water. A clear ZOC (mm) around the colony was determined and recorded. The experiment was carried out in triplicate for each bacterial isolate.

Amylase

The amylase production was tested on starch agar according to the methods of Alkahtani et al. (2020) with little modifications. A 24-h bacterial culture was spot-inoculated on sterilized starch agar medium composed of peptone 5 g, magnesium sulfate ($MgSO_4$) 0.5 g, yeast extract 5 g, iron sulfate ($FeSO_4$) 0.01 g, soluble starch 10 g, agar 15 g, and sodium chloride (NaCl) 0.01 g in 1,000 ml sterile water and then incubated at 37°C for 48 h. After that, Lugol's iodine solution (iodine 0.4%, potassium iodide 0.8%, distilled water—200 ml) was poured on the plates for 10 min. The formation of a ZOC (mm) around each bacterial isolate on the plate indicated amylase production. The un-inoculated plate served as control. The experiment was carried out in triplicate for each bacterial isolate.

Xylanase

Screening of bacterial isolates for xylanase production on mineral salt medium (MSM) supplemented with 0.5% xylan (beechwood) was performed according to the methods described by Alkahtani et al. (2020) with minor modifications. The MSM composition include, agar 2%, peptone 0.5%, yeast extract 0.3%, and sodium chloride (NaCl) 0.5%. The media solution was adjusted to pH 9 before sterilization at 121°C for 15 min. The media were allowed to cool and pour plating. Plates were inoculated with fresh 24-h old bacterial culture by straight streak at the mid-point of the plates and then incubated at 28°C for 24 h. After that, plates were flooded with 0.4% Congo red and incubated for 10 min, then washed with 1 M NaCl to determine the ZOC. The ZOC around the bacterial isolates on each plate was considered positive for xylanase production. The un-inoculated plate served as control. The experiment was carried out in triplicate for each bacterial isolate.

Protease

The primary screening for each endophytic bacterium for protease production was performed on LB agar plates supplemented with skim milk powder according to the methods described by Alkahtani et al. (2020) with little modifications. The media composition (g/L) includes, skim milk powder 28, dextrose 1, casein 5, yeast extract 2.5, and agar 15 at pH 7. The media were prepared, sterilized, and then allowed to cool before pour-plating. Consequently, fresh bacterial culture was inoculated on each plate and incubated at 28°C for 48 h. The bacterial isolates exhibiting a circular ZOC (mm) indicated a positive result for protease production. An un-inoculated plate served as control. The experiment was performed in triplicate.

Indole Acetic Acid

The IAA production was tested according to the modified method of Gutierrez et al. (2009). Ten milliliters of LB broth supplemented with tryptophan were aseptically inoculated with 0.1 ml freshly grown bacterial culture (10^6 CFU/ml) and incubated at 28°C for 7 days at 120 rpm in a rotary shaker (SI-600, LAB Companion, Korea). The bacterial cultures were cold centrifuged at 4°C for 10 min at 8,000 rpm. IAA from the crude extract was measured by transferring 1 ml of the supernatant into a clean tube and one drop of orthophosphoric acid (10 mM) and Salkowski reagent (2 ml) (1:30:50 ratio of 0.5 M $FeCl_3$ solution: 95% w/w sulfuric acid: distilled water) was added. The mixture was allowed to stand (incubation) for 10 min at room temperature. The appearance of pink coloration in the tubes after incubation in the dark indicated a positive result. An un-inoculated plate served as control. Color development by the bacterial strains was grouped as low, average, and high. The IAA production of the reacting mixture after incubation was determined at 530 nm using UV-spectrophotometer (ThermoFisher Scientific, USA). The IAA concentration of each bacterial isolate was evaluated from the IAA gradient standard curve (SC). The experiment was performed in triplicate for mean value calculation. Furthermore, three bacterial isolates were optimized under different growth conditions: nitrogen source, carbon source, pH, and temperature.

Media Preparation and Optimization Process for IAA Production

Varied concentrations of carbon and nitrogen sources were supplemented into an IAA production medium (IPM) composed of (g/L); yeast extract 6, L-tryptophan 1, peptone 10, and NaCl 5 at pH 7.6 (Chandra et al., 2018). Other optimized conditions include incubation time, temperature, and pH. For incubation time, a 200 ml IPM inside 500 ml conical flasks was sterilized at 121°C for 15 min, allowed to cool, and then inoculated with fresh 24-h grown *S. indicatrix* BOVIS40, *B. cereus* T4S, and *S. maltophilia* JVB5 with 0.5 optical density at 630 nm. The culture medium was incubated at 37°C, and 180 rpm for 11 days.

TABLE 1A | Biochemical characterization of endophytic bacteria from sunflowers.

Strain	SP	GR	CT	Gel	OX	SH	H_2S	VP	MR	MT	NT		IND	CS	UR	Isolation sources
											Clr	Gas				
B. cereus SFR35	Rod	+	+	+	+	+	+	+	+	+	+	−	+	−	−	Root (Ten (10) bacterial isolates)
B. wiedmannii FTL29	Rod	+	−	+	+	+	+	+	+	+	+	−	−	+	−	Root
Bacillus sp. CAL14	Rod	+	+	−	+	+	+	+	+	+	−	+	−	+	−	Root
B. cereus T4S	Rod	+	+	−	+	+	+	+	+	+	+	−	−	+	−	Root
P. lini BS27	Rod	−	+	−	+	+	+	+	+	+	+	−	−	+	−	Root
S. indicatrix BOVIS40	Rod	−	+	−	+	−	+	+	+	+	+	−	−	−	−	Root
S. maltophilia JVB5	Rod	−	+	−	+	+	+	+	+	+	+	−	−	+	−	Root
B. albus TSN29	Rod	+	+	−	+	+	+	+	+	+	+	+	−	+	−	Root
B. cereus BLBS20	Rod	+	+	−	+	+	+	+	+	+	−	+	−	−	−	Root
B. thuringiensis SFL02	Rod	+	+	−	+	+	+	+	+	+	+	+	+	+	−	Root
S. maltophilia PK60	Rod	−	+	+	+	+	+	+	+	+	+	−	+	+	−	Stem (Ten (10) bacterial isolates)
B. subtilis VS52	Rod	+	+	+	+	+	+	+	+	+	+	−	−	+	−	Stem
B. thuringiensis BAAG44	Rod	+	+	+	+	−	+	+	+	+	+	−	−	−	−	Stem
B. pseudomycoides SFS19	Rod	+	−	−	+	−	+	+	+	+	+	−	−	+	−	Stem
B. toyonensis OLT2020	Rod	+	−	−	+	−	+	+	+	+	+	+	+	+	−	Stem
B. thuringiensis BSA123	Rod	+	+	+	+	+	+	+	+	+	−	+	−	+	−	Stem
B. paramycoides LS11	Rod	+	−	+	+	+	+	+	+	+	−	+	+	+	−	Stem
P. saponiphilia J4R	Rod	−	−	+	+	+	+	+	+	+	+	+	−	+	−	Stem
B. cereus VEJU7080	Rod	+	+	+	+	−	+	+	+	+	+	−	−	−	−	Stem
Pseudomonas sp. FOBS21	Rod	−	+	−	+	+	+	+	+	+	+	−	−	+	−	Stem

+, positive; –, negative; SP, shape; GR, Gram reaction; Clr, color; CT, citrate; Gel, gelation; OX, oxidase; SH, starch hydrolysis; H2S, hydrogen sulfide; VP, vogue Proskauer; MR, methyl red; NT, nitrate; IND, indole; CS, casein; UR, urease; MT, motility.

One ml of the cultured medium was constantly withdrawn at 24-h intervals and assayed for IAA production by Salkowski's reagent. Furthermore, similar incubation conditions were used to monitor the effects of other parameters under the same IAA assay conditions. The ability of bacterial isolates to utilize carbon as substrate and their effects on IAA production was tested using 5 sugars; namely, maltose, fructose, sucrose, glucose, and galactose, at different concentrations (1, 3, and 5%) as described by Khan et al. (2020). Additionally, the ability of bacterial isolates to utilize nitrogenous-base compounds, such as peptone, potassium nitrate, casein, yeast extract, and urea as substrates were tested at different concentrations of 1, 3, and 5% (Chandra et al., 2018). The pH of the medium ranging from 4 to 10 was examined. The pH of the IAA-producing medium was adjusted using 1 M of NaOH or HCl. IPM was optimized at varied temperatures; 25, 30, 37, 45, and 60°C. After sterilization, the IPM for each optimized parameter was allowed to cool, inoculated with 0.1 ml (10^6 CFU/ml) of each selected bacterium, and incubated at 28 ± 2°C for 7 days on a rotary incubator machine at 180 rpm. After incubation, the supernatant was subjected to an IAA assay and IAA concentration was measured at 630 nm for pH and temperature, and 590 nm for carbon and nitrogen, respectively, using a spectrophotometer (Thermo Spectronic; Meck, South Africa). The common reagents used for the plant growth-promoting screening were procured from Merck Chemicals (Pty) Ltd, Gauteng, South Africa, and Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

Molecular Identification of Plant Growth-Promoting Endophytic Bacteria

DNA Extraction Process

The genomic content of pure bacterial isolates was extracted using a commercial Quick-DNATM Miniprep Kit specific for fungi or bacteria (Zymo Research, Irvine, CA, USA; Cat. No. D6005), following the manufacturer's guide. The quantity of the extracted DNA (ng/μl) was measured using a NanoDrop ND-2000 UV-Vis spectrophotometer (ThermoFisher Scientific, USA) and stored at −80°C.

Polymerase Chain Reaction and Sequence Analysis

The determination of 16S rRNA nucleotide sequences of the identified bacterial isolates was achieved using the amplified PCR products. The specific forward and reverse primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5′-TACGGTTACCTTGTTACGACTT-3') were purchased from Inqaba Biotechnological Industrial (Pty) Ltd, Pretoria, South Africa. A total of 25 μl reaction volume for each bacterial isolate composed of 12.5 μl OneTaq 2X MasterMix with the Standard Buffer, 1 μM for each primer, ~5 ng genomic DNA, and 9.5 μl nuclease-free water were used for PCR amplification on DNA Engine DYAD™ Peltier Thermal Cycler

TABLE 1B | Sugar utilization by endophytic bacteria from sunflowers.

Isolate code.	Sugars used									Isolation sources
	Mal	Gal	Glu	Arab	Suc	Fru	Raff	Mann	Xyl	
B. cereus SFR35	+A	+AG	+Ag	+A	+Ag	+A	+A	+A	+A	Root
B. wiedmannii FTL29	+A	+Ag	+Ag	+A	+Ag	+A	–a	+A	+A	Root
Bacillus sp. CAL14	+A	+Ag	+AG	+A	+Ag	+A	+A	+A	+A	Root
B. cereus T4S	+A	+AG	+AG	+A	+Ag	+A	+A	+A	+A	Root
Pseudomonas lini BS27	+A	+Ag	+AG	+A	+Ag	+A	+A	+A	+A	Root
S. indicatrix BOVIS40	+A	+Ag	+Ag	+A	+Ag	+A	–a	+A	+A	Root
S. maltophilia JVB5	+A	+Ag	+Ag	+A	+Ag	+A	+A	+A	+A	Root
B. albus TSN29	+A	+Ag	+AG	+A	+Ag	+A	–a	+A	+A	Root
B. cereus BLBS20	+A	+AG	+Ag	+A	–ag	+A	+A	+A	+A	Root
B. thuringiensis SFL02	+A	+Ag	+Ag	+A	+Ag	+A	+A	+A	+A	Root
										Ten (10) bacterial isolates
S. maltophilia PK60	+A	+Ag	+Ag	+A	+Ag	+A	+A	+A	+A	Stem
B. subtilis VS52	+A	+AG	+AG	+A	+AG	+A	–a	+A	+A	Stem
B. thuringiensis BAAG44	+A	–ag	+AG	+A	+Ag	+A	+A	+A	+A	Stem
B. pseudomycoides SFS19	+A	–ag	+Ag	+A	–ag	+A	+A	+A	+A	Stem
B. toyonensis OLT2020	+A	+AG	+AG	+A	+AG	+A	–a	+A	+A	Stem
B. thuringiensis BSA123	+A	+AG	+AG	+A	+Ag	+A	+A	+A	+A	Stem
B. paramycoides LS11	+A	+Ag	+Ag	+A	+Ag	+A	+A	+A	+A	Stem
P. saponiphilia J4R	+A	–ag	+Ag	+A	+Ag	+A	+A	+A	+A	Stem
B. cereus VEJU7080	+A	+AG	+AG	+A	+Ag	+A	+A	+A	+A	Stem
Pseudomonas sp. FOBS21	+A	+AG	+Ag	+A	+Ag	+A	+A	+A	+A	Stem
										Ten (10) bacterial isolates

ag, no acid and gas production; AG, acid and gas production; +, positive; –, negative; Suc, sucrose; Glu, glucose; Fru, fructose; Arab, arabinose; Mann, mannose; Xyl, xylose; Mal, maltose; Raff, raffinose.

(BIO-RAD, USA, C1000 TouchTM). The PCR cycle parameters were programmed as follows: initial denaturation at 94°C for 5 min; 35 cycles of amplification. Also, the denaturation for 30 s at 94°C, annealing for 30 s at 50°C, extension for 1 min at 68°C; and a final overall extension for 10 min at 68°C. After running PCR, the PCR product was determined on agarose gel electrophoresis. Subsequently, the gel was carefully removed, and confirmation of the expected size of the product was visualized on a UV trans-illuminator. The resulting outcome was captured in a Chemidoc^{TM} imaging system (BIO-RAD Laboratories, California, USA). Finally, 20 µl of the PCR product for each bacterial isolate was placed in an ice-box pack and sent for sequencing at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria of South Africa. 16S rRNA sequences for each bacterium were submitted to GenBank on the NCBI online server and were assigned with accession numbers. The twenty identifiable endophytic bacteria deposited on GenBank of the National Center for Biotechnology Information (NCBI) web server can be accessed from the links provided in the data availability section.

Sequence Alignment and Construction of Phylogenetic Tree

The Basic Local Alignment Search Tools (BLAST) program of the nucleotide sequences on the National Center for Biotechnology Information (NCBI) was employed to determine bacterial isolate sequence similarities and identities. The sequenced data were further analyzed by subjecting to multiple sequence alignment by ClustalW using a Bio-Edit program. MEGA-X online program was used to construct the phylogenetic tree from the resulting ClustalW sequences and the maximum likelihood method of the taxa with the Tamura-Nei model. The phylogeny test of the aligned sequences was achieved by the bootstrap method (Tamura et al., 2013).

Sunflower Seed Inoculation and *in vitro* Effect of Endophytic Bacteria on Seedling Growth

The effectiveness of sunflower seed inoculation was performed based on the methods described by Ullah et al. (2017). The bacterial inoculum size in LB broth at 24-h incubation was standardized to 0.5 (10^6 CFUml^{-1}) at OD_{600}. The three bacterial isolates, namely, *S. indicatrix* BOVIS40, *B. cereus* T4S, and *S. maltophilia* JVB5 were selected based on the most promising plant growth-promoting properties. A seed inoculation assay was used to facilitate bacterial adherence to the disinfected sunflower seeds. Cleaning of the seeds was performed by washing in sterile distilled water to remove floating-unhealthy seeds and dirt, and disinfected in 70% ethanol for 3 min, followed by 3% hypochlorite for 3 min, then immersed in 70% alcohol for 30 s, and lastly rinsed 5 times with sterile distilled water. Prepared LB broth inoculated with fresh bacterial culture was incubated at room temperature in a rotary incubator machine (SI-600, LAB

TABLE 2 | Identification of endophytic bacteria based on 16S rRNA gene sequences.

Strain	Identity	% Similarity	GBAN	Homologous accessions	Isolation sources
SFR35	*B. cereus*	100	MW265416	MW092893	Root
FTL29	*B. wiedmannii*	99	MW265418	MZ292345	Root
CAL14	*Bacillus* sp.	95	MW265422	MK554656	Root
T4S	*B. cereus*	100	MW265423	MW115619	Root
BS27	*Pseudomonas lini*	94	MW265425	JQ833637	Root
BOVIS40	*S. indicatrix*	100	MW265419	MW116366	Root
JVB5	*S. maltophilia*	100	MW265431	MT605498	Root
TSN29	*B. albus*	99	MW265420	MT636856	Root
BLBS20	*B. cereus*	100	MW265427	MT543036	Root
SFL02	*B. thuringiensis*	99	MW265413	MK743981	Root
PK60	*S. maltophilia*	97	MW265415	MK588914	Stem
VS52	*B. subtilis*	100	MW265429	MT613731	Stem
BAAG44	*B. thuringiensis*	98	MW265424	MK743981	Stem
SFS19	*B. pseudomycoides*	99	MW265430	MK999393	Stem
OLT2020	*B. toyonensis*	100	MW265417	MT605503	Stem
BSA123	*B. thuringiensis*	100	MW265426	JX994096	Stem
LS11	*B. paramycoides*	100	MW265414	MW090883	Stem
J4R	*P. saponiphilia*	100	MW265421	MT501808	Stem
VEJU7080	*B. cereus*	99	MW265428	MH231418	Stem
FOBS21	*Pseudomonas* sp.	100	MW261910	MT561438	Stem

GBAN, GenBank accession number.

Companion, Korea) at 180 rpm for 24 h. The bacterial cells in the broth culture were harvested by centrifugation at 8,000 × g for 10 min to obtain the pelletized cells and then washed in 0.85% normal saline solution. The centrifugation and washing of the pellets were performed under sterile conditions. The surface-sterilized seeds were suspended in a bacterial suspension containing 1% (v/w) carboxymethyl cellulose (CMC) as an adhesive (binder) in a 250 ml flask for 60 min. The seeds suspended in sterile distilled water containing 1% (v/w) CMC without bacterial inoculum served as control.

Under sterile conditions, 10 coated seeds (of each bacterial strain) were placed inside Petri dishes lined with moistened sterile absorbent cotton and sealed with parafilm. The plates were kept at 28°C in the growth chamber and seedling growth was monitored daily for 5 days. Each treatment was performed in triplicates. The plates were carefully taken out for seedling growth assessment. The fresh weight and dry weight of seedlings after oven drying at 60°C were measured. The seedling's fresh and dry weight obtained was expressed in gram (g) per triplicate.

Statistical and Data Analysis

The analysis of data from this study was performed using SPSS - Statistical Package for the Social Sciences (version 6.0) and Microsoft Excel. A significant difference among the treatment groups was calculated using ANOVA - one-way analysis of variance. The mean difference was determined by Duncan's tests at a 5% level of significance. Data obtained were presented as mean ± standard deviation. All experiments were performed in triplicates.

Data Availability

The sequenced dataset associated with this study can be accessed at https://www.ncbi.nlm.nih.gov/nuccore/MW261910, and https://www.ncbi.nlm.nih.gov/nuccore/?term=MW265413:MW265431[accn].

RESULTS

Bacterial Endophytes Isolation and Biochemical Characterization

A total of twenty-seven bacterial isolates from the roots and twenty-three bacterial isolates from the stems were isolated and characterized. However, ten (10) bacteria isolated from the stems and ten (10) bacteria isolated from the roots were further characterized and selected for plant growth-promoting screening based on the distinct morphological characterization, Gram staining, and biochemical tests (see **Table 2** below). The cultural, biochemical characterization, and sugar utilization by endophytic bacteria from sunflowers were presented in **Tables 1A**,**B**.

Based on Gram reaction, 70% of the bacterial isolates were Gram-positive, whereas 30% were Gram-negative. The Gram-negative bacterial endophytes identified include *Pseudomonas* sp. FOBS21, *S. maltophilia* PK60/JVB5, *S. indicatrix* BOVIS40, *P. saponiphilia* J4R, and *P. lini* BS27, while Gram-positive bacterial isolates include the genus *Bacillus*. All the bacterial isolates were rod-like with positive results for oxidase, hydrogen sulfide production, Voges-Proskauer, and methyl red. *B. thuringiensis* BSA123 was positive for the citrate test, while nine isolates were positive for gelatin liquefaction. *B. cereus* SFR35 utilizes indole

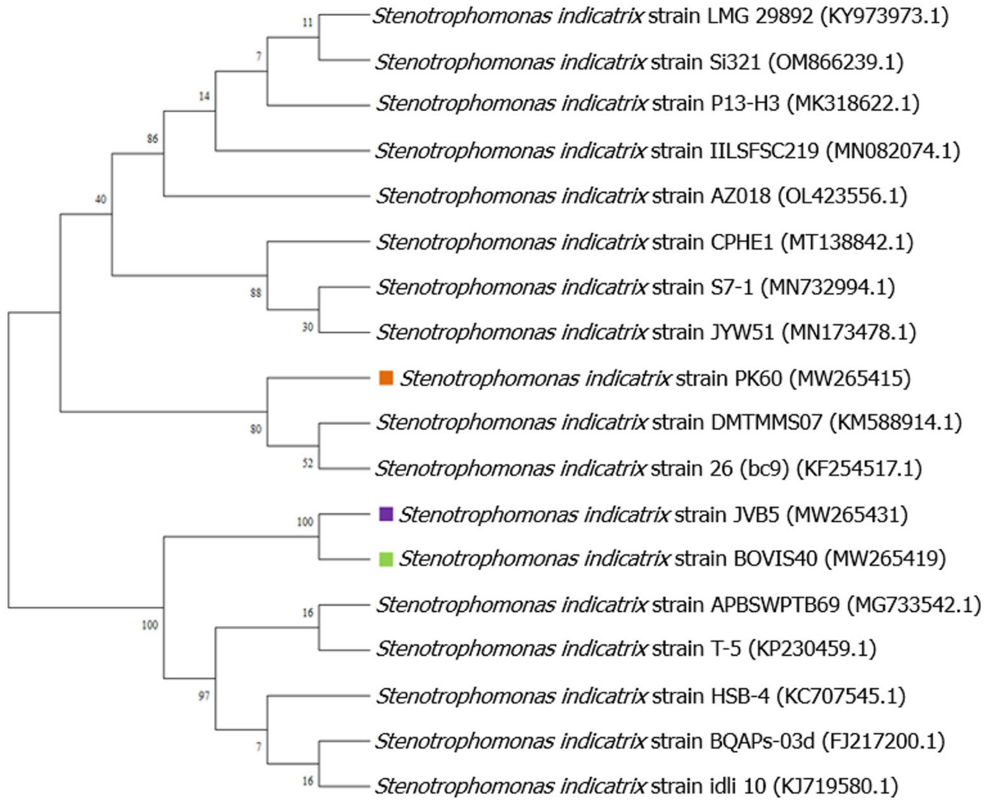

FIGURE 1 | Evolutionary relationships of taxa tree based on partial 16S rRNA sequences using maximum likelihood-based on the Tamura-Nei model showing relationships between the endophytic *Stenotrophomonas* species and its closely related strains from NCBI GenBank.

while *B. thuringiensis* BSA123 showed a positive reaction to casein hydrolysis. For nitrate utilization, eight bacterial isolates produced gas (N_2), while sixteen reduced nitrates. All the isolates were urease negative. For sugar fermentation tests, all bacterial isolates fermented glucose, fructose, arabinose, mannitol, xylose, and maltose, respectively. Based on molecular identification, *Bacillus* species were the most common identifiable bacteria in the stem and root samples (**Table 2**). Each bacterial strain was designated as SFR35, FTL29, T4S, CAL14, BS27, BOVIS40, JVB5, TSN29, BLBS20, SFL02, PK60, VS52, BAAG44, SFS19, OLT2020, BSA123, LS11, J4R, VEJU7080 and FOBS21 (**Table 2**).

Identification of Selected Endophytic Bacteria by 16S rRNA Gene Sequencing and Phylogeny Analysis

The identification of endophytic bacteria based on 16S rRNA gene sequences was presented in **Table 2**. The phylogeny information of the identifiable bacteria genera and bacterial sequences of related genera recovered from the GenBank database is shown in **Figures 1–3**.

Plant Growth-Promoting Traits

The plant growth-promoting traits of endophytic bacteria from sunflowers are presented in **Table 3**. The qualitative screening revealed the ability of the bacterial isolates to produce ammonia, siderophore, exopolysaccharide, hydrogen cyanide, IAA, and solubilize phosphate. Nine bacterial isolates exhibited high siderophore production, while five exhibited medium and six exhibited low activity for the siderophore production. The quantitative results revealed a high siderophore value of 87.73 % by *B. cereus* T4S.

Screening of Extracellular Enzymes

The qualitative screening of endophytic bacteria for enzyme production; namely, amylase, cellulase, xylanase, mannanase, and protease was presented in **Table 4**. *S. indicatrix* BOVIS40, *B. weidmannii* FTL29, *B. subtilis* VS52, and *B. thuringiensis* BSA123, exhibited a positive reaction to all enzymes assayed. Except for amylase, bacterial isolates *B. cereus* VEJU7080 and *B. cereus* T4S were positive for other screened enzymes. Summarily, bacterial isolated designated *B. weidmannii* FTL29, *B. albus* TSN29, *B. thuringiensis* BSA123, and *B. thuringiensis* BAAG44 displayed amylase, xylanase, mannanase, and protease production tendencies, respectively.

Optimization of Process Parameters for IAA Production

All the bacterial isolates displayed varied IAA activities at different L-tryptophan concentrations. The medium

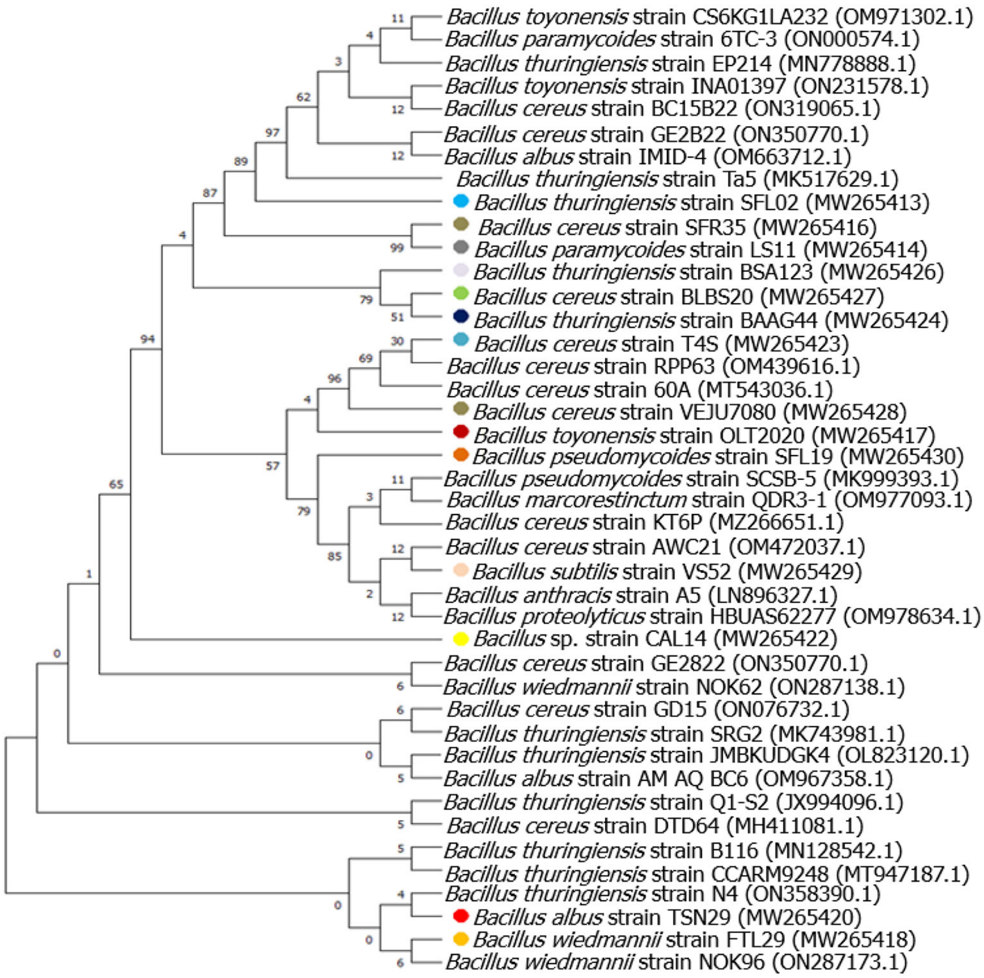

FIGURE 2 | Evolutionary relationships of taxa tree based on partial 16S rRNA sequences using maximum likelihood-based on the Tamura-Nei model showing relationships between the endophytic *Bacillus* species and its closely related strains from NCBI GenBank.

supplemented with L-tryptophan yielded higher IAA production compared to the control (**Figure 4**). Bacterial strains, *S. indicatrix* BOVIS40, *B. cereus* T4S, and *S. maltophilia* JVB5 from the roots of sunflower exhibited high IAA production compared with other isolates. In contrast, the lowest IAA production was observed in *B. cereus* SFR35 compared with the control.

Effect of Incubation Time and pH on IAA Production

The time course for IAA production by the bacterial isolates was presented in **Figure 5**. An increase in IAA production with an increase in incubation time was recorded. Optimum IAA production was attained at 168 h and beyond this point, there was a decline. The optimum IAA production of 16.94, 11.76, and 9.92 µg/ml at 168 h of incubation were recorded by *S. maltophilia* JVB5, *B. cereus* T4S, and *S. indicatrix* BOVIS40, respectively. The results of IAA produced by the bacterial isolates monitored between pH 4–10 were presented in **Figure 6**. The IAA production increased from pH 4–7, and beyond this point, there was a decline from pH 8–10. *S. indicatrix* BOVIS40 showed maximum IAA production of 25.36 µg/ml, followed by *B. cereus* T4S of 12.34 µg/ml and *S. maltophilia* JVB5 of 5.46 µg/ml at pH 7. Similarly, maximum IAA production of 11.83 µg/ml by *S. maltophilia* JVB5 at pH 6, 10.74 µg/ml at pH 8, with the least IAA production of 2.83 µg/ml at pH 4 were obtained. At pH 9 and 10, no significant difference was observed in the IAA production of *B. cereus* T4S and *S. maltophilia* JVB5.

Effect of Temperature on IAA Production

The effect of incubation temperature on IAA production by endophytic bacteria ranging from 25 to 60°C was presented in **Figure 7**. Maximum IAA production of 34.40 µg/ml by *B. cereus* T4S at 37°C was recorded. The amount of IAA produced at temperatures 45 and 60°C was lower compared with other temperatures. Bacterial strains showed an increase in IAA production from temperature 25–37°C before it declined. At 30 and 37°C, there was no significant difference in the IAA production by *S. maltophilia* JVB5.

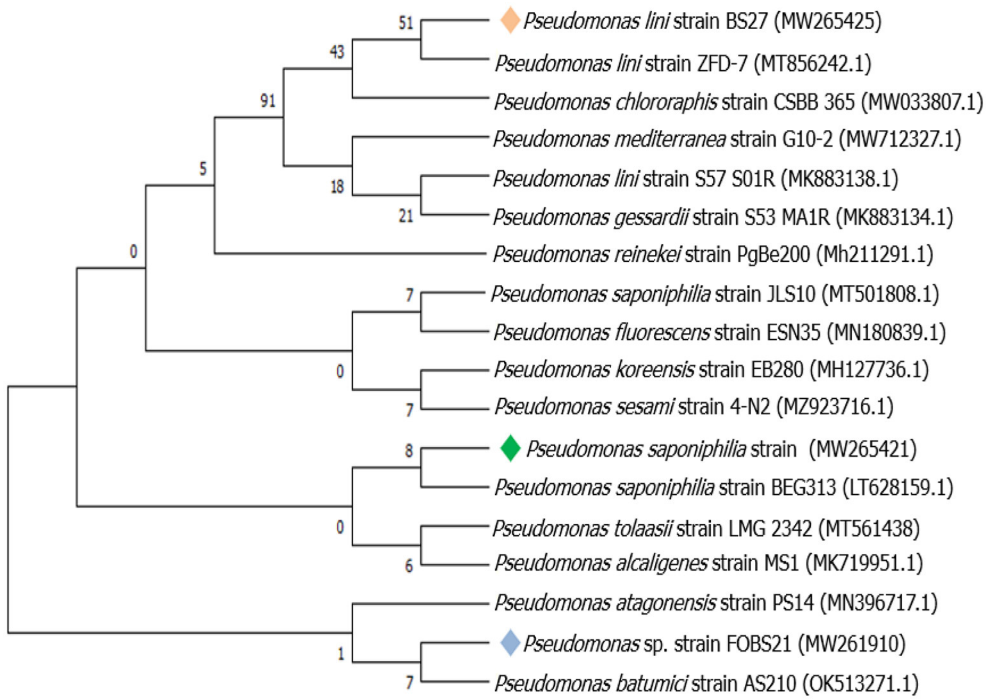

FIGURE 3 | Evolutionary relationships of taxa tree based on partial 16S rRNA sequences using maximum likelihood-based on the Tamura-Nei model showing relationships between the endophytic *Pseudomonas* species and its closely related strains from NCBI GenBank.

Effect of Carbon Source on IAA Production

Figure 8 showed the effect of carbon source on IAA production by endophytic bacteria from sunflowers. The amount of IAA produced in the growth medium varied with the sugar concentration. A maximum IAA production of 23.36 and 20.72 µg/ml were recorded from *S. maltophilia* JVB5 and *B. cereus* T4S at 5% sucrose and 3% glucose, respectively.

Effect of Nitrogen Source on IAA Production

The effect of nitrogen source on IAA production by endophytic bacteria was presented in **Figure 9**. All the bacterial isolates exhibited IAA production >20 µg/ml in a medium amended with casein and yeast extracts. Maximum IAA production of 19.31 and 17.70 µg/ml were recorded from *S. maltophilia* JVB5 at 3 and 5% peptone. Similarly, *B. cereus* T4S exhibited a maximum IAA production of 17.94 µg/ml at 3% peptone. A high IAA production of 14.97 µg/ml was obtained from *B. cereus* T4S at 5% potassium nitrate. There was no significant difference in the IAA production by *S. maltophilia* JVB5 at 5% yeast extract. Similar results were obtained of *S. indicatrix* BOVIS40 and *B. cereus* T4S at 1% and *B. cereus* T4S and *S. maltophilia* JVB5 at 3% yeast extract, respectively. An increase in the amount of IAA production with an increase in yeast extract concentration was recorded. *S. indicatrix* BOVIS40 exhibited an IAA production increase from 21.71 to 45.34 µg/ml, while *B. cereus* T4S showed an increase in IAA production from 21.00 to 42.89 µg/ml and *S. maltophilia* JVB5 from 25.58 to 45.82 µg/ml, respectively. There was no significant difference in the amount of IAA produced by *S. maltophilia* JVB5 at 1 and 5% urea. *B. cereus* T4S displayed high IAA production of 5.30 µg/ml at 1% urea.

In vitro Effect of IAA-Producing Endophytic Bacterial Isolates on Sunflower Seedling Growth

The effect of IAA-producing bacteria *S. indicatrix* BOVIS40, *B. cereus* T4S, and *S. maltophilia* JVB5 on sunflower seeds by inoculation were tested (**Table 5**). The percent increase of the number of lateral roots of 5.13, 6.97, and 1.58% were obtained from the inoculated sunflower seedling with bacterial strain *S. indicatrix* BOVIS40, *B. cereus* T4S, and *S. maltophilia* JVB5 compared to the un-inoculated sunflower seeds (control). A significant difference in the shoot and root length of sunflower seedlings compared with the control was recorded. The percentage increase of 9.09, 3.7, and 20.88% of root length and 6.23, 5.23, and 2.88% of shoot length were obtained from the inoculated sunflower seedlings compared with the un-inoculated sunflower seeds.

DISCUSSION

The need to ensure a safe environment for improved crop production has been a major concern, as insights into plant-microbe interactions remain crucial in developing eco-friendly agriculture. Due to the complex dynamics of biodiversity in the plant root environment, this can facilitate the recruitment

TABLE 3 | Plant growth-promoting traits of endophytic bacteria.

	Qualitative						Quantitative		
Strain	**HCN**	**AM**	**SDR**	**IAA**	**EPS**	**PS**	**PSI (mm)**	**%PS (v/v)**	**%SDR (v/v)**
B. cereus SFR35	++	+	+	+	++	+	4.09 ± 0.08^{l}	29.96 ± 0.03^{e}	64.93 ± 0.04^{k}
B. wiedmannii FTL29	+	+	++	+	+++	+	2.65 ± 0.04^{e}	30.96 ± 0.03^{e}	15.30 ± 0.04^{d}
Bacillus sp. CAL14	++	+	++	+	+++	+	3.14 ± 0.05^{j}	33.83 ± 0.04^{g}	52.96 ± 0.04^{i}
B. cereus T4S	++	+	+++	+	+++	+	2.62 ± 0.02^{e}	30.54 ± 0.05^{e}	87.73 ± 0.05^{q}
P. lini BS27	++	+	+	+	++	+	3.07 ± 0.12^{hij}	30.62 ± 0.03^{e}	36.90 ± 0.02^{f}
S. indicatrix BOVIS40	++	+	+++	+	+++	+	2.10 ± 0.10^{a}	32.81 ± 0.18^{g}	77.33 ± 0.05^{n}
S. maltophilia JVB5	++	+	+++	+	+++	+	2.95 ± 0.05^{g}	32.20 ± 0.17^{g}	79.81 ± 0.17^{o}
B. albus TSN29	++	+	+	+	+	+	3.50 ± 0.02^{k}	26.11 ± 0.03^{d}	80.50 ± 0.02^{p}
B. cereus BLBS20	+	+	+	+	+	+	2.50 ± 0.04^{d}	31.82 ± 0.14^{f}	0.53 ± 0.03^{a}
B. thuringiensis SFL02	+	+	+++	+	+	+	2.41 ± 0.01^{c}	26.48 ± 0.03^{c}	77.72 ± 0.04^{n}
S. maltophilia PK60	++	+	+++	+	+++	+	3.03 ± 0.06^{ghi}	33.48 ± 0.03^{g}	58.50 ± 0.02^{j}
B. subtilis VS52	++	+	+++	+	+	+	2.82 ± 0.01^{f}	33.43 ± 0.04^{g}	17.73 ± 0.04^{e}
B. thuringiensis BAAG44	++	+	+++	+	++	+	2.43 ± 0.03^{cd}	15.76 ± 0.21^{a}	70.12 ± 0.03^{m}
B. pseudomycoides SFS19	+	+	+++	+	+++	+	2.17 ± 0.03^{a}	27.64 ± 0.04^{d}	68.89 ± 0.09^{l}
B. toyonensis OLT2020	+	+	+	+	++	+	3.01 ± 0.02^{gh}	22.64 ± 0.03^{b}	5.72 ± 0.02^{b}
B. thuringiensis BSA123	++	+	++	+	++	+	2.79 ± 0.03^{f}	24.95 ± 0.04^{c}	64.92 ± 0.04^{k}
B. paramycoides LS11	++	+	++	+	+	+	2.80 ± 0.03^{f}	29.75 ± 0.22^{e}	44.53 ± 0.03^{g}
P. saponiphilia J4R	++	+	+	+	+	+	3.11 ± 0.02^{ij}	33.24 ± 0.05^{g}	12.91 ± 0.01^{c}
B. cereus VEJU7080	++	+	+++	+	+	+	2.66 ± 0.02^{e}	24.55 ± 0.04^{c}	46.10 ± 0.02^{h}
Pseudomonas sp. FOBS21	+	+	++	+	+	+	2.31 ± 0.02^{b}	31.32 ± 0.02^{b}	53.34 ± 0.04^{i}

IAA, indole-3-acetic acid; AM, ammonia; HCN, hydrogen cyanide; EPS, exopolysaccharide; %PS, percentage of phosphatase; %SDR, percentage of siderophore production. The values of triplicate readings represented as mean ± standard deviation with different alphabets down the column show a significant difference.

of soil-root microbes to established microbial biomass in the endosphere (Liu et al., 2021). Plants harbor diverse agriculturally important endophytic microbes with notable plant growth-promoting traits and their exploration has been proven efficient in enhancing crop yield (Alkahtani et al., 2020). The ability of endophytic microbes to withstand drought stress or climate-induced abiotic stress for plant survival and nutrition can suggest their future exploration as a suitable candidate for formulating bioinoculants for sustainable agriculture (Khalil et al., 2021). The presence of endophytic bacteria in host plants and their ability to synthesize growth hormones can significantly enhance seedling growth, development, and elongation of lateral roots and cell differentiation (Shahzad et al., 2017).

In this study, the combination of culturing and molecular techniques in the characterization of sunflower roots and stem-associated endophytic bacteria has been reported (Tiwari and Thakur, 2014; Bahmani et al., 2021; Shah et al., 2022). The biochemical characterization reflected the most identifiable Gram-positive compared to the Gram-negative bacterial isolates. The screened twenty bacterial isolates showed multifunctional PGP traits. The bacteria identified in this study showed similarities to the previous studies by Ambrosini et al. (2016) and Schmidt et al. (2021), who reported similar bacteria from sunflowers. A study by Forchetti et al. (2007) reported the isolation of *Bacillus* from sunflower plants. Recent genomics has revealed plant growth promotion and stress tolerance attributes of *Stenotrophomonas* strain 169 (Ulrich et al., 2021). The bacteria identified agreed with the findings of Ambrosini et al. (2012), who isolated Stenotrophomonas spp. and Pseudomonas spp. from the root of sunflower.

The ability of endophytic bacteria to solubilize phosphate was evident from the work of researchers (Khamwan et al., 2018; Sánchez-Cruz et al., 2019; Alkahtani et al., 2020). Several endophytic bacterial genera, such as *Stenotrophomonas, Bacillus,* and *Pseudomonas* have been reported as phosphate solubilizers (Pandey et al., 2013). In this study, all the bacterial isolates displayed phosphate-solubilizing traits. Hence, these bacteria with greater potential can be harnessed as bio-input in both present and future agriculture. Diverse phosphate-solubilizing endophytic bacteria have been reported to increase phosphate levels in the soil (Alkahtani et al., 2020; Varga et al., 2020). Shahid et al. (2015) reported phosphate-solubilizing endophytic *Bacillus* sp. Ps-5 and *Alcaligenes faecalis* Ss-2, contribute to the sunflower yield. The results obtained from this study corroborate with Pandey et al. (2013) and Vandana et al. (2021), who reported phosphate-solubilizing endophytic *Stenotrophomonas*, *Bacillus,* and *Pseudomonas* from the root of sunflower, and soybean. The phosphate solubilization potential of bacterial isolates might depend on suitable growth conditions, genetic make-up, and limited nutrient supply (Youseif, 2018). Furthermore, the results here compared to previous studies confirmed the phosphate-solubilizing potential of sunflower-associated endophytic bacteria with promises in ensuring the bioavailability of soluble minerals in soils for plant nutrition.

TABLE 4 | Qualitative screening of endophytic bacteria for enzyme production.

Isolate	Zone of clearance (mm)				
	AM	XL	PR	CL	MN
B. cereus SFR35	0.00 ± 00[a]	0.00 ± 00[a]	36.24 ± 0.40[m]	44.03 ± 0.06[a]	43.06 ± 0.05[g]
B. wiedmannii FTL29	20.05 ± 0.05[c]	40.02 ± 0.03[f]	18.03 ± 0.03[e]	47.06 ± 0.05[b]	42.04 ± 0.04[f]
Bacillus sp. CAL14	0.00 ± 00[a]	0.00 ± 00[a]	10.06 ± 0.06[b]	59.05 ± 0.05[g]	0.00 ± 00[a]
B. cereus T4S	0.00 ± 00[a]	41.02 ± 0.04[g]	35.05 ± 0.04[l]	57.02 ± 0.02[e]	45.00 ± 0.01[h]
P. lini BS27	0.00 ± 00[a]	32.96 ± 0.06[c]	30.00 ± 0.11[i]	58.07 ± 0.11[f]	18.02 ± 0.55[b]
S. indicatrix BOVIS40	44.05 ± 0.05[e]	35.04 ± 0.04[e]	16.03 ± 0.02[d]	50.10 ± 0.10[d]	43.05 ± 0.05[g]
S. maltophilia JVB5	0.00 ± 00[a]	0.00 ± 00[a]	25.06 ± 0.05[d]	58.08 ± 0.11[f]	0.00 ± 00[a]
B. albus TSN29	0.00 ± 00[a]	0.00 ± 00[a]	0.00 ± 00[a]	48.04 ± 0.05[c]	54.04 ± 0.05[k]
B. cereus BLBS20	0.00 ± 00[a]	0.00 ± 00[a]	0.00 ± 00[a]	69.10 ± 0.10[i]	0.00 ± 00[a]
B. thuringiensis SFL02	0.00 ± 00[a]	47.05 ± 0.05[i]	0.00 ± 00[a]	60.03 ± 0.03[h]	30.05 ± 0.06[e]
S. maltophilia PK60	18.04 ± 0.04[b]	0.00 ± 00[a]	31.01 ± 0.03[j]	59.03 ± 0.06[g]	18.04 ± 0.55[b]
B. subtilis VS52	20.05 ± 0.04[c]	35.04 ± 0.04[d]	22.03 ± 0.06[f]	59.02 ± 0.07[g]	51.06 ± 0.06[j]
B. thuringiensis BAAG44	0.00 ± 00[a]	0.00 ± 00[a]	30.06 ± 0.06[i]	58.06 ± 0.06[f]	18.02 ± 0.04[b]
B. pseudomycoides SFS19	0.00 ± 00[a]	0.00 ± 00[a]	0.00 ± 00[a]	58.02 ± 0.02[f]	0.00 ± 00[a]
B. toyonensis OLT2020	0.00 ± 00[a]	43.05 ± 0.06[h]	0.00 ± 00[a]	60.03 ± 0.03[h]	20.05 ± 0.05[c]
B. thuringiensis BSA123	18.01 ± 0.05[b]	40.06 ± 0.06[f]	35.02 ± 0.03[l]	47.04 ± 0.05[b]	42.02 ± 0.04[f]
B. paramycoides LS11	25.04 ± 0.04[d]	0.00 ± 00[a]	29.04 ± 0.04[h]	58.04 ± 0.05[f]	45.03 ± 0.04[h]
P. saponiphilia J4R	0.00 ± 00[a]	0.00 ± 00[a]	33.10 ± 0.10[l]	59.08 ± 0.10[g]	18.06 ± 0.06[b]
B. cereus VEJU7080	0.00 ± 00[a]	32.03 ± 0.03[b]	15.03 ± 0.05[c]	48.04 ± 0.04[c]	50.01 ± 0.02[i]
Pseudomonas sp. FOBS21	0.00 ± 00[a]	0.00 ± 00[a]	32.03 ± 0.03[k]	50.24 ± 0.40[d]	25.02 ± 0.03[d]

AM, amylase; CL, cellulose; XL, xylanase; MN, mannanase; PR, protease. The values of triplicate readings represented as mean ± standard deviation with different alphabets down the column show a significant difference.

Siderophore-producing microbes can protect plants by mitigating the effect of induced biotic and abiotic stresses (Ferreira et al., 2019). In this study, endophytic bacteria displayed varied siderophore production. The differences observed may be due to the bacterial viability and genetic make-up. Siderophore producing ability of endophytic bacteria associated with *Vitis vinifera* has been reported to increase mineral elements in the soil (Andreolli et al., 2016). Pourbabaee et al. (2018) reported the potential contribution of siderophore-producing bacteria to the growth and Fe ion concentration of sunflower under water stress.

The HCN production by bacteria can inhibit cell metabolism and electron transport chain, thus causing cell death. The HCN and siderophores production by endophytic bacteria can provide a competitive advantage by exploring them as biocontrol agents in plant disease suppressiveness (Igiehon et al., 2019). The results obtained in this study revealed HCN production by the endophytic bacteria. The ability of endophytic bacteria to produce ammonia with the underlining antibiosis activities has been reported (Khan et al., 2020). All the bacterial isolates produce ammonia and HCN, thus suggesting their possible use as a biocontrol agent. The HCN and ammonia production by the bacterial isolates conformed with the findings of Pandey et al. (2013) and Moin et al. (2020) who reported HCN, ammonia, and volatile antifungal metabolites biosynthesis by the endophytic bacterium *Pseudomonas* isolated from healthy sunflower plants. Additionally, the production of exopolysaccharides, signal molecules, multilayered cell wall structures, extracellular enzymes, and stress-resistant endospores by *Bacillus* spp., however, can contribute to their survival and ecological functions in diverse environments (Lyngwi et al., 2016).

Endophytic microbes can stand as a potential source of extracellular enzymes for industrial purposes due to catalytic activity, thermostability, low cost, organic substrates availability, etc. (Toghueo and Boyom, 2021). Screening of extracellular enzymes, such as cellulases, proteases, xylanases, chitinases, and xylanases from plant microbes has been documented (Alkahtani et al., 2020; Blibech et al., 2020). The substrate level and growth conditions may influence the enzyme production ability of the bacterial isolates in the growth medium (Yadav, 2017). With the biotechnological views, sunflower endophytic bacteria can be harnessed as a source of enzymes in the degradation of complex organic compounds and derivation of desirable bio-products.

IAA is considered the most important phytohormone that enhances plant root development and the rate of nutrient absorption for plant growth promotion (Ahmad et al., 2020). The ability of microbes to produce growth hormones can underline their multifunctional effects on improving agricultural productivity (Choudhury et al., 2021). Different bacterial species have been implicated in the synthesis of IAA depending on their ability to utilize the precursory substance L-tryptophan in the growth medium (Mustafa et al., 2018). The increase in the amount of IAA produced by the bacterial isolates in the IAA production medium (IPM) conformed with the findings

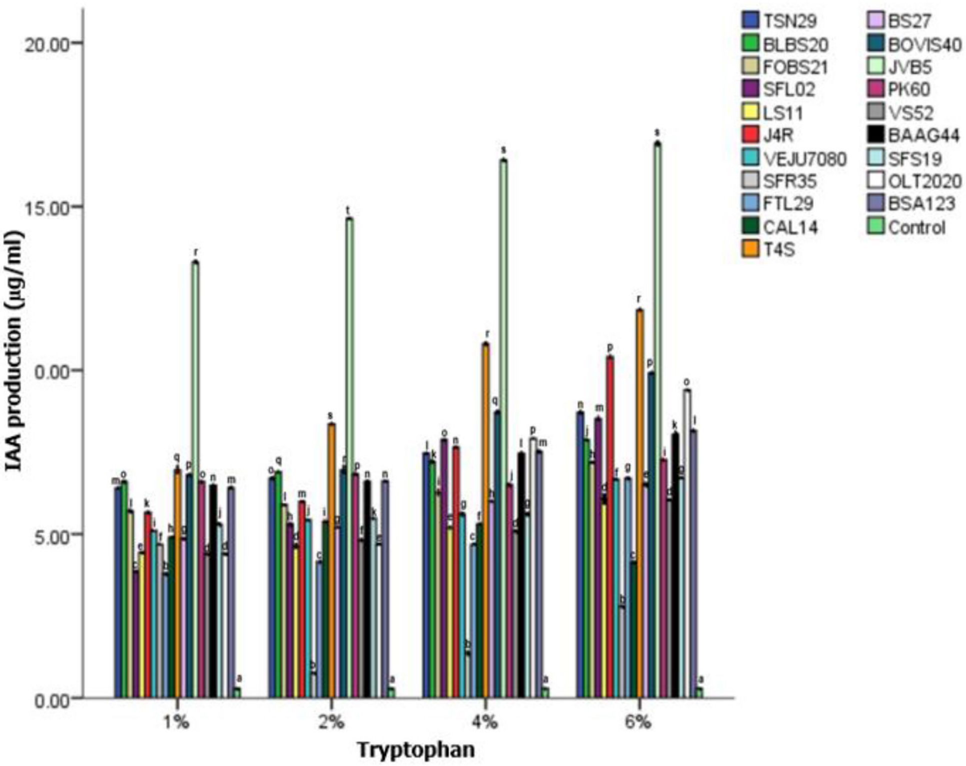

FIGURE 4 | Qualitative screening of endophytic bacteria for indole acetic acid production. Bacterial isolate codes are represented in **Table 2** and different alphabets indicate significant differences in triplicate readings. SFR35, *B. cereus*; FTL29, *B. wiedmannii*; CAL14, *Bacillus* sp.; T4S, *B. cereus*; BS27, *P. lini*; BOVIS40, *S. indicatrix*; JVB5, *S. maltophilia*; TSN29, *B. albus*; BLBS20, *B. cereus*; SFL02, *B. thuringiensis*; PK60, *S. maltophilia,* VS52, *B. subtilis,* BAAG44, *B. thuringiensis,* SFS19, *B. pseudomycoides,* OLT2020, *B. toyonensis,* BSA123, *B. thuringiensis,* LS11, *B. paramycoides,* J4R, *Pseud. saponiphilia*; VEJU7080, *B. cereus*; FOBS21, *Pseudomonas* sp.

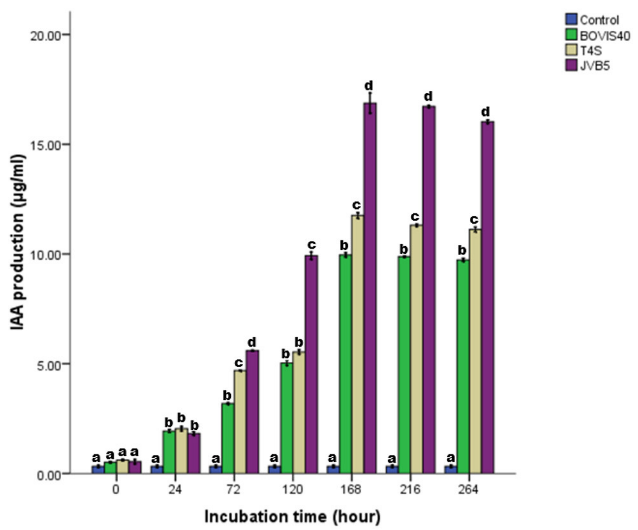

FIGURE 5 | IAA production by endophytic bacteria in the growth medium after 11 days of incubation. The different alphabets indicate significant differences in triplicate readings.

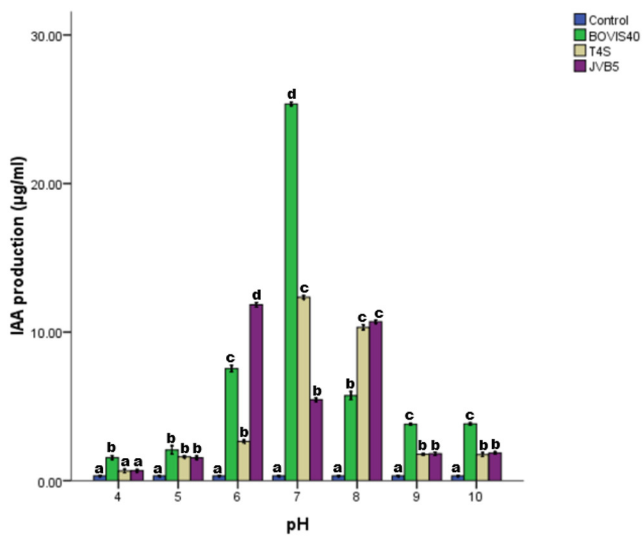

FIGURE 6 | Effect of pH on IAA production by endophytic bacteria. The different alphabets indicate significant differences in triplicate readings.

of Chukwuneme et al. (2020), who reported the enhancement of IAA production by the addition of tryptophan to the IPM. IAA production by *B. amyloliquefaciens* FZB42 in a tryptophan-dependent medium and its effect on plant growth promotion

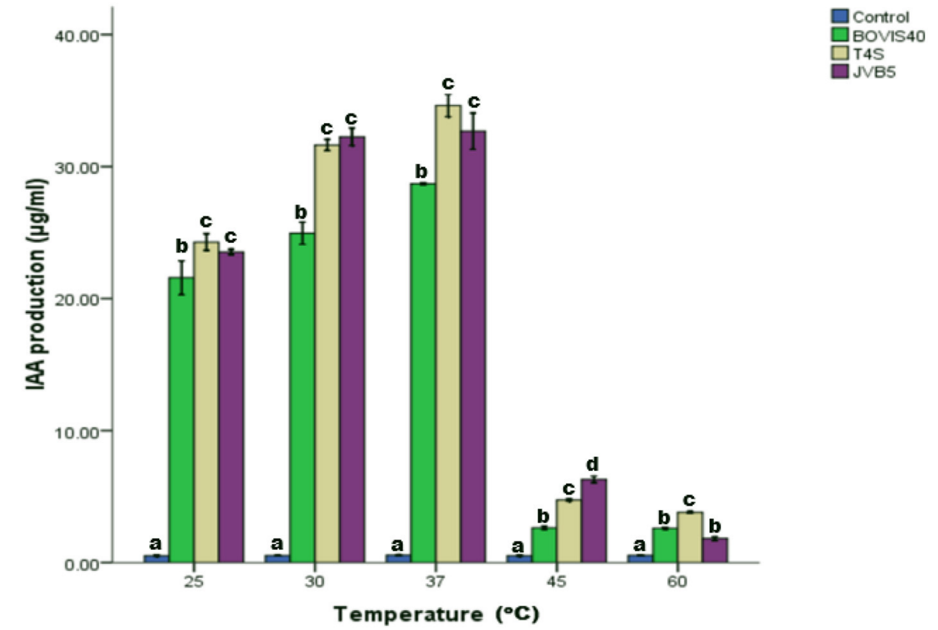

FIGURE 7 | Effect of temperature on IAA production by endophytic bacteria. The different alphabets indicate significant differences in triplicate readings.

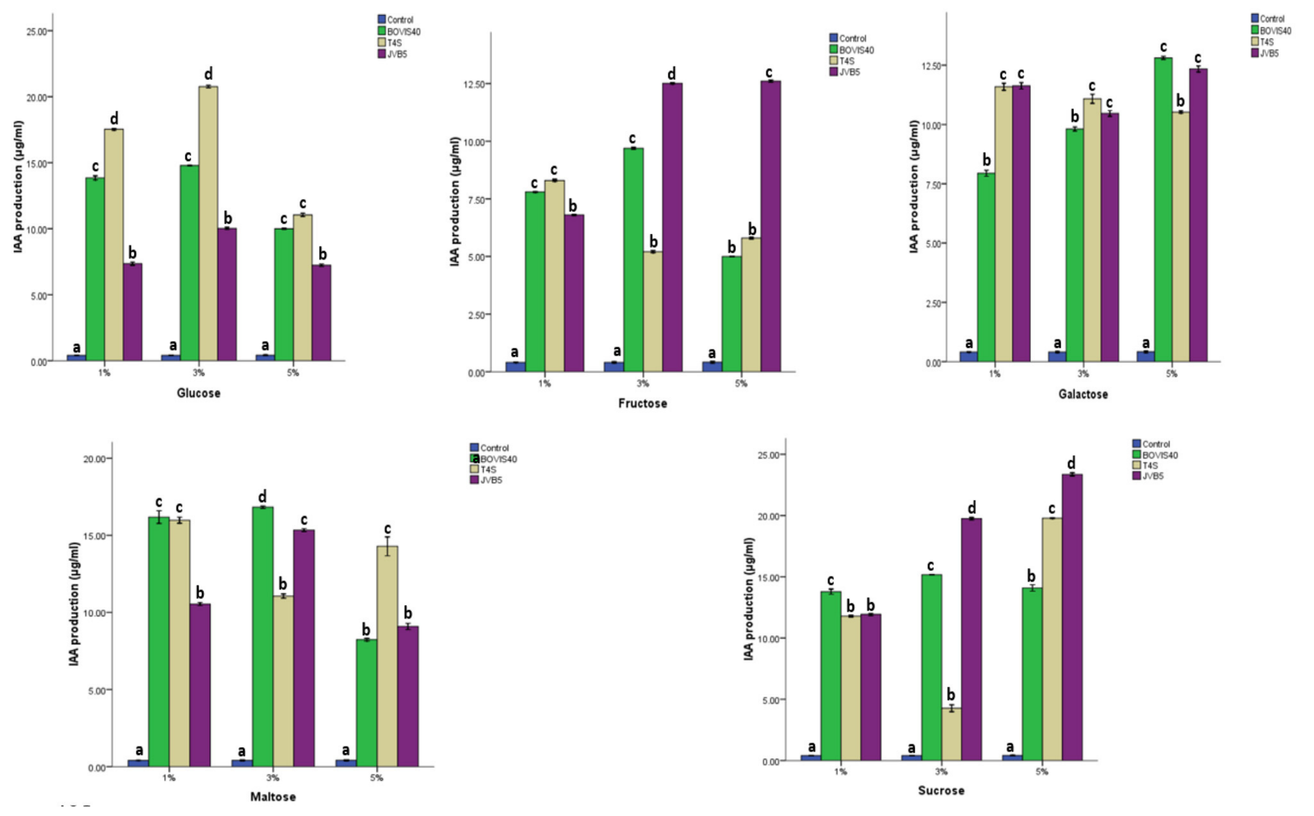

FIGURE 8 | Effect of different carbon source on IAA production by endophytic bacteria. The different alphabets indicate significant differences in triplicate readings.

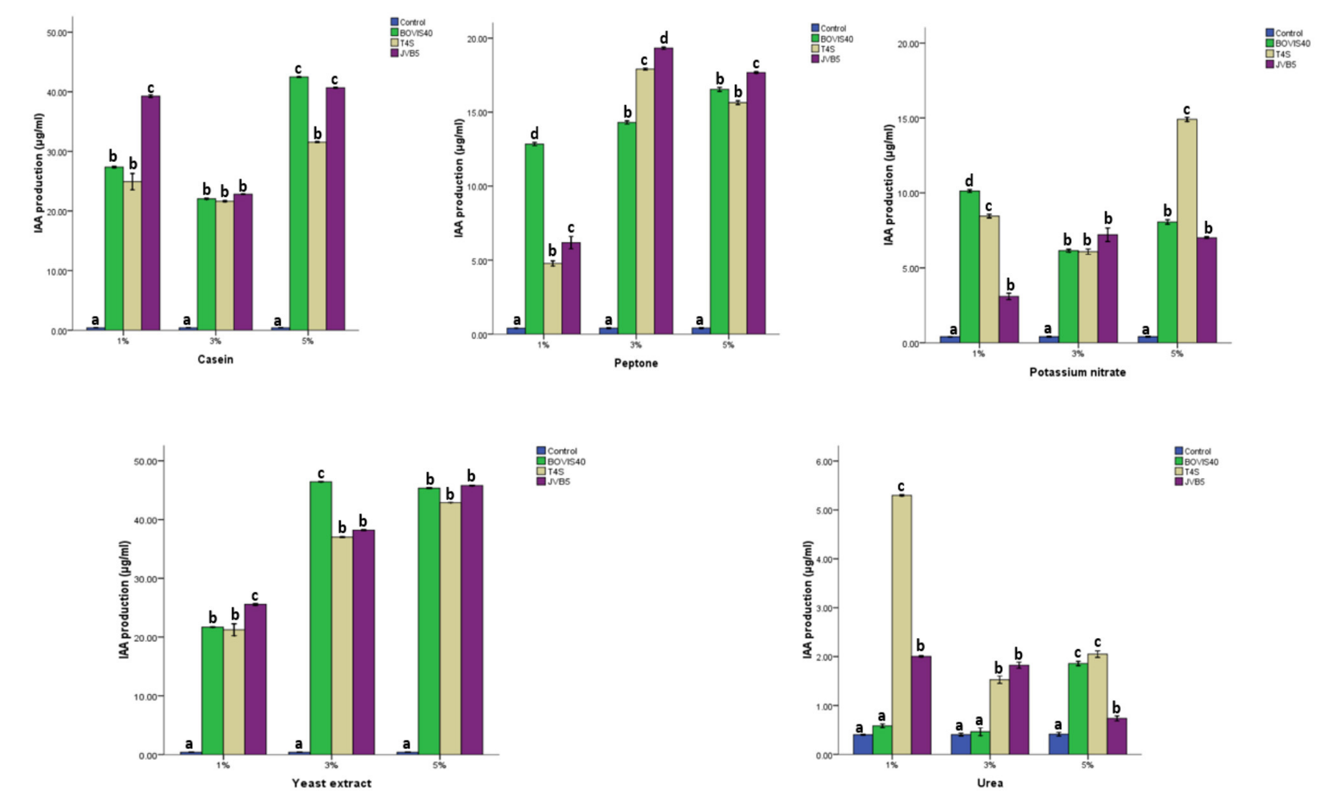

FIGURE 9 | Effect of different nitrogen sources on IAA production by endophytic bacteria. The different alphabets indicate significant differences in triplicate readings.

have been reported (Idris et al., 2007). The IAA potential displayed by the bacterial isolates corroborates the findings of Bashir et al. (2020), who reported IAA production by *Bacillus* spp. isolated from sunflowers. Furthermore, endophytes, *P. stutzeri, B. subtilis, S. maltophilia, B. cereus,* and *B. thuringiensis* native to the sunflower with IAA-producing potential have been reported to improve sunflower growth, seed germination, root elongation, and crop yield (Pandey et al., 2013; Singh et al., 2019).

Importantly, the time monitoring in a culture medium for metabolite biosynthesis is crucial in determining the biological activity of endophytic bacteria in the growth medium. Growing bacterial isolates in a medium amended with L-tryptophan as a precursor enhanced the IAA production based on their ability to utilize substrate in the medium through diverse IAA metabolic pathways (Hoseinzade et al., 2016). The geometric increase in IAA concentration with incubation time can be attributed to the ability of bacterial isolates to adjust and metabolize the substrate in the growing medium for maximum productivity. At low concentrations, a limited supply of substrate in the growth medium may affect the IAA-producing ability of the endophytic bacteria. In this study, a strong correlation between bacterial biomass and IAA production exists. A decrease in IAA concentration beyond the optimum level might be linked to the reduction in the amount of substrate or synthesis of lytic enzymes, such as IAA peroxidase and oxidase in the growing medium (Lebrazi et al., 2020). Nevertheless, bacterial growth under shaking conditions may influence IAA production due to agitation that allows the free flow of oxygen in the medium. Oxygen availability in the medium facilitates the conversion of tryptophan into auxins. Research findings on sunflower root endophytic bacteria and their optimization with incubation time have not been documented. The results obtained corroborate the conclusions of Myo et al. (2019) who reported IAA production of 82.36 µg/ml by *Streptomyces fradiae* NKZ-259 after 6 days of incubation. Interestingly, endophytic *Rhizobium* spp., *Bacillus subtilis* KA(1)5r and *Pseudomonas mosselii* with high IAA production at 216 and 96 h incubation have underlined their ability in promoting the growth of the medicinal herb *Aconitum heterophyllum* and wheat (*Triticum* spp.) (Emami et al., 2019; Lebrazi et al., 2020; Minakshi et al., 2020). Furthermore, IAA synthesis by actinomycetes in an IPM supplemented with suitable precursor L-tryptophan has been reported to occur *via* a tryptophan-dependent pathway or other similar pathways (Samaras et al., 2020).

The pH is an important factor that influences growth and microbial metabolism. At low or high pH, microbial activities may be affected (Alkahtani et al., 2020). Adjustment of pH in the growth medium to suitably favor bacterial growth can facilitate IAA biosynthesis. The differences observed in IAA production can be attributed to the pH of the medium and media composition (Widawati, 2020). A study by Myo et al. (2019) has reported maximum IAA production by *Pantoea glomerans* PVM,

TABLE 5 | Growth parameters of inoculated and un-inoculated sunflower seedlings.

Bacterial strain	Root length (mm)	Shoot length (mm)	Number of lateral roots	Fresh weight (g)	Dry weight (g)
Un-inoculated (control)	16.00 ± 0.0a[a]	22.03 ± 0.06[b]	5.09 ± 0.19[a]	0.14 ± 0.00[b]	0.03 ± 0.00[a]
S. indicatrix BOVIS40	25.00 ± 0.02[c]	27.98 ± 0.08[c]	10.17 ± 0.17[c]	0.15 ± 0.00[b]	0.06 ± 0.00[a]
B. cereus T4S	19.66 ± 0.05[b]	26.99 ± 0.02[c]	11.99 ± 0.01[c]	0.17 ± 0.00[b]	0.04 ± 0.00[a]
S. maltophilia JVB5	36.67 ± 0.02[d]	24.66 ± 0.03[a]	6.65 ± 0.05[b]	0.18 ± 0.00[b]	0.05 ± 0.00[a]

Values are represented as means ± standard deviation in replicates. The different alphabets down the column show a significant difference.

Klebsiella pneumoniae K8, and *Streptomyces viridis* CMU-H009 at pH ranging between pH 7 and 8. Here, the results obtained were similar to the findings of Widawati (2020), who reported optimum IAA production by *B. siamensis* at pH 7 and 8, respectively. Furthermore, changes in the temperature of the growth medium may influence IAA synthesis. A study by Emami et al. (2019) reported optimum IAA production of 23.62 μg/ml by *Pseudomonas mosselii* isolated from the root of wheat at 32°C.

Different carbon sources amended in the IAA-producing medium can serve as energy sources to enhance the overall efficiency of recycling co-factor in the cells for IAA biosynthesis (Myo et al., 2019). The differences observed in the IAA production by the bacterial isolates can be attributed to the carbon source, concentration, and utilization of the substrate (Khan et al., 2020). Usually, a growing medium amended with monosaccharide sugar compared to di-or-polysaccharides can contribute to high IAA production based on the ability of endophytic microbes to assimilate. In addition, the utilization of monosaccharide sugars by most bacteria has been linked to high IAA production (Emami et al., 2019). However, the results from this study revealed high IAA concentration in IPM amended with sucrose, thus suggesting sucrose as a sole carbon source. The differences observed in the IAA concentrations may depend on the sugar source and the ability of the bacteria to utilize them for growth (Oliveira et al., 2021). An increase in IAA production in the IPM amended with sucrose agrees with the findings of Huu et al. (2015) and Payel et al. (2017), who reported an increase in IAA production by *B. subtilis* and *Pantoea agglomerans* on sucrose amended media. Also, results from this study corroborate the findings of Bharucha et al. (2013) who reported maximum IAA production by *P. putida* UB1 in a medium amended with sucrose. Lactose and glucose have also been reported as preferred sugars for maximum IAA production by *Enterobacter* sp. and *Rhizobium* (Basu and Ghosh, 2001; Nutaratat et al., 2017). Similarly, reports on maximum IAA production by root endophytic bacteria, such as *Rhizobium* P2, *Bacillus* spp., *Pantoea* spp., and *Pseudomonas mosselii* in a medium amended with sucrose, glucose, and maltose have been documented to enhance IAA production (Apine and Jadhav, 2011; Kucuk and Cevheri, 2016; Emami et al., 2019).

The addition of various nitrogen sources increased IAA production compared to the control medium. The soluble nitrogen source in the growth medium remains the key factor for bacterial growth and metabolite biosynthesis (Khan et al., 2017). The addition of various nitrogen sources to the IPM influences the rate of IAA production (Shahzad et al., 2017). Casein and yeast extract yielded high IAA production (>20 μg/ml) compared to other nitrogen sources. Like other parameters tested, varying concentrations of nitrogen source added to the growing medium can influence the amount of IAA biosynthesis (Emami et al., 2019). IAA production by rhizobacteria inhabiting the root of leguminous plants in a growing medium amended with glutamic acid and L-asparagine as a nitrogen source has been reported (Zhao et al., 2020). The results from this study corroborate the findings of Balaji et al. (2012), who reported yeast extract as the best nitrogen source for *Pseudomonas* species with an IAA concentration of 210 μg/ml. Also, Emami et al. (2019) reported IAA production of 23.66 μg/ml by *Pseudomonas* in a yeast extract amended medium. Furthermore, the addition of tryptone, beef extract, and peptone with varied IAA production can contribute to the bacterial lifestyle in the synthesis of phytohormones (Widawati, 2020).

In this study, endophytic bacteria with promising phytostimulant activities, i.e., *B. cereus* T4S, *S. maltophilia* JVB5, and *S. indicatrix* BOVIS40 were selected and their *in vitro* effect was assessed on sunflower seedlings growth. Inoculation of *Sesbania aculeate, Brassica campestris, Vigna radiate,* and *Pennisetum americanum* with endophytic bacteria *Azotobacter* spp., *Bacillus* spp., *Azospirillum brasilense,* and *Pseudomonas putida*, which increase adventitious root development, shoot, root length, and chlorophyll pigmentation has experimented (Khan Latif et al., 2016). The observed variation in the weight, root, and shoot length of rice and maize inoculated with *Bacillus* and *Pseudomonas* has been presumed to be influenced by IAA production (Karnwal, 2017, 2018). Most *Bacillus* spp. isolated from the root endosphere has been implicated in nitrogen fixation in legumes with a positive influence on seedling's growth (Bertani et al., 2016). An increase in rice shoot, root, and leaf length due to phytohormone production by the bacterial isolates contributes to crop production. Furthermore, the rooting potential of *Agrobacterium rhizogenes* in jujube's root has been reported (Lebrazi et al., 2020).

CONCLUSIONS

This study provides information on the *in vitro* screening of endophytic bacteria associated with sunflower. The PGP traits, such as IAA, ammonia production, exopolysaccharide, hydrogen cyanide, siderophore, and enzyme production exhibited by the endophytic bacteria, can underline their potential in plant growth promotion and protection from the biotic and abiotic stresses. The IAA production by the identifiable endophytic bacteria can contribute to sunflower rooting for nutrient and water absorption from the soil. The significant differences observed in the inoculated sunflower seedling compared to un-inoculated showed the tendencies of these bacteria in plant growth promotion. Hence, based on the high siderophore potential of *B. cereus* T4S among the screened bacterial isolates with multifunctional attributes, this bacterium can be explored for sunflower cultivation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accession: MW265413-MW265431 and MW261910).

AUTHOR CONTRIBUTIONS

BSA, ASA, and OOB designed the study. BSA managed the literature searches, carried out the laboratory work, interpreted the results, wrote the first draft of the manuscript, and revised and formatted the manuscript. ASA assisted in the result analysis and review of various drafts. OOB provided academic input, thoroughly critiqued the manuscript, proofread the draft, and secured funds for the research. All authors approved the article for publication.

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Backyard poultry farming with improved germplasm: Sustainable food production and nutritional security in fragile ecosystem

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Approximately 3 billion people were unable to afford a healthy diet in 2019 because of poverty and inequality. Most of these people live in Asia and Africa. Furthermore, 30% of the world population was affected by moderate to severe food insecurity in 2020, and most of this population lives in low- and middle-income countries. The world is at a critical juncture, and there is an urgent need for transformative food systems that ensure the empowerment of poor and vulnerable population groups, often smallholders with limited access to resources or those living in remote locations, as well as the empowerment of women, children, and youth (FAO, 2018). The backyard poultry production system (BPPS), as practiced by 80% of the world's rural population, can be that transformative change in low- and middle-income countries. Although the BPPS has low productivity, it still plays an important role in the food and nutritional security of rural people living in fragile ecosystems. Backyard poultry has been recognized as a tool for poverty alleviation and women empowerment besides ensuring food and nutritional security for rural poor. Poultry meat and eggs are the cheapest and best source of good quality protein, minerals, and vitamins. The introduction of improved backyard poultry germplasm has improved the productivity of this system in resource-poor settings and thereby improved the income and nutritional security of poor households. With these birds, the availability, access, utilization, and stability of food security have improved at household and national levels. Diseases, predation, non-availability of improved germplasm, lack of access to markets, and lack of skills are the major constraints to the adoption of improved backyard poultry. These constraints can be addressed by involving a network of community animal service providers. The improved backyard poultry germplasm will dominate the backyard poultry production system in the future and will be a tool for ensuring food and nutritional security on a sustainable basis, more particularly in low- and middle-income countries.

KEYWORDS

backyard poultry production system, improved germplasm, food and nutritional security, women empowerment, sustainability

Introduction

Around the world, more than 780 million people live in extreme poverty with <$1.90 per person per day, an amount that is impossible to support a healthy livelihood in any part of the world (https://www.actionagainsthunger.org/). As a result of the high cost of healthy diets, coupled with persistently high levels of income inequality, ∼3 billion people were unable to afford a healthy diet in 2019. Most of these people live in Asia (1.85 billion) and Africa (1.0 billion). In addition, the number of undernourished people in the world continued to rise in 2020. More than half of the world's undernourished are found in Asia (418 million) and more than one-third in Africa (282 million). Also, approximately 720–811 million people in the world faced hunger in 2020. Furthermore, 30% of the world population was affected by moderate to severe food insecurity in 2020, and most of this population lives in low- and middle-income countries (FAO et al., 2021). The world is at a critical juncture, and there is an urgent need for transformative food systems that ensure the empowerment of poor and vulnerable population groups, often smallholders with limited access to resources or those living in remote locations, as well as the empowerment of women, children, and youth (FAO, 2018). The backyard poultry production system, as practiced by 80% of the world's rural population (Wong et al., 2017), can be a transformative change in low- and middle-income countries.

Poultry is the world's primary source of animal protein (FAO, https://www.fao.org/poultry-production-products/products-processing/zh/). Globally, poultry meat is expected to represent 41% of all the protein from meat sources in 2030. In lower income developing countries, poultry meat is cheap as compared with other meats, while in high-income countries, poultry meat is preferred because white meat is considered a healthier food choice (OECD-FAO Agricultural Outlook 2021–2030).

In poultry production, the most primitive (BPPS) and most advanced (highly mechanized and integrated system) production systems exist side by side (Thieme et al., 2014). The latter uses the latest innovation and technologies and is capital intensive, whereas the former is a low-input and low-output system. Backyard poultry production systems, mostly composed of chickens, account for the majority of the poultry population in low- and middle-income countries (Gilbert et al., 2015; Wong et al., 2017; Rajkumar et al., 2021). Although the BPPS has low productivity, it still plays an important role in the food and nutritional security of rural people living in fragile and resource-poor ecosystems (Wong et al., 2017; Chaiban et al., 2020; FAO and IFAD, 2022). Because of its low-input and low-output nature, a considerable yield gap exists in the BPPS.

Backyard poultry is being practiced in all developing countries and plays a crucial role in poor rural households (Alexander et al., 2004; Alders, 2012). Backyard poultry is a source of scarce animal protein in the form of meat and eggs (FAO, 2013). Besides, they can be sold or bartered to meet emergency family needs such as medicine, clothes, and school fees (Alders et al., 2018). Backyard poultry helps in pest control, provides manure, converts kitchen waste into good-quality protein, and is required for religious and social ceremonies. In resource-poor regions, backyard poultry is owned and managed by women and is often essential element of female-headed households (Alders and Pym, 2009; Bagnol et al., 2013). Backyard poultry is an available and accessible form of livestock in rural and resource-poor areas and, therefore, is a significant source of economic, nutritional, and food security for the poorest of households (Alders and Pym, 2009; Wong et al., 2017). In particular, it significantly improves the livelihood and food security of women, children, the elderly, and the chronically ill (Kumaresan et al., 2008; Wong et al., 2017).

The productivity in terms of meat and eggs of backyard poultry is lower than that of commercial poultry, and traditional backyard poultry production systems are unable to meet the demand (Alders, 2012; Singh et al., 2018b). Chaiban et al. (2020) observed that backyard poultry production systems are highly heterogeneous in terms of size, age, accessibility, management, opportunities, and challenges. The farm location affects market access and influences opportunities available to farmers, resulting in further diversity in farm profiles. Furthermore, with the increasing human population and industrialization, there will be an increase in demand for sustainable animal source foods for human consumption.

Backyard poultry farming with improved productivity through appropriate interventions can be a source of a sustainable food production system (Singh et al., 2018a; Rajkumar et al., 2021). One such intervention is the introduction of improved backyard-type stock in rural and tribal areas. In the recent past, there has been much focus on improved poultry varieties suitable for backyard production in Africa and Asia. These varieties, with higher production potential even on a low plane of nutrition, were developed specifically for backyard production in resource-poor areas and fragile ecosystems (Singh et al., 2018a; Rajkumar et al., 2021). Other interventions include skill enhancement, health prophylaxis measures, implementation of on-farm biosecurity, and efficient market linkages. Through the education and empowerment of farmers, the farmer field school (FFS) approach can contribute to strengthening the knowledge of holistic agroecosystem management, improving decision-making skills, facilitating group collaboration, and encouraging local innovation, particularly by women and young people (FAO and IFAD, 2022).

The present review is an attempt to appraise the status of the backyard poultry production system vis-a-vis improved backyard poultry germplasm and its impact on nutritional and food security, women empowerment, and sustainability. Furthermore, major constraints for the expansion of improved backyard poultry production systems are also discussed.

Backyard poultry production system

Backyard poultry production systems are integrated with human livelihoods for thousands of years, providing income, and food and nutrition security to the rural poor (Alders and Pym, 2009; Wong et al., 2017). Backyard poultry constitutes 50–80% of total poultry in several developing countries. Local poultry constitutes 80% of poultry production in sub-Saharan countries (Desha et al., 2016), with Nigeria known to have 180 million local chickens (Pym and Guerne-Bleich, 2006). In India, backyard poultry is 317 million, and it has increased by 45% in the last decades and now contributes 35% of the total poultry population (20th Livestock Census, Government of India). Backyard poultry farming contributes around 70–80% of the total poultry population in China. In Vietnam, a majority of poor people keep poultry for their meat as well as subsidiary income (Epprecht et al., 2007). Backyard poultry converts waste material such as kitchen waste, vegetable waste, green grass, earthworms, and insects, into high-quality animal protein for human consumption (Alders et al., 2018). Backyard poultry is recognized as an entry point into the livestock production system, which is associated with breaking out of poverty traps (Gueye, 2000; Thieme et al., 2014; Wong et al., 2017).

Backyard poultry is characterized by the rearing of native or indigenous or improved poultry in the backyard (Kumaresan et al., 2008; Chaiban et al., 2020). The number of birds varies depending upon the natural feed base available. Supplementary feeding is also being practiced as and when available (Thieme et al., 2014; Wong et al., 2017). Birds are housed at nighttime only in the locally made chicken coup, whereas in the daytime, chickens are let free for scavenging (Alders et al., 2018). Backyard poultry production is commonly associated with the integrated farming system model with crops, vegetables, fisheries, and other livestock species (Alders and Pym, 2009; Wong et al., 2017). In this system, animal health prophylaxis and biosecurity are minimally applied (Conan et al., 2012; Samanta et al., 2018). There is high mortality because of diseases and predation (Alders, 2012; Chaiban et al., 2020). Chickens are consumed by households, and surplus birds are sold locally. Surplus male birds are consumed or sold in the market at 1.5–2 kg body weight, whereas females are reared for further propagating the flocks. Indigenous female poultry lays 30–80 eggs in three to four clutches in a year (Singh et al., 2018b). The brooding efficiency of native or indigenous birds is very high and incubating 15–20 eggs at one time. In general, the production of indigenous birds is low and further constrained by diseases and predation (Alders and Pym, 2009; Wong et al., 2017).

Backyard poultry production is classified into small-scale extensive scavenging, scavenging, semi-intensive, and small-scale intensive (FAO, 2014) systems. Rajkumar et al. (2021) classified the backyard poultry production system in India into traditional backyard system (<20 birds with little or no input), semi-intensive farming (50–200 birds under semi-scavenging conditions), small-scale intensive farming (200 or more birds with improved birds under a high-input system), and native chicken farming (indigenous birds with a run area and complete ration). Thieme et al. (2014) classified the backyard poultry production system into small extensive scavenging (1–5 adult birds), extensive scavenging (5–50 birds), semi-intensive (50–200 birds), and small-scale intensive production (>200 broilers or >100 layers). The type of backyard poultry production system is based on the availability of poultry germplasm, marketing avenues, availability of natural food base resources, food habits of the population, etc. (Thieme et al., 2014; Chaiban et al., 2020).

Importance of backyard poultry farming

1. Backyard poultry can survive in harsh and inclement climatic conditions. They are resilient to climate change and better adapt to different environments.
2. Backyard poultry birds convert waste material such as kitchen waste, vegetable waste, and green grass into high-quality animal protein.
3. Backyard poultry farming involves minimal initial investment.
4. It provides employment to the rural poor farmers, women, unemployed youth, and old members of the family along with subsidiary income.

5 Eggs and meat from backyard poultry farming fetch a high price as compared to those from commercial poultry farming.
6 Produce of backyard poultry is a source of good-quality animal protein and hence a source of food and nutritional security to vulnerable communities.
7 Backyard poultry may well-integrate with other agricultural operations such as poultry–fish integrated farming system.
8 Manure from backyard poultry is a rich source of soil nutrients and can be utilized to enhance soil fertility.
9 Women empowerment: Backyard poultry are generally owned and managed by women of the household. the sense of ownership and income from backyard poultry empowers rural women.
10 Conservation of biodiversity: Backyard poultry consists of native or indigenous birds, which are well-adapted to the local climate and are resistant to diseases. There is high genetic and phenotypic diversity in indigenous chickens. this can be utilized as a base resource for further improving the productivity of backyard chickens.

Backyard poultry production with improved germplasm

The productivity of the backyard poultry production system can be improved by the introduction of improved germplasm (Table 1) or by adopting improved management practices (Singh et al., 2018b; Chaiban et al., 2020). In the case of improved poultry germplasm, there is a need to develop birds with genetic potential for enhanced growth and egg production. Also, the birds should resemble the indigenous birds with multicolored plumage, longer shanks, higher productivity, adaptability to varied agroclimatic conditions, and better immunity (Kumaresan et al., 2008; Rajkumar et al., 2021). In addition, the improved dual-purpose birds should be able to perform on a low plane of nutrition in the backyard production system. Also, the flavor and texture of meat should be similar to local chicken. Improved poultry germplasm suitable for the backyard production system can be developed either through selective breeding in native or indigenous birds or through crossbreeding of indigenous birds with exotic germplasm. The former method is slow, but changes in production will be permanent without losing the peculiar character of indigenous birds (Padhi, 2016). Also, once selected for higher growth and egg production, further propagation can be carried out at the farmer level. In the case of crossbreeding, improved and native germplasm are crossed, and the heterosis of two breeds is exploited, which results in higher productivity. Although it has been successfully used to enhance the productivity of backyard poultry in Asia and Africa mainly because of the shorter time required for evolving improved germplasm (Singh et al., 2018b; Rajkumar et al., 2021), however, there are inherent problems of crossbreeding. There is segregation of genes, which results in a decrease in productivity in a future generation; therefore, farmers depend on suppliers for regular supply of these birds. Also, the introduction of crossbred poultry in native breeding tacks of indigenous poultry poses serious threats to them and may lead to dilution or erosion of native germplasm. Nonetheless, with due care and a suitable breeding policy, improved dual-purpose poultry has played a significant role in the improvement of food and nutritional security of rural farmers, particularly in low- and middle-income countries (Singh et al., 2018a; Rajkumar et al., 2021). In India, the breeding policy of the Government of India and ICAR envisages avoiding the introduction of the improved varieties in the home tracts of the recognized chicken breeds, which will prevent the genetic erosion of native breeds (Rajkumar et al., 2021). There are several improved poultry germplasms developed in different countries for the backyard production system. Rajkumar et al. (2021) reported that high-yielding poultry varieties, which resemble native poultry, transformed backyard poultry farming into a highly remunerative farming activity in India. Chaiban et al. (2020) reported that because of the increase in poultry meat demand, backyard poultry farms are transforming themselves into semi-intensive (50–200 birds) backyard farms mainly with the help of improved birds and commercial feed.

In our previous study (Singh et al., 2017), we reported that Vanaraja, dual-purpose improved backyard poultry, performs well in sub-tropical to the sub-temperate climate in the Indian Himalayan ecosystem. In this study, body weight at 24 weeks varied from 1.7 to 2.7 kg in different climatic and production systems. Similarly, 72-week hen day egg production varied from 90 eggs to 112 eggs. Singh et al. (2018a) reported that the Vanaraja chick's survivability up to 4 weeks was 96% in the summer season and 83% in the winter season in sub-temperate climatic conditions.

Further, Singh et al. (2018b) found 95% survival of the chicks of improved dual-purpose backyard birds Vanaraja and Srinidhi in a hot humid sub-tropical climate. Also, the hen day egg production was 140 eggs and 195 eggs for Vanaraja and Srinidhi birds, respectively. The eggs and meat of these birds reared in the backyard farming fetches premium prices due to high consumer acceptability even in the urban sectors, where plenty of eggs and poultry meat from commercial units are available. Besides a stable supply of high-quality animal food, backyard poultry production promotes income opportunities, particularly for the weaker sections in the tribal areas. Backyard farming fulfills a wide range of functions, e.g., the provision of meat and eggs, food for special festivals, chicken for traditional ceremonies, pest control, and petty cash, utilizing minimum inputs, minimum human attention, and causing less environmental pollution (Singh et al., 2018b). Furthermore, Singh et al. (2019) reported that the net income per bird was significantly higher (Rs. 995.97 only) in Vanaraja than in local birds (Rs. 287.22 only). In

TABLE 1 Growth and egg production performance of improved backyard poultry germplasm.

SI No.	Improved backyard poultry	Type (egg/meat/ dual)	Body weight female	Body weight male	Egg production	References
1.	Kuroiler	Dual	953–1,766 gram at 20 weeks age	1,109–1,785 gram at 20 weeks age	98–115 up to 45 weeks of laying	(Kassa et al., 2021)
2.	Sasso	Dual	1,052–1,748 gram at 20 weeks age	889–2,111 gram at 20 weeks age	98–112 up to 45 weeks of laying	
3.	Sasso-R	Dual	903–1,330 gram at 20 weeks age	913–1,624 gram at 20 weeks age	86–100 up to 45 weeks of laying	
4.	Sasso	Dual	2,730 gram at 20 weeks age	2,980 gram at 20 weeks age	229 eggs per hen per year	(Aman et al., 2017)
5.	Fayoumi	Egg	1,215 gram at 26 weeks age	–	150 eggs per hen per year	(Samson et al., 2013)
6.	Vanaraja	Dual	1,613 gram at 20 weeks age	2,216 gram at 20 weeks age	137 eggs per hen per year	(Singh et al., 2021)
7.	Srinidhi	Dual	981 gram at 20 weeks age	2,288 gram at 20 weeks age	202 eggs per hen per year	
8.	Sonali	Egg	1,180 gram at 20 weeks age	–	156 eggs per hen per year	(Rahman et al., 2017)
9.	Gramapriya	Egg	1,780 gram at 20 weeks age	–	256 eggs per hen per year	(Rajkumar et al., 2018)
10.	Rainbow rooster	Dual	1,650 gram at 20 weeks age	–	163 eggs per hen per year	(Islam et al., 2017)

another study in India, Vanaraja poultry farming was found more profitable than native poultry, with 46.78% more net returns from a unit of 20 birds with a benefit-to-cost ratio of 2.84 in the backyard production system (Baruah and Raghav, 2017). Kumaresan et al. (2008) found that village poultry is an important income source for household expenses in India and that improved dual-purpose birds can be employed to improve traditional free-range poultry production.

Da Silva et al. (2017) proposed the identification, selection, and introduction of tropically adaptable semi-scavenging dual-purpose poultry breeds to improve the productivity of BPPS in Tanzania. Currently, efforts are being made to introduce those dual-purpose breeds with higher genetic potential for growth and egg production and adaptability to varied agroclimatic conditions in the backyard production system (Guni et al., 2021). Dana et al. (2010) reported that although farmers preferred native poultry for rearing because of their adaptation to the local climate, however, low productivity of indigenous poultry warrants the development of improved dual-purpose poultry based on native germplasm. Similarly, Desta (2021a) stated that the indigenous village poultry production system has low productivity; however, it has the potential to achieve profitable and sustainable production through the genetic improvement of indigenous chicken. Desta (2021b) proposed enhanced management, selection strategies, and genetic crosses including the crossing of commercial chickens with red jungle fowl to sustainably intensify the indigenous village chicken production system. In Uganda, a dual-purpose chicken, Kuroiler, has been successfully evaluated under on-farm conditions in scavenging management systems (Galukande et al., 2016).

Kuroiler and Sasso, two improved dual-purpose poultry for the backyard production system, are getting popular in Tanzania compared to the local chicken because of more meat and egg production performance (Sharma, 2011; Getiso et al., 2017). The Kuroiler breed is a cross of several pure genetic lines of chickens, including White Leghorn, Rhode Island Red, colored broiler, and local Desi chickens, followed by selection for high production performance and ability to thrive in the village environment under scavenging or semi-scavenging rearing systems (Sharma, 2011). Kuroiler birds recorded higher body weight gain than indigenous chickens raised under scavenging conditions by rural households in Uganda (Sharma et al., 2015). Sasso breed of poultry was developed in France for extensive production systems through an intensive selection of traditional colored lines of chickens (SASSO, 2014). In Tanzania, Andrew et al. (2019) reported that the net present value, net cash farm income, and the highest probability of attaining economic return were highest in rearing Sasso strain, followed by Kuroiler, and the local chicken was economically least viable. Their study recommends that the improved poultry birds should be promoted for adoption to increase household income for improved livelihood along with education on technical know-how on good farming practices; feed formulations, medication; and shelter for improved productivity (Andrew et al., 2019). In eastern Tanzania, Guni et al. (2021) revealed that the performance traits of the Kuroiler and Sasso breeds are different in lowland and highland ecology, and therefore, knowledge of breed performance in relation to agroecological differences is critical when introducing improved poultry breeds to a different agroclimatic zone. Rajkumar et al. (2021) reported several improved poultry varieties suitable for backyard rearing developed in India. These include Vanaraja, Gramapriya, Srinidhi, Giriraja, Kuroiler, Rainbow, and Rooster. These varieties lay 110–180 eggs in one laying cycle in backyard conditions. The success of these varieties has been reported in India and Africa by several studies (Singh et al., 2018a,b; Andrew et al., 2019; Sanka et al., 2020; Guni et al., 2021; Rajkumar et al., 2021).

Nutrition and food security

The high cost of healthy diets coupled with persistently high levels of income inequality puts healthy diets out of reach for ~3 billion people, especially the poor, in every region of the world in 2019. Most of these people live in Asia (1.85 billion) and Africa (1.0 billion), although a healthy diet is also out of reach for millions living in Latin America and the Caribbean (113 million) and Northern America and Europe (17.3 million). The number of undernourished people in the world continued to rise in 2020. Approximately 720–811 million people in the world faced hunger in 2020. More than half of the world's undernourished are found in Asia (418 million) and more than one-third in Africa (282 million). Now, moderate to severe food insecurity affects more than 30% of the world population, and most of this population lives in low- and middle-income countries. Poverty and inequality are underlying structural causes of food insecurity and malnutrition. Income inequality in particular increases the likelihood of food insecurity, especially for socially excluded and marginalized groups (FAO et al., 2021).

Severe energy deficiency has been reported in 34% of the human population in South Asia and 59% in sub-Saharan Africa. People in these regions obtained their energy mostly from staple foods (cereal grains, grain legumes, starchy roots, and tubers) and consume a small quantity of low-quality protein. The per capita consumption of egg and animal protein in these regions is low as compared to the world average (FAO, 2013). There is a need for a transforming food system that can provide nutritious and affordable food for all and become more efficient, resilient, inclusive, and sustainable. The food systems need to provide decent livelihoods for the people who work within them, in particular for small-scale producers in developing countries.

Because of its low rearing cost, backyard poultry is being reared by the poorest of the poor households for their food and nutritional requirement. In general, poultry meat and eggs are consumed globally without any religious or social taboo. Backyard poultry converts kitchen or agricultural waste into quality animal protein for human consumption, which is much needed by poor households in developing countries (FAO, 2013). Poor households generally consume cereals that have less bioavailable protein and are deficient in vital minerals and vitamins. Poultry egg has 87 net protein utilization (NPU), an index of quality protein, and poultry egg and meat are rich sources of essential amino acids (FAO, 2013). Besides fulfilling the protein requirement of humans, eggs and poultry meat are concentrated sources of micronutrients and, therefore, are valuable food for alleviating under-nutrition and malnutrition in developing countries.

Meat and eggs from backyard poultry are the cheapest source of high-quality animal-based food, densely packed with essential macro- and micronutrients (Wong et al., 2017). Poultry meat is a valuable source of highly digestible proteins of good nutritional quality, B-group vitamins (mainly thiamin, vitamin B6, and pantothenic acid), and minerals (like iron, zinc, and copper) (Bruyn et al., 2015; Réhault-Godbert et al., 2019). Foods with high bioavailability of nutrients are important for infants and young children, pregnant and lactating women, and elderly and ill people (Olaoye, 2011).

Eggs are a rich source of essential nutrients and vitamins (except vitamin C) to meet human nutrition requirements (Vizard, 2000; Réhault-Godbert et al., 2019). The egg has a balanced and diversified nutrient content with high bioavailability, which makes it high-valued basic food for consumption (Réhault-Godbert et al., 2019). Eggs have been recognized as the lowest cost source of protein, vitamin A, vitamin B12, riboflavin, iron, and zinc (Drewnowski, 2010; Réhault-Godbert et al., 2019) and are also a good source of folate, selenium, vitamin D, and vitamin K (Applegate, 2000; Abeyrathne and Ahn, 2015). Besides, the egg is a good source of bioactive compounds, which are essential for human health.

Singh et al. (2018b) reported that improved backyard poultry contributed significantly to the food and nutritional security of tribal farmers in mountainous regions of northeast India. Wong et al. (2017) reported that backyard poultry contributes directly and indirectly to the food and nutritional security of poor rural households. Backyard poultry are available in vulnerable areas and are a rich source of the nutrient. Additionally, backyard poultry does not compete with humans for feed, thereby improving the availability of densely packed nutritious food to rural poor at a minimum cost (Wong et al., 2017). Poultry meat and eggs provide more protein than swine, cow milk, beef, and lamb per unit of intake. Thus, greater availability and affordability of poultry meat and eggs could contribute to enhanced nutrition for poor rural people, particularly in vulnerable ecology. Rajkumar et al. (2021) emphasized the importance of improved backyard poultry farming for the nutritional and livelihood security of rural farmers in India. It was earlier reported that animal source food improves the nutritional status and linear growth of children (Murphy and Allen, 2003). Thus, the overall benefits of backyard poultry in resource-poor regions are much greater than being an available food source alone. Therefore, increased backyard poultry production with improved germplasm could help improve the nutritional status of rural communities as poultry products are often the only source of animal protein for resource-poor households (Gueye, 2000).

Women empowerment and sustainability

More than 30% of women in Africa and Asia were affected by anemia, compared with only 14.6% of women in North America and Europe. At the global level, the prevalence of moderate or severe food insecurity was 10% higher among women than among men in 2020 (FAO et al., 2021). Backyard poultry is a valuable enterprise because of its role in alleviating poverty, securing food supply, and promoting women empowerment (Rajkumar et al., 2021). Backyard poultry in low- and middle-income countries are mainly managed and owned by women of the households in rural areas (FAO and IFAD, 2022). The fact that women own a large proportion of backyard poultry emphasizes its importance as a means of improving their livelihoods. Income from the sale of poultry products is often the main source of income for female-headed households, whereas male-headed households usually have multiple income sources. Women's income often contributes more to improvements in household health, education, and nutrition status than men's income and has a positive impact on household food security (FAO and IFAD, 2022). In Africa, most women have access to backyard poultry but do not have full control over ownership and decision-making, thereby depriving them of economic benefits (Gueye, 2000). In view of this, Gueye (2000) recommended that backyard poultry development programs should be more women-friendly in order to facilitate women's participation. In India, the rearing of Haringhata black (native poultry) with improved management practices empowered the tribal women economically (Gupta et al., 2021). Also, the position and involvement of women farmers in family affairs have got positive and significant improvement. The adoption of improved management practices of backyard poultry has resulted in increased flock size, increased household income, increased household food security, and increased decision-making power for women (Alders and Pym, 2009). In Africa, women were able to purchase goats and cattle by selling excess poultry, thereby empowering them with the resources that were previously denied to them. In Bangladesh, the rearing of improved hens for table egg purposes under the backyard production system improved the economic status of rural women folk (Alam, 1997). Similarly, in Bhutan, backyard chickens also act as a source of protein for the female members of the household during pregnancy and post-parturition periods (Tashi and Dorji, 2014). This will help in reducing food insecurity, alleviate poverty, and will promote gender equality. Therefore, the greater empowerment of women through backyard poultry farming may contribute significantly to alleviating poverty, enhancing food security, and promoting gender equality (Alders et al., 2018; FAO and IFAD, 2022).

The BPPS is low-input-based and utilizes feed that is not used for human beings, thereby making it economically sustainable, although its productivity is low. However, backyard poultry has more environmental impact in terms of greenhouse gas emissions and manure production because of the long life cycle of backyard poultry compared to broilers (Gerber et al., 2013). Still, the other aspects of backyard poultry, including nutrient recycling, pest management, and improvement in soil fertility, were not considered in environmental impact studies. It was reported that long-term poultry manure application benefited crop yield, soil health, and farm economics (Hoover et al., 2019). Also, backyard poultry reduces environmental pollution by converting kitchen waste into animal proteins. The production of eggs and chicken locally will reduce transportation-related carbon emissions and thereby minimize the carbon footprint of the backyard poultry production system (Samanta et al., 2018). However, there is no study documenting the environmental impact of these improved backyard poultry. Nonetheless, improvement in management and productivity of BPPS with improved germplasm will further lower the adverse environmental impact.

Constraints and challenges to improving the backyard poultry production system

1. Non availability of improved germplasm: Backyard poultry is reared by rural poor farmers in remote and disadvantageous regions. These regions are generally the least developed and also experience extreme weather conditions. Also, the produce from backyard poultry is less than commercial poultry. Thus, it does not attract investment from industry, thereby; leaving the farmers to depend on government institutions for the supply of chicks. As the rural farmers are not equipped with good infrastructure including electricity in these regions, there is high mortality of chicks during unfavorable weather conditions. Therefore, a timely supply of improved germplasm will go a long way to improving the productivity of the BPPS across the globe as is the case in

India (Singh et al., 2018b). In the author's experience, the survival of the birds in the BPPS increased with the supply of grown-up chicks (4–6 weeks of age) to the farmers. Also, on-farm research should be undertaken on improved germplasm before introduction in farmers' fields. It is important to mention here that improved germplasm should not be introduced in core breeding tracts of native or indigenous poultry.

2 Skill deficiency: Although backyard poultry is being practiced by farmers for ages, there is a constant need to upgrade the knowledge and skills of rural farmers. improved poultry germplasm requires scientific management practices to realize its full genetic potential. The success of the Bangladesh model to improve backyard poultry production was largely attributed to the skill enhancement of farmers before the introduction of improved poultry (Alam, 1997). Therefore, farmers, particularly women, should be exposed to different training modules, including brooding, housing, nutrition, and health management.

3 Diseases, predation, and biosecurity threats: In developing countries, backyard poultry represents a majority of stocks reared by farmers with minimum input. Birds are reared with minimum biosecurity, and they are exposed to wild birds, vermin, and predators and, therefore, are predisposed to disease outbreaks (Conan et al., 2012; Samanta et al., 2018). Also, some diseases such as Newcastle Disease (ND) or Highly Pathogenic Avian Influenza (HPAI) are zoonotic in nature and can have fatal consequences for poultry as well as humans (Conan et al., 2012; Wong et al., 2017). Alders et al. (2010) reported that ND is the most common cause of mortality in the BPPS, which can sometimes result in 100% mortality. Similarly, HPAI was found to have adverse effects on backyard flock size, livelihoods, and food security of households (Alders et al., 2013). Also, in the BPPS, predation accounts for the loss of chicks and adult birds, and losses can be sometimes as high as 50–70% (Ahlers et al., 2009). To reduce the disease burden of ND in the BPPS, vaccination by trained community animal health workers was proposed as a key strategy (Alders et al., 2010; Bagnol et al., 2013). Although it is very difficult to implement full biosecurity measures in the backyard poultry production system, disease knowledge, vaccination, and proper housing can significantly reduce the losses to the households (Conan et al., 2012). In India, Samanta et al. (2018) proposed the biosecurity strategy for backyard poultry including daily cleaning of the utensils with ash, offering potable drinking water to birds, preparation of feed with boiled water, daily change of drinking water in the trough, a sprinkling of detergent water left after washing of clothes in the scavenging area, disposal of carcasses by garden burial, washing of the eggs, and storage of the eggs in a cold temperature maintained by indigenous structures.

4 Lack of veterinary health services: Although the requirement for veterinary health services in BPPS Is low, it is not easily available when required. In developing countries, because of a lack of resources and infrastructure in remote areas, cold chain facilities and vaccines are also not available to the farmers. All these adversely impact farmers' access to information regarding disease outbreaks, biosecurity measures, and timely availability of medicines and vaccines (Alders et al., 2010). To address these issues, it was suggested to form networks of community animal health workers, where training and information are exchanged between veterinarians and communities regarding vaccinations and disease control (Alders et al., 2010; Bagnol et al., 2013). Involving women in skill and training programs can have a positive impact on disease control and vaccination in the backyard poultry production system.

5 Lack of access to market: Backyard poultry production is mostly practiced in rural areas which are far away or poorly connected to the market. Although the produce from this system is natural or organic in nature, lack of access to the market prohibits the premium price to the farmers. The poultry and eggs from this system are generally sold in the local market in villages or towns where farmers do not get a better price. In the author's experience, when improved poultry germplasm was introduced for backyard production in a village, the availability of meat and eggs was considerably increased; however, the market price declined because every household has surplus produce. In this context, the co-operative model of marketing or making self-help groups and linking them with the urban market is a viable alternative. if market innovations are not adopted, there are chances that the BPPS will face fierce competition from commercial poultry producers as was the case in Thailand (NaRanong, 2007). Therefore, projects on improvement in productivity of backyard poultry must invariably include the forward market linkage of the producers.

6 Backyard poultry and zoonosis: Infectious diseases such as Highly pathogenic avian influenza A (H5N1) can be transmitted from poultry to humans and can cause lethal infection in humans (Shanta et al., 2017). Besides, poultry is a source of several pathogenic enteric bacteria that are of zoonotic importance; however, very little is known about the occurrence of zoonotic pathogens in backyard poultry. Pohjola et al. (2016) Reported that backyard chickens are a reservoir of *Campylobacter Jejuni* strains and also carry *L. monocytogenes*, *Campylobacter coli*, *Yersinia pseudotuberculosis*, and *Salmonella enterica* and non-pathogenic *Yersinia enterocolitica*. Backyard chickens have free access to the outdoors, which can increase the

risk of contact with zoonotic pathogens transmitted from wild birds and other animals. furthermore, the birds live in close contact with humans and other livestock and therefore increase the chance of direct or indirect spread of infection (Behravesh et al., 2014). Batz et al. (2012) Reported that *Salmonella*, *Campylobacter*, and *E. Coli* are the most important poultry and poultry meat-related foodborne biological hazards to public health. To reduce the risk of diseases spread from backyard poultry, it is important to educate and make aware all the stakeholders.

7 Backyard poultry as a source of antimicrobial resistance: Antimicrobial resistance (AMR) remains a growing threat to human and animal health. globally, over 70% of antimicrobials produced are used in food and animal production systems (Van Boeckel et al., 2019; Hedman et al., 2020). As backyard poultry production systems are generally practiced in resource-poor setting which involve zero to low input. In these resource-poor setting, antibiotics are not easily available, thereby minimizing the chance of their use (FAO, 2015, 2021; Wong et al., 2017). However, if available, there are high chances of indiscriminate use of antibiotics in these settings as farmers are not well-aware, and veterinary extension services are also poor (Barroga et al., 2020; Hedman et al., 2020). The use of antimicrobials can increase with an increase in intensification of the backyard production system and subsequent linkage with the market (Samanta et al., 2018). Similar findings were reported from other south asian countries (Coyne et al., 2019). In the philippines, Barroga et al. (2020) reported the use of critically important antimicrobials on backyard poultry farms. These family-operated micro-enterprises could potentially promote the risk of AMR and zoonosis exposure to community members due to the close proximity of production animals and surrounding human populations (Wuijts et al., 2017; Hedman et al., 2019). Hence, there is a need to educate the farmers regarding the use of antimicrobials in the backyard production system along with strengthening the animal health services. moving forward, a supportive environment will be needed, which includes regulations controlling use, improved systems for monitoring use, financial incentives, raising healthy chicks in stress-free environments, and minimum use of antimicrobials (Lhermie et al., 2017; FAO et al., 2021).

Conclusion

Backyard poultry farming provides food and nutritional security besides generating income and employment for the most vulnerable communities in developing countries. For them, backyard poultry is the first and last asset to be used in times of distress. In particular, they significantly improve the livelihood and food security of women, children, and the disabled. Backyard poultry production systems are known for their low productivity, which can be improved through the implementation of scientific measures in management, improvement in genetics, or improvement in health management. In recent times, the introduction of improved backyard poultry germplasm has revolutionized backyard poultry farming in Asia and Africa. These improved backyard poultry systems have characteristics similar to the native birds and are, therefore, preferred by rural farmers. The improved backyard poultry germplasm has given a ray of hope to the rural poor; however, there exist several constraints for these birds to realize their full potential. This includes but is not limited to the non-availability of improved germplasm, lack of skill, disease outbreaks, poor market linkages, and absence of veterinary health services. There is a need to focus on ecology-specific technology and to avoid the introduction of improved backyard germplasm in breeding tracts of native poultry. Involving the local community at every step of backyard poultry farming is the best approach to gain maximum from new technologies.

Author contributions

MS, RM, RP, and NP conceptualize and design the work. MS and RY wrote the first draft of manuscript. RY, VS, RKa, RKu, CS, MB, and SB review and edited the manuscript. DR and VM supervise the project and reviewed the manuscript. All authors viewed and approved the final draft of manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Revisiting the oldest manure of India, *Kunapajala*: Assessment of its animal waste recycling potential as a source of plant biostimulant

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India's oldest documented manure, most commonly referred to as *Kunapajala*, has a long history of over 1,000 years in crop cultivation. *Kunapajala* is primarily an *in-situ* decomposition technology of animal waste and can potentially provide an eco-friendly pipeline for recycling bio-waste into essential plant nutrients. This traditional animal manure, in addition, also contains dairy excreta (e.g., feces and urine), dairy products (e.g., milk and ghee), natural resources (e.g., honey), broken seeds or grains, and their non-edible by-product waste. Here, we aimed to assess the waste recycling and plant biostimulant potential of *Kunapajala* prepared from livestock (e.g., Black Bengal goats) or fish (e.g., Bombay duck) post-processed wastes over different decomposition periods, e.g., (0, 30, 60, and 90-days). In this study, an *in-situ* quantification of livestock- (*lKPJ*) and fish-based *Kunapajala* (*fKPJ*) reveals a dynamic landscape of essential plant primary nutrients, e.g., (0.70 > NH_4-N < 3.40 g•L^{-1}), (100.00 > P_2O_5 < 620.00 mg•L^{-1}), and (175.00 > K_2O < 340.00 mg•L^{-1}), including other physico-chemical attributes of *Kunapajala*. Using correlation statistics, we find that the plant-available nutrient content of *Kunapajala* depicts a significant ($p < 0.0001$)

transformation over decomposition along with microbial dynamics, abundance, and diversities, delineating a microbial interface to animal waste decomposition and plant growth promotion. Importantly, this study also reports the indole 3-acetic acid (IAA) content (40.00 > IAA < 135.00 mg•L^{-1}) in *Kunapajala*. Furthermore, the bacterial screening based on plant growth-promoting traits and their functional analyses elucidate the mechanism of the plant biostimulant potential of *Kunapajala*. This assay finally reports two best-performing plant growth-promoting bacteria (e.g., *Pseudomonas chlororaphis* and *Bacillus subtilis*) by the 16S ribotyping method. In support, *in-planta* experiments have demonstrated, in detail, the bio-stimulative effects of *Kunapajala*, including these two bacterial isolates alone or in combination, on seed germination, root-shoot length, and other important agronomic, physio-biochemical traits in rice. Together, our findings establish that *Kunapajala* can be recommended as a source of plant biostimulant to improve crop quality traits in rice. Overall, this work highlights *Kunapajala*, for the first time, as a promising low-cost microbial technology that can serve a dual function of animal waste recycling and plant nutrient recovery to promote sustainable intensification in agroecosystems.

KEYWORDS

***Kunapajala*, animal waste, waste recycling, nutrient recovery, plant growth regulators (PGRs), plant growth-promoting bacteria (PGPB)**

1. Introduction

To meet the increasing global demands for better quality food, intensified crop production in combination with large-scale livestock farming has been contributing to on-farm residue generation, including animal waste, at an astonishingly rapid growth rate in the modern era. India, as an example, produces about 683 million tones of crop residues per year of 10 major Indian crops (Bhattacharjya et al., 2019), while rice and wheat alone contributed around 300 million tones of residue generation recorded in the year 2017–2018 (Venkatramanan et al., 2021). The data on livestock, on the other hand, estimates ∼135 million tones of animal excreta generation per year in India, of which more than 90% are from cattle and buffalo sources (Bhattacharjya et al., 2019). In addition, India also accounts for 11% of goat meat and nearly 8% of fish production, ranking as the world's second and third largest producers, respectively (Norris and Smith, 2020). According to the 20th Livestock census data, livestock industries, including fisheries, contribute more than 25% of the total agriculture GDP in India, with an overall increase of 4.6% in production in the last 7 years. The post-processed by-products generated daily in these growing livestock and fish industries in India and the rest of the world, in turn, have led to a gigantic bio-waste accumulation of serious environmental concerns and seek immediate attention for careful management. Recent studies evaluated and discussed the scope of available biomass residues, including leftover agricultural residues and animal and municipal waste, in bioenergy production in India (Singh et al., 2022). In addition, biomass conversion into manures and compost can be an alternate mode of bio-waste management with a possible application in agriculture. Several studies confirmed that bio-waste, including crop residues and animal excreta, are potential sources of essential plant nutrients (e.g., N, P, K) and plant biostimulants (e.g., plant growth-promoting microbes, phytohormones, protein hydrolysates) and can be suitably recycled back into the agroecosystems (Huang et al., 2021). Therefore, the technological services in agriculture and allied sectors have become increasingly instrumental in transforming the circular economy associated with bio-waste management in recent times (Paes et al., 2019; Bakan et al., 2022). At present, advanced technologies, such as anaerobic digestion (AD), composting, algal-based sewage treatment and resource recovery systems (STaRR), are commercially available to promote the recycling of "waste-turn-into-wealth" and are reported to have tremendous potential to minimize the menace of waste hazards, posing environmental and public health risks (Onwosi et al., 2017; Abeysiriwardana-Arachchige et al., 2021; Cremonez et al., 2021). On a similar goal, the technologies that recycle crop residues, animal wastes, and human excreta can provide alternate options to convert bio-degradable solid waste into agricultural inputs in a sustainable way (Ahuja et al., 2020; Kelova et al., 2021; Greff et al., 2022). The main aim of these technologies, in general, is to maximize nutrient recovery and the fertilizer potential of derived by-products. In addition, there are also reports of indirect application-based robust methods for safe recycling in domestic sewage treatment, municipal, and agro-industrial bio-waste for further use in agriculture (Gross et al., 2021; Lin et al., 2021; Ravindran et al., 2021). These technologies, in summary, promote the waste recycling process, create an alternate nutrient cycle, and maintain soil health in agricultural lands, and as a result, can significantly reduce the adverse loads of synthetic fertilizers and pesticides in the agroecosystems.

In India, several traditional formulations have been documented for their application in crop farming for over a 1,000 years. *Kunapajala* (a Sanskrit word meaning filthy fluid) is an ancient innovation of animal waste recycling into agricultural inputs. This liquid animal manure, narrated originally in "*Vrikshayurveda*" by Surapala around 1,000 AD, is a formulation of decomposed animal waste such as bones, viscera, fins, and scales from fish waste or waste of crushed bones, skins, and flesh derived from livestock including cattle, goats, pigs, and sheep (Nene, 2018). In addition, locally available resources such as dairy excreta (e.g., cow dung and cow urine), broken or damaged pulse seeds (e.g., green gram or black gram), crop residues, or non-edible by-products obtained after oil extraction (e.g., rice husks and oil cakes), natural or forest resources (e.g., honey), dairy products (e.g., milk and ghee) are also used either as raw or processed materials and most commonly considered as enrichment agents or bulking materials in the *Kunapajala* preparation (Nene, 2012). Based on end-product trend analyses of animal waste decomposition (Ahuja et al., 2020; Brod and Øgaard, 2021), this low-cost animal manure is assumed to provide a rich source of plant nutrients, the majority of which are in the form of N, P, and K (Chakraborty et al., 2019). Hence, this technology of great potential can serve a dual purpose of sustainable animal waste management and an alternate mode of nutrient cycling in agriculture (Sorathiya et al., 2014). On the other hand, manure composition is dynamic and varies with changing farming circumstances and higher eco-social requirements (e.g., conventional vs. organic farming) (He et al., 2016). Thus, updated knowledge of the oldest manure of India is needed to optimize its recycling potential.

Kunapajala is also an abundant source of plant growth-promoting bacteria (PGPB) that could offer various benefits to its host plants, including nutrient availability, plant growth promotion, and control of pests and diseases (Chakraborty et al., 2019). Therefore, it has been recommended widely as a foliar spray or soil drenching for several crops, such as rice (*Oryza sativa*), mustard (*Brassica campestris*), and black gram (*Vigna mungo*), however with more emphasis on vegetables in India, such as okra (*Abelmoschus esculentus*), tomato (*Solanum lycopersicum*), chili (*Capsicum annuum*), and cowpea (*Vigna unguiculata*) (Mishra, 2007; Ali et al., 2012; Deshmukh et al., 2012; Sarkar et al., 2014; Kavya and Ushakumari, 2020). In addition, the *Kunapajala* formulation also showed a significant impact on the growth, physiological, biochemical, yield, and quality attributes of medicinal plants, *Ashwagandha* (*Withania sominifera*), and *Kalamegha* (*Andrographis paniculata*) (Ankad et al., 2017, 2018). However, despite in-depth physico-chemical and microbiological characterization of *Kunapajala* (Jani et al., 2017; Chakraborty et al., 2019) and its positive effect on plant growth, no attempt has been made, to date, to understand the population dynamics of microbes in *Kunapajala* and their functions in animal waste decomposition, nutrient recycling, and mineralization processes. In addition, functional screening of the PGPB isolates, owing to their combinatorial, bio-stimulative impact on the *Kunapajala* formulation in relation to plant growth and development, remains largely unexplored.

The present study describes the trend analyses of livestock- (*lKPJ*) and fish-based *Kunapajala* (*fKPJ*) throughout decomposition (e.g., 0, 30, 60, and 90-days) to maximize the recovery of resources in terms of available plant nutrients, plant growth regulator content, and as a source of the microbial niche contributing to plant growth-promoting traits such as IAA-production, N-fixation, P- and K-solubilization. We then screened bacteria for their efficiency in plant growth promotion and reported the best-performing isolates using the standard 16S ribotyping method. Finally, this study also assessed the plant biostimulant potential of *Kunapajala*, including these two bacterial isolates, on different growth stages of rice in a pot-based assay.

2. Materials and methods

2.1. Livestock and fish waste-derived *Kunapajala* preparation

In order to assess and compare various quality attributes, the preparation of *Kunapajala* and its components used in this study has been adopted based on the recommendation of Nene, a modified version of the ancient formulation (Jani et al., 2017; Nene, 2018). Here, the *Kunapajala* samples were prepared separately from two different sources of waste: livestock waste (e.g., Black Bengal goats: *Capra hircus*) collected from the slaughterhouse; and fish waste (e.g., Bombay duck: *Harpadon nehereus*) from a local fish market. Subsequently, these wastes were boiled and mixed with other ingredients to prepare the *Kunapajala* formulation (see Supplementary Table A1 for details). Briefly, livestock and fish waste (having a fresh weight of about 1.25 kg) with crushed bones, fins, skins, and marrows were boiled in 2.5 L of water at 100°C for an hour in two separate containers. After cooling down, the liquid residue was added to 5.0 L of water along with rice husks, available oil cakes (e.g., Mustard: *Brassica campestris*), and broken or damaged pulse seeds (e.g., Green gram: *Vigna radiata*). The other ingredients, such as cow dung, cow urine, cow milk, ghee, and honey, were then serially added in the amounts specified to these preparations and were adjusted with water to 50.0 L. The mixture was then kept in the container under shade at room temperature (25–30°C), stirred twice daily during the incubation, and the mouth of the container was further closed with a cloth to facilitate aeration, the prevention of houseflies from laying eggs, and uniform decomposition of the components. Finally, the liquid manures were collected with the help of a fine net and diluted to 100.0 L to prepare the *Kunapajala* formulation. In this study, the samples were collected for analysis at 30-day intervals, starting from 0-day to 90-days of incubation, e.g., (0, 30, 60, and 90-days).

2.2. The pH, electrical conductivity, organic C, total soluble protein, and macro-nutrient concentrations in the *Kunapajala* manure

The physico-chemical attributes of the *Kunapajala* preparation were determined by following the standard methods. In brief, after mixing with deionized water at a 1:100 (v/v) ratio, the pH and the electrical conductivity (EC) of the *Kunapajala* samples were measured using pH meter (Systronics Digital) and conductivity electrode, respectively. It is important to note that the deionized water has poor conductivity, typically in the range of 10^{-5}-10^{-6} mS•cm^{-1}, and is therefore assumed to have no influence on the EC measurement. The wet oxidation (Walkley and Black, 1934) and the Lowry (Waterborg, 2009) methods were used to estimate oxidizable organic C and total soluble protein (TSP) content, respectively.

The available N (NH_4-N) of *Kunapajala* was then determined using the standard alkaline-based Kjeldahl technique (Subbiah and Asija, 1956). Similarly, available P (P_2O_5) and K (K_2O) were determined by Olsen et al.'s (1954) and by the flame photometric (Jackson, 1973) methods, respectively.

2.3. The diversity analysis of the microbial community

To determine the microbial population, *Kunapajala* was serially diluted at the desired concentration and plated with three replications per Petri plate on appropriate culture media so that microbes grow as distinct colonies. Then, the colonies were counted according to standard microbiological norms to study and calculate the microbial dynamics (Mukherjee et al., 2022). The specific growth media for a diverse group of microbes, including their standard cultural conditions, are further enumerated in Supplementary Table A2. The bacteria that appeared as single colonies on specific growth media were streaked further to ensure the purity of cultures for various plant beneficial trait assays.

2.4. The efficacy assay of free-living N-fixers and P-solubilizing bacteria

Free-living N-fixers (FNFs) and P-solubilizing bacteria (PSB), thus obtained as pure colonies on culture plates, were cultured again in nutrient broth to study their plant beneficial traits. Now, to assess the ability of FNFs to fix atmospheric N, the bacteria were grown on a Luria-Bertani culture medium overnight, and then the freshly grown culture was inoculated again to Jansen's media. Finally, 8 mL of grown culture was collected after 7-days of incubation at 30°C for acid digestion to estimate the total N content by the Kjeldahl method (Subbiah and Asija, 1956). On the other hand, to determine the efficiency of PSB in solubilizing insoluble P sources, the diameter of the P-solubilization zone was taken as an indicative measurement during their growth on Pikovskaya's agar media at 30°C (Pikovskaya, 1948; Mukherjee et al., 2022). Furthermore, the efficiency of P-solubilization was again validated quantitatively by Olsen's extraction method (Olsen et al., 1954).

The best-performing FNF and PSB among these two classes of beneficial bacteria obtained in this screening were scored and compared based on their highest N-fixing and P-solubilizing attributes, respectively, and characterized further by subsequent 16S ribotyping and *in-planta* experiments.

2.5. Estimation of IAA production

The IAA content of the *Kunapajala* formulation was detected and quantified by the high-performance thin-layer chromatography (HPTLC) method (Goswami et al., 2015). Briefly, the *Kunapajala* extractants were first isolated using the ethyl acetate solvent. Then, the extractants were loaded on the TLC plate and air-dried. The spots thus formed on the TLC plate were scanned using Scanner 3 (Camag) in absorbance-reflectance mode at a 256 nm wavelength. In the case of the plant beneficial microbes, the bacterial isolates were grown on Luria-Bertani culture media with 0.10 g•L^{-1} of L-tryptophan. Then, 10 mL of grown culture was collected after 6- and 9-days of incubation to determine the total indole content by the standard Salkowski method (Salkowski, 1885).

2.6. Molecular identification of plant beneficial bacteria

Genomic DNA was extracted from two best-performing plant beneficial bacteria by the lysozyme-mediated lysis method (Moore et al., 2004) with a minor modification. This

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protocol, however, did not include a preheated NaCl/CTAB solution. Then, using the primers [Forward primer (27F): 5′AGAGTTTGATYMTGGCTCAG3′; Reverse primer (1492R): 5′TACCTTGTTAYGACTT3′], PCR was performed to amplify the 16S rDNA region of the selected bacteria (Edwards et al., 1989). Next, the PCR-amplified products were gel purified according to the manufacturer's protocol (QIAGEN). The purified rDNA fragments thus obtained were sequenced by the Sanger di-deoxy method, and the generated nucleotide sequences were further run to search for homology by the NCBI Nucleotide BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.7. A plant-based bioassay of *Kunapajala* and derived bacterial isolates of plant beneficial traits

Seeds of rice (*Oryza sativa*) from the Shatabdi (IET4786) variety were used in this experiment. Notably, Shatabdi is a popular, high-yielding rice variety cultivated in different agro-climatic zones of West Bengal, India. Studies on germination percentage and root-shoot length were executed by employing a plant-based assay based on the *Kunapajala* formulation and derived bacterial isolates. In this experiment, the selected seeds were first subjected to surface sterilization by stepwise washing with 70% ethanol and 0.5% hydrogen peroxide solutions for 3 min each, followed by 5 rinses in sterile distilled water. Next, to determine the effect of *Kunapajala* on germination and other agronomic parameters, sterilized seeds were further soaked and treated with seed priming agents for 6 h. The seed priming agents used in this study were 1% *lKPJ* and *fKPJ* formulations after different days of decomposition (e.g., 0, 30, 60, and 90-days), best FNF, and PSB isolated from the *Kunapajala* preparation, and sterile distilled water as a control. In the case of the best FNF and PSB, a fixed number of bacterial cells was counted ($\sim 3 \times 10^{10}$ cells in 30 mL of sterilized distilled water) and used to treat the seeds. It is important to note that the experiment comprises five replications with 100 seeds per replication. The study on germination percentage was performed on Petri plates under 10 h of light and 14 h of darkness for 7 consecutive days at 25–30°C. The emergence of root-shoot length, including the germination percentage, was carefully observed, recorded, and measured during this period.

The plant growth-promoting abilities of *Kunapajala* and derived bacterial isolates were evaluated on transplanted rice seedlings in a pot-based experiment. In this experiment, three healthy rice seedlings with a root length of about 3 mm were selected randomly from Petri plates and transplanted further in each earthen pot filled with 2.5 kg of air-dried and autoclaved soil. The details of the nutrient content and physico-chemical and microbiological properties of the agricultural soils used in this study are available in Supplementary Table A3. In this study, we followed a foliar spray of the *Kunapajala* formulation (e.g., @1% v/v) every 15-day interval up to the flowering stage of rice while considering the plant nutrient content and an earlier recommendation of *Kunapajala* (Mishra, 2007).

2.8. Agronomic and physico-biochemical attributes of rice seedlings

Shoot height, root length, fresh weight, and dry weight of shoots and roots of a tiller were recorded at every 15-day interval starting from 45-days after transplanting to till harvesting. In addition, other standard agronomic attributes such as the number of tillers per plant, the number of active tillers per plant, the number of grains per panicle, the chaffy: filled grain ratio, the 1,000 seed weight, and yield per plant after harvest were also examined and recorded.

Further, the content of the photosynthetic pigments, such as chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content, was estimated using the standard spectrophotometric method. Briefly, 50 mg of freshly chopped leaves were dipped in 80% acetone in dark conditions for 72 h at 10°C, followed by their intensity measurement at 645 and 663 nm wavelengths. All the quantitative calculations to estimate chlorophyll content follow the standard formula (Tao et al., 2022).

On the other hand, to determine the total carbohydrate and soluble protein content of different plant parts, roots, shoots, and leaves were collected and finely chopped into smaller pieces. Then, 50 mg of finely chopped plant parts were further acid-hydrolyzed to estimate the carbohydrate content by the standard colorimetric method (Jain et al., 2017). In the case of total protein content estimation, the same amount of plant parts was dissolved in phosphate-buffered saline (PBS) and further analyzed by following the Lowry method (Lowry et al., 1951).

2.9. Statistical analysis

Samples collected after different incubation periods were analyzed, with $n = 3$ in each case unless otherwise specified in the *in-planta* experiment (i.e., $n = 5$). The study used Dunnett's tests as a multiple comparison procedure for a significant One-way ANOVA. When the data breached test assumptions (e.g., non-normal data), we employed the non-parametric Kruskal-Wallis test as an alternative to One-way ANOVA. In that case, pairwise comparisons using Dunn's test were used to compare statistical differences among treatments. Significance values were further adjusted by the Bonferroni correction for multiple tests. Analyses were performed in SPSS for Windows 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Similarly, Pearson's correlation coefficient analysis was performed by a computer-based STAR program (http://bbi.irri.org/products).

3. Results

3.1. Biomass-degrading bacteria: An implication in the *Kunapajala* technology to recycle animal waste

To explore the microbial action of livestock and fish waste degradation in *Kunapajala*, we initially studied the microbial dynamics of proteolytic, lipolytic, and starch-hydrolyzing bacteria, henceforth named biomass-degrading bacteria, based on their colony-forming unit (CFU) on appropriate nutrient media (Supplementary Table A2). The idea to examine the dynamics of the starch-hydrolyzing bacterial isolates is due to the crop residues, including rice husks and cow dung, which are present in the *Kunapajala* formulation. In this study, we observed an elevated level of the biomass-degrading bacterial population (Table 1). However, till 90-days of incubation, there was no reduction observed in the microbial population specifically for this class of bacteria, except for the starch-hydrolyzing bacteria, which showed a decline in the population (Table 1). Overall, our data reveal that the trend in the population dynamics of the biomass-degrading bacteria is amazingly similar in *lKPJ* and *fKPJ*.

To monitor the decomposition process and depolymerization of organic matter subsequently, organic C and TSP were estimated at every 30-day interval till 90-days of incubation. The result indicates that the level of organic C is gradually depleted significantly ($p < 0.0001$) over time, at least up to 60-days, in both *lKPJ* and *fKPJ* (Table 1). It is important to note that the initial concentration of the organic C (in percent value) in two different *Kunapajala* formulations is in nearly identical ranges (*lKPJ*: 1.95 ± 0.01%, and *fKPJ*: 1.79 ± 0.03%). Similarly, the initial TSP content ($g{\bullet}L^{-1}$) of the *lKPJ* and *fKPJ* formulations ranges between 3.58 ± 0.02 and 3.98 ± 0.14, respectively, and it also shows a similar trend of gradual depletion over a period of incubation. The correlation statistics further establish a positive interaction between microbial growth and animal waste decomposition in the *Kunapajala* technology (Supplementary Table A4). Together, these data indicate a possible role of microbial action as evident in the abundance of biomass-degrading bacteria in the initial steps of animal waste degradation in *Kunapajala*.

Besides, other factors such as pH, EC, moisture content, aeration, and temperature are critical in regulating microbial activity and thus may determine the waste decomposition rate in the *Kunapajala* system (Rastogi et al., 2020). The study revealed that the pH in both of these formulations increased significantly ($p < 0.0001$) from the initial acidic pH (*lKPJ*: 4.70 ± 0.01, and *fKPJ*: 4.47 ± 0.02) to nearly neutral (*lKPJ*: 7.17 ± 0.06, and *fKPJ*: 6.87 ± 0.06) over decomposition (Table 1). The EC data ($mS{\bullet}cm^{-1}$) in *lKPJ* and *fKPJ*, on the other hand, range from 0.31 ± 0.003 to 1.21 ± 0.03 and 0.36 ± 0.01 to 1.29 ± 0.01, respectively, and show no adverse fluctuation to a higher value over incubation (Table 1). Based on physico-chemical and microbiological analyses of *Kunapajala*, this study highlights for the first time a comprehensive nutrient-microbe network and further reports an amazingly uniform decomposition pattern in *Kunapajala*, irrespective of animal waste source and heterogeneity.

3.2. *Kunapajala* is the source of essential plant nutrients: An *in-situ* resource recycling mode to promote the nitrogen, phosphorus, and potash cycles in agroecosystems

In this study, we further assessed the bio-fertilizer potential of *Kunapajala* in terms of its plant nutrient concentration. An earlier report indicates *Kunapajala* formulation as a rich source of plant nutrients based on 40-days of decomposition (Chakraborty et al., 2019). Here, we have extended the earlier observation and evaluated, in particular, the plant nutrient content (e.g., available N, P, and K) of *Kunapajala* at every 30-day sample till 90-days of incubation. Our data indicate that the concentration of NH_4-N and P_2O_5 increases significantly ($p < 0.0001$) at 60-days of waste decomposition in both the *lKPJ* and the *fKPJ* (Table 1). In comparison over different incubation periods, our study established that the overall concentration of NH_4-N, P_2O_5, and K_2O reaches its optimum range at 60-days of incubation in *lKPJ* and *fKPJ* (Table 1). These data, in fact, correlate with the uniform animal waste decomposition, including the population dynamics of biomass-degrading bacteria (Supplementary Table A4). These results support a previous report (Chakraborty et al., 2019) that the *Kunapajala* manure is a rich source of plant nutrients, especially as a plant-available N, P, K (0.70 > NH_4-N < 3.40 $g{\bullet}L^{-1}$; 100.00 > P_2O_5 < 620.00 $mg{\bullet}L^{-1}$; and 175.00 > K_2O < 340.00 $mg{\bullet}L^{-1}$). In summary, this study presents *Kunapajala* as a natural resource restoration technology that may offer enormous potential to provide an alternate source of plant primary nutrients in modern-day agriculture, considering an incremental mining operation followed by the gradual exhaustion of natural resource-based rock phosphate and potash mines and their limited availability in recent years.

TABLE 1 The microbiological and physico-chemical properties of *Kunapajala* after different days of incubation.

Treatment	*lKPJ*				*fKPJ*			
	0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days
I. Microbiological parameters								
Total bacteria ($\times 10^{10}$ CFU•ml^{-1})	3.33 ± 0.09a	2.77 ± 0.15b	3.27 ± 0.19a	2.80 ± 0.17b	0.80 ± 0.21c	0.93 ± 0.09c	0.33 ± 0.13d	0.33 ± 0.03d
Total proteolytic bacteria ($\times 10^{9}$ CFU•mL^{-1})	0.45 ± 0.01h	0.69 ± 0.01f	0.79 ± 0.01d	0.84 ± 0.01c	0.52 ± 0.01g	0.72 ± 0.01e	0.91 ± 0.01b	0.94 ± 0.003a
Total lipolytic bacteria ($\times 10^{8}$ CFU•mL^{-1})	0.22 ± 0.01e	0.66 ± 0.003b	0.77 ± 0.01a	0.76 ± 0.01a	0.14 ± 0.01f	0.54 ± 0.01d	0.66 ± 0.01b	0.61 ± 0.01c
Total starch-hydrolyzing bacteria ($\times 10^{9}$ CFU•mL^{-1})	0.10 ± 0.01f	0.47 ± 0.02c	0.60 ± 0.02a	0.29 ± 0.01e	0.12 ± 0.01f	0.36 ± 0.01d	0.55 ± 0.01b	0.40 ± 0.003d
Total FNFs ($\times 10^{8}$ CFU•mL^{-1})	1.00 ± 0.12e	2.23 ± 0.09b	2.93 ± 0.12a	3.20 ± 0.001a	0.23 ± 0.07g	0.67 ± 0.09f	1.70 ± 0.17c	1.37 ± 0.09d
Total *Rhizobium* bacteria ($\times 10^{6}$ CFU•mL^{-1})	2.67 ± 0.67cd	8.33 ± 1.45b	12.33 ± 0.89a	3.00 ± 0.58cd	1.33 ± 0.33d	2.33 ± 0.33cd	4.00 ± 0.58c	4.67 ± 0.33c
Total PSB ($\times 10^{8}$ CFU•mL^{-1})	0.70 ± 0.06cd	0.97 ± 0.03bcd	1.30 ± 0.12b	1.37 ± 0.03b	0.60 ± 0.12d	1.13 ± 0.09bc	2.03 ± 0.09a	2.40 ± 0.31a
Total KSB ($\times 10^{5}$ CFU•mL^{-1})	0.00 ± 0.00d	0.37 ± 0.03bc	3.43 ± 0.18a	0.43 ± 0.03b	0.00 ± 0.00d	0.33 ± 0.03bc	0.43 ± 0.03b	0.17 ± 0.03cd
Total *Pseudomonas* bacteria ($\times 10^{9}$ CFU•mL^{-1})	0.30 ± 0.06d	0.60 ± 0.06c	3.07 ± 0.18b	3.40 ± 0.12a	0.08 ± 0.003d	0.16 ± 0.01d	0.19 ± 0.003d	0.24 ± 0.02d
Total actinomycetes ($\times 10^{10}$ CFU•mL^{-1})	0.46 ± 0.01b	0.16 ± 0.02c	0.08 ± 0.01de	0.13 ± 0.01cd	0.53 ± 0.05a	0.16 ± 0.02c	0.07 ± 0.003de	0.05 ± 0.01e
Total fungi ($\times 10^{6}$ CFU•mL^{-1})	2.20 ± 0.12b	4.27 ± 0.09a	0.60 ± 0.06de	0.27 ± 0.03e	1.23 ± 0.07c	1.37 ± 0.07c	1.47 ± 0.20c	0.70 ± 0.15d
II. Physico-chemical parameters								
Organic C (%)	1.95 ± 0.01a	1.81 ± 0.02b	1.72 ± 0.01c	1.68 ± 0.003c	1.79 ± 0.03b	1.71 ± 0.01c	1.68 ± 0.02c	1.61 ± 0.03d
TSP (g•L^{-1})	3.58 ± 0.02b	3.00 ± 0.09cd	2.74 ± 0.02d	2.38 ± 0.03e	3.98 ± 0.14a	3.47 ± 0.11b	3.03 ± 0.08c	2.96 ± 0.06cd
pH	4.70 ± 0.01f	5.79 ± 0.01e	7.01 ± 0.02b	7.17 ± 0.06a	4.47 ± 0.02g	5.96 ± 0.02d	7.25 ± 0.003a	6.87 ± 0.06c
EC (mS•cm^{-1})	0.31 ± 0.003f	0.42 ± 0.02d	1.21 ± 0.03b	0.42 ± 0.003d	0.41 ± 0.001d	0.46 ± 0.01c	1.29 ± 0.01a	0.36 ± 0.01e
Available N (g•L^{-1})	0.88 ± 0.07e	2.35 ± 0.05d	2.69 ± 0.05c	2.91 ± 0.04b	0.77 ± 0.05e	2.58 ± 0.04c	2.73 ± 0.05c	3.25 ± 0.07a
Available P (mg•L^{-1})	247.24 ± 14.93e	500.99 ± 14.55b	613.88 ± 2.53a	489.62 ± 22.81b	109.63 ± 5.18f	263.60 ± 8.52e	367.00 ± 8.31d	414.93 ± 12.83c
Available K (mg•L^{-1})	265.44 ± 1.80c	219.90 ± 1.15d	222.50 ± 1.43d	190.08 ± 1.65e	329.77 ± 3.16a	265.16 ± 1.12c	319.69 ± 0.44b	178.74 ± 1.02f

Duncan's multiple range test was used to analyze the data, which are represented as the mean ± standard error of samples ($n = 3$), followed by different letters indicating that the difference was statistically significant ($p < 0.05$). The results with the same letters were not significantly different ($p > 0.05$).

3.3. Screening and performance of plant beneficial bacteria isolated from the *Kunapajala* formulation

We next sought to study the growth dynamics of PGPB, such as FNFs and symbiotic N-fixers (e.g., *Rhizobium* species), along with PSB and K-solubilizing bacteria (KSB). We observed that the PGPB population ranges between 10^7 and 10^8 CFU•mL^{-1} in the *Kunapajala* formulation, except for the KSB (Table 1). The population density of *Pseudomonas* species was reported to be in the 10^8-10^9 CFU•mL^{-1} range, while the KSB and *Rhizobium* species, on the other hand, were in the ranges of 10^4-10^5 and 10^6 CFU•mL^{-1} count, respectively (Table 1). In most cases, there is a significant ($p < 0.0001$) and highest bacterial population observed at 60-days of decomposition, irrespective of the source of animal waste. In addition, our data also confirm that the microbial population of plant beneficial traits exhibits temporal variation in the effects of animal waste source and their decomposition over different days of incubation (Table 1).

The plant beneficial bacteria that appeared were further purified and screened based on their abilities to fix atmospheric N and P-solubilization. We also assume that these PGPB isolates are the most abundant species, given their appearance on their respective culture media after serial dilutions of at least the 10^{-7} range. In this study, 16 different bacteria (e.g., 9 FNFs and 7 PSB) were screened and selected based on their N-fixation or P-solubilization and evaluated further for their performance owing to other plant growth promotion abilities. We observed that 6 FNFs (designated as KP49, KP52N, KP60, KP82A, KP85, and KP113) showed the highest level of N-fixation ($1.05 \pm 0.18 >$ NH_4-N $< 2.87 \pm 0.12$ mg•g^{-1}), and the rest 3 bacterial isolates (e.g., KP51, KP109A, and KP109B), however, failed to fix above 1.00 mg•NH_4-N•g^{-1} of sugar consumed (Figure 1). In the case of the PSB, 4 bacterial isolates, such as KP2, KP52P, KP38, and KP19, show a maximum level of inorganic P-solubilization *in vitro* in both qualitative and Olsen's methods. These bacterial isolates can solubilize inorganic P within the range of $46.39 \pm 6.25 >$ P_2O_5 $< 240.69 \pm 18.28$ mg•mL^{-1} after 14 days of their growth in the culture media (Figure 1). Finally, we report two best-performing PGPB (e.g., KP85 and KP19) among these 16 isolates based on their highest N-fixing and P-solubilization abilities. These data, together, reflect a microbial pool of plant beneficial bacteria and their contribution to the plant nutrient niche of *Kunapajala*.

3.4. *Kunapajala* and derived plant beneficial bacterial isolates are efficient sources of IAA production

In this study, we report that *Kunapajala* is also a potent source of IAA ($41.00 \pm 1.00 >$ IAA $< 132.33 \pm 1.53$ mg•L^{-1}), and the concentration of IAA in the *lKPJ* and *fKPJ* formulations reached the highest peak after 30- and 60-days of incubation, respectively (Figure 2). The dynamic plot revealed that the IAA concentration depletes sharply after 30-days of decomposition in *lKPJ*. In the case of *fKPJ*, however, the concentration of IAA gradually increases up to 60-days of incubation and then declines upon further incubation (Figure 2). Further, we also observed that the IAA concentration and its dynamics are not directly related to the soluble protein content of *Kunapajala* and the population of proteolytic bacteria that decompose proteins into peptides and free amino acids (Supplementary Table A4).

The other source of IAA in the *Kunapajala* formulation could be microbial *in-vivo* IAA synthesis, as bacteria and fungi can also produce IAA besides plants (Spaepen et al., 2007). To elucidate the role of bacteria in IAA production in the *Kunapajala* formulation, we studied the selected FNFs and PSB to evaluate their ability to synthesize IAA. All 16 bacterial isolates (designated KP49, KP51, KP52N, KP60, KP82A, KP85, KP109A, KP109B, and KP113 from FNFs; and KP2, KP15, KP17, KP19, KP38, KP52P, and KP72 from PSB) were confirmed to have indolic compound synthesis ability *in vitro* (Figure 2). However, upon screening 16 different bacterial isolates, there was no bias in the distribution of the magnitude of the IAA-production ability, irrespective of their N-fixation and P-solubilization attributes. These bacterial isolates were studied further for their ability to produce IAA (in µg•mL^{-1}) after 6- and 9-days of growth in LB culture media with 0.10 g•L^{-1} of L-tryptophan (see section Materials and methods). It confirms an overall improvement in IAA synthesis (either $p < 0.05$ or $p < 0.01$) after 9-days of growth in most bacterial strains (Figure 2). These results strongly indicate that the bacterial isolates from the class of FNFs and PSB also contribute to the IAA reservoir of the *Kunapajala* formulation.

3.5. Molecular identification of the best FNF and PSB isolated from *Kunapajala*

The two best-performing bacterial isolates mentioned earlier were identified further by the 16S rDNA amplicon sequencing technique (Table 2). In this method, the Sanger sequencing reads were obtained for their high-quality sequence coverage by both primers running reversibly. The sequence data thus obtained were joined together to have at least 1.3 kb rDNA sequence coverage by assembling overlapping sequence reads. Finally, the NCBI BLAST program revealed that the best FNF (KP85) and PSB (KP19) show strong sequence homologies with *Pseudomonas chlororaphis* subsp. *aurantiaca* and *Bacillus subtilis* subsp. *subtilis*, respectively (Table 2).

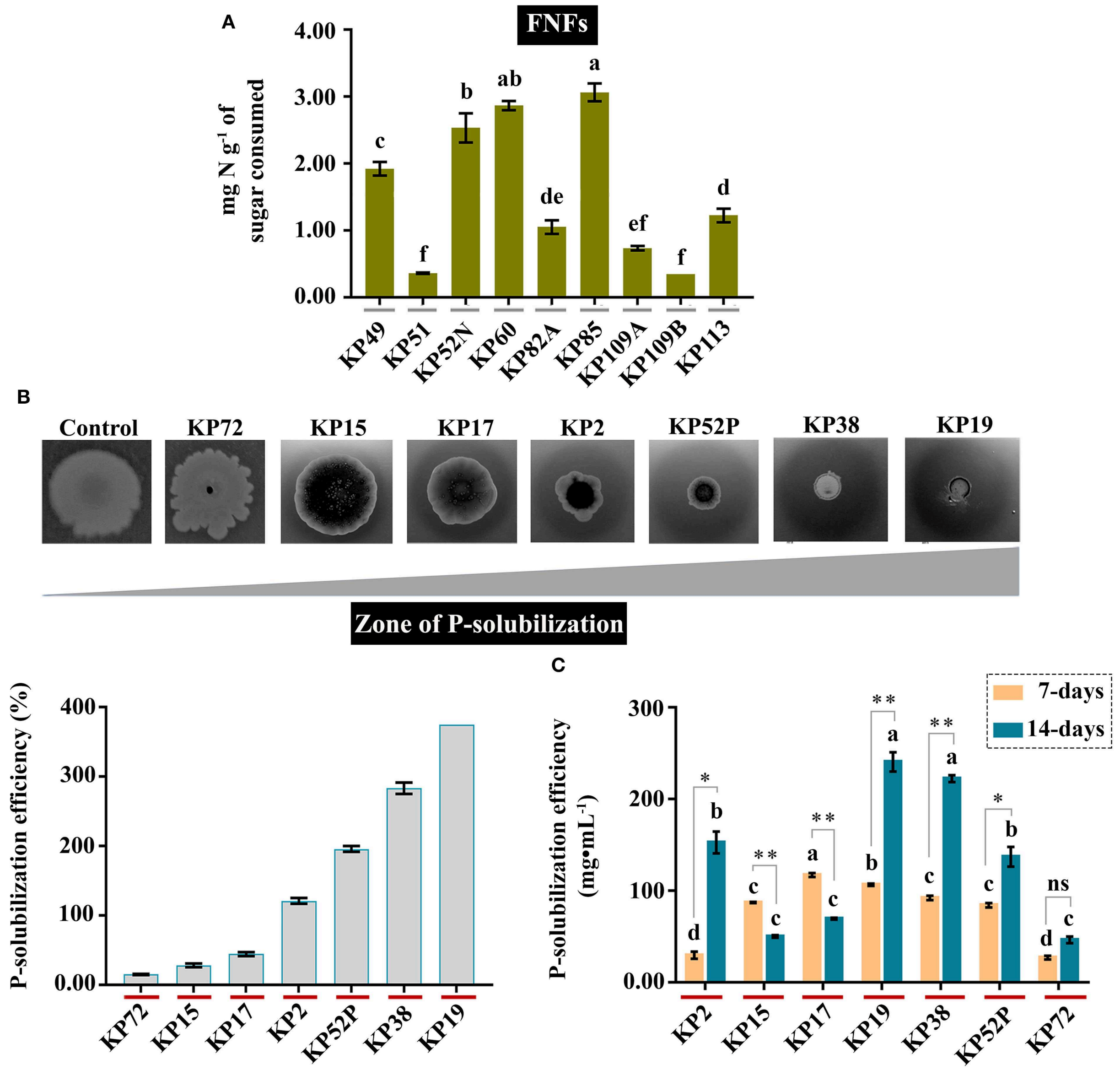

FIGURE 1
An assay to determine the N-fixing and P-solubilizing efficiency of PGPB isolates from the *Kunapajala* formulation. **(A)** The ability of various FNFs to fix N from the atmosphere. The bacterial isolates were grown in Jensen's media and measured further for their N-fixing efficiency by the Kjeldahl method. The *y*-axis denotes the amount of total N fixed in mg•g^{-1} of sucrose consumed. **(B, C)** The P-solubilization ability of PSB. **(B)** The bacterial isolates were spotted on Pikovskaya's media and incubated for 21 days to measure the zone of P-solubilization. The zone of P-solubilization efficiency (in percent value) was calculated further and plotted. The colonies in the figure are not drawn in scales. **(C)** The bacterial isolates were grown in liquid Pikovskaya's media, and their P-solubilizing efficiency was measured by the standard spectrophotometric method. The *y*-axis denotes the amount of phosphorus in mg•mL^{-1}. Here, different letters indicate a statistically significant difference ($p < 0.05$), and asterisks indicate statistical significance. *$p < 0.05$; **$p < 0.01$; ns, non-significant.

3.6. The biostimulant effects of the *Kunapajala* formulation on rice seedlings: An agronomic and physio-biochemical trait analysis approach

This study discussed the *Kunapajala* technology so far in terms of its roles in waste recycling, contribution to nutrient cycles, as a source of plant nutrients, plant growth regulators, and plant beneficial bacteria. These observations stimulate immediate follow-up experiments on the biostimulant effects of this technology on the whole-plant and tissue levels, addressing various agronomic and physio-biochemical traits of rice seedlings. Here, we analyzed, in total, 11 agronomic and 3 physio-biochemical traits in rice, including

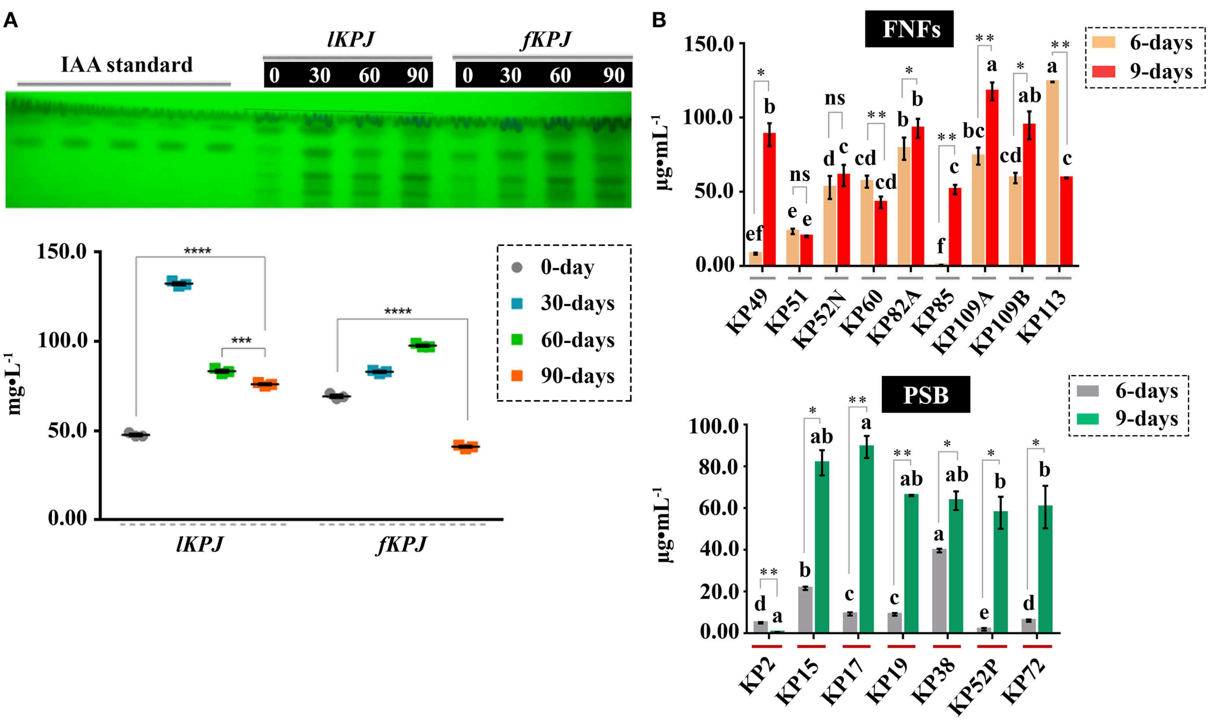

FIGURE 2
Kunapajala may act as a potential source of IAA. **(A)** A dynamic plot of the IAA content of *Kunapajala*. The IAA concentration (in mg•L^{-1}) was estimated from the *Kunapajala* formulation after different incubation periods by the HPTLC technique. The *y*-axis denotes the value of three replicates with a standard error of the mean (SEM). **(B)** The indolic compound synthesis ability of FNFs and PSB. The total indolic compound (in µg•mL^{-1}) was analyzed with three replicates by growing the FNFs and PSB on LB media with 0.10 g•L^{-1} of tryptophan after 6- and 9-days of incubation. In this figure, different letters indicate a statistically significant difference ($p < 0.05$), and asterisks indicate statistical significance. *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$; ****$p < 0.0001$; ns, non-significant.

germination percentage, root-shoot height and weight, yield, total carbohydrates, total proteins, and chlorophyll content. The results revealed a significant difference ($p < 0.0001$) observed among the treatments of the *lKPJ* and *fKPJ* formulations prepared during different decomposition periods (Table 3). We further showed that rice seedlings treated with *Kunapajala* formulations after 60-days of incubation perform better in their crop quality traits, including yield (Table 3). A trend in the dynamics of almost 14 agronomic and physio-biochemical traits in rice, irrespective of animal waste source, reflects a gradient in the plant biostimulant potential of *Kunapajala* formulations at least up to 60-days of incubation. This study, in support, indicates a uniform decomposition pattern and microbial dynamics in both *lKPJ* and *fKPJ*, owing to their combinatorial impacts on plant biostimulant potential. However, the *fKPJ* formulation, in essence, acts better to improve the agronomic traits (Table 3). We next sought to study the plant biostimulant potential of the two best-performing bacterial isolates from the *Kunapajala* formulation. These isolates, alone or in combination, show the best result in crop yield and further quality improvement in rice (Table 3). In conclusion, our study establishes that the *Kunapajala* formulation, in addition to the derived bacterial strains, could be recommended as a source of plant biostimulants in rice fields.

4. Discussion

4.1. Biodegradable solid waste management and the nutrient potential of *Kunapajala*

The biomass of livestock and fish tissues is in huge global demand as a high-value nutritive source in the human diet. However, the vast amounts of waste generated due to the processing of meat or fish products, either in the fish market or slaughterhouses, respectively, is a growing problem in the urban-based modern world. It recently appeared that 70 and 60% of processed fish and meat products lead to abattoir waste not fit for human consumption (Natalia et al., 2022). The waste management practice generally employs a segregation strategy that converts a substantive portion of this animal waste into the rendering process as poultry or fish feed (Ominski et al., 2021). The remaining waste, in general, is often disposed of in

TABLE 2 Molecular identification of the best performing bacterial isolates reported from the *Kunapajala* formulation.

Bacterial isolate	GenBank accession code	Fragment length (bp)	Closest bacterial strains	Sequence identity (%)
KP85	OM698823	1,384	*Pseudomonas chlororaphis* subsp. *aurantiaca*	99.85
KP19	OM698822	1,341	*Bacillus subtilis* subsp. *subtilis*	100.00

incineration or landfills, concerning serious hygienic issues and public health. An estimation of over 40 tones of animal waste of daily disposal in India seeks an immediate strategic plan to address the daunting task of solid waste management. In this paper, we have explored the benefits of microbial application in animal waste recycling while revisiting the oldest *Kunapajala* manure technology. Our results have shown that the presence of biomass-degrading bacteria in the *Kunapajala* technology speeds up the animal waste degradation process, causing 10–14% organic C depletion and 25–35% TSP decomposition after 90-days of incubation. To support this further, we also observed an overall consistent enrichment of biomass-degrading bacteria within the 10^7-10^8 range (CFU•mL^{-1}) throughout the decomposition period. This bacterial niche is, in fact, likely to release several hydrolytic enzymes (e.g., starch-hydrolyzing, proteolytic, and lipolytic) to favor decomposition (Table 1). In support, *Bacillus subtilis* subsp. *subtilis*, isolated and characterized from *Kunapajala* in this study, has been previously reported to secrete amylases, proteases, and lipases (Latorre et al., 2016; Su et al., 2020). Together, it reflects the microbial degradative capacity in *Kunapajala*, which has turned out to be crucial at the initial stages of the decomposition process. Based on these data, we assume that the *Kunapajala* technology might provide promising applications in animal waste management.

Animal wastes in the *Kunapajala* preparation, as stated in other animal manures (Chen et al., 2019), should include complex biomasses of carbohydrates, proteins, lignin, and fat. Consequently, the hydrolysis of these complex bio-molecular fractions often generates a valuable and concentrated source of water-soluble plant nutrients and biologically stable humic substances, which can be added directly to agricultural fields as soil amendments (Huang et al., 2021). In this study, we report the bio-fertilizer potential of the *Kunapajala* manure in terms of plant-available nutrient conversion recovery and its overall content. In addition, the EC profile in *Kunapajala*, along with neutral pH after 60-days of decomposition, indicates that it may positively stimulate soil processes, biological activity, and overall impact on ecological health when applied to soils (Kumar and Sharma, 2020). This technology, therefore, could provide an eco-friendly pipeline to create a nutrient cycle both *in-situ* and *ex-situ* (Figure 2). The nutrient cycle, in general, is a natural mode of resource management that becomes crucial to balance nutrient recycling and ecosystem health (Harindintwali et al., 2021). Not surprisingly, nutrient mineralization, mobilization, further uptake by crops, and return to soil organic matter provide a foundation for soil health and sustainable crop cultivation. According to IFOAM-Organics International (https://www.ifoam.bio/sites/default/files/2020-03/poa_english_web.pdf), organic farming practices nurture these naturally occurring cycles and boost their ecological benefits, either through agronomic practices or various improved technologies, to achieve sustainable nutrient management (Lorenz and Lal, 2023). The *Kunapajala* technology as a low-cost valorization approach of animal waste into high-value plant biostimulant may thus appear to act significantly in maintaining soil health and promoting sustainable agriculture.

The safety of the direct application of animal manure to agricultural fields also depends on the toxicity level of heavy metal contamination. Studies predict a high chance of heavy metals cross-transfer to crops, as the concentration above the threshold level could be an alarming issue in animal waste management (da Rosa Couto et al., 2018; Zheng et al., 2022). However, the bio-availability of heavy metals in composts and manures was reportedly lesser in concentration than in anaerobic digestate (Zheng et al., 2022). Therefore, immediate follow-up studies are needed to explore at least two practical aspects of the *Kunapajala* formulation: (i) periodic monitoring of the quality testing parameter of harmful substances, in particular, heavy metal contamination in the *Kunapajala* samples; and (ii) accelerate the decomposition rate of animal waste in combination with physical, chemical, or biological treatments. In continuation, future studies may look at the possibility of the black soldier (*Hermetia illucens*) fly larvae (Lalander et al., 2019; Bortolini et al., 2020) as a biological tool in the *Kunapajala* formulation to speed up the decomposition process.

4.2. The plant biostimulant potential of *Kunapajala* and modes of plant growth promotion

The eco-friendly approach of crop cultivation to improve plant biomass and yield is a top priority worldwide in modern-day agriculture. The use of microbial plant biostimulants, such as the beneficial PGPB, to promote climate-resilient

TABLE 3 The effects of the *Kunapajala* formulation and two bacterial isolates on various agronomic and physio-biochemical traits of rice.

Treatment		Control	*lKPJ*				*fKPJ*				FNF and PSB		
			0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days	KP85	KP19	KP85+ KP19
GP (%)		64 ± 2.45de	54 ± 2.45e	54 ± 2.45e	74 ± 2.45cd	68 ± 2.00cd	64 ± 2.45de	72 ± 2.00cd	86 ± 2.45ab	70 ± 4.47cd	74 ± 5.10cd	94 ± 2.45a	78 ± 5.83bc
SL (cm)	7 DAS	0.54 ± 0.01g	0.54 ± 0.01g	0.63 ± 0.01f	0.77 ± 0.01d	0.64 ± 0.02f	0.58 ± 0.03g	0.71 ± 0.02e	0.85 ± 0.01c	0.67 ± 0.01ef	0.92 ± 0.02b	0.82 ± 0.03cd	0.99 ± 0.02a
	14 DAS	1.98 ± 0.05h	1.86 ± 0.06h	2.54 ± 0.02ef	2.84 ± 0.04c	2.40 ± 0.08f	2.14 ± 0.07g	2.66 ± 0.04de	3.42 ± 0.07b	2.74 ± 0.04cd	3.46 ± 0.04b	2.70 ± 0.03cd	3.70 ± 0.06a
	21 DAS	2.27 ± 0.01hi	2.25 ± 0.01hi	2.34 ± 0.05gh	2.80 ± 0.03e	2.18 ± 0.06i	2.41 ± 0.05fg	2.52 ± 0.04f	3.94 ± 0.04c	3.05 ± 0.07d	4.21 ± 0.03b	4.13 ± 0.05b	4.50 ± 0.02a
	28 DAS	2.62 ± 0.01h	2.56 ± 0.01h	3.00 ± 0.05f	3.41 ± 0.05d	2.92 ± 0.05fg	2.82 ± 0.05g	2.96 ± 0.02fg	4.15 ± 0.09c	3.18 ± 0.07e	4.44 ± 0.01b	4.42 ± 0.01b	5.01 ± 0.02a
RL (cm)	7 DAS	1.91 ± 0.04f	1.28 ± 0.04h	1.60 ± 0.08g	1.88 ± 0.04f	1.22 ± 0.05h	2.26 ± 0.04e	2.52 ± 0.07d	3.44 ± 0.15a	2.24 ± 0.05e	2.84 ± 0.02c	2.72 ± 0.04c	3.24 ± 0.07b
	14 DAS	1.98 ± 0.05h	1.86 ± 0.06h	2.54 ± 0.02ef	2.84 ± 0.04c	2.40 ± 0.08f	2.14 ± 0.07g	2.66 ± 0.04de	3.42 ± 0.07b	2.74 ± 0.04cd	3.46 ± 0.04b	2.70 ± 0.03cd	3.70 ± 0.06a
	21 DAS	2.99 ± 0.03e	2.25 ± 0.01ij	2.34 ± 0.05hi	2.80 ± 0.03f	2.18 ± 0.06j	2.41 ± 0.05gh	2.52 ± 0.04g	3.94 ± 0.04d	3.05 ± 0.07e	5.14 ± 0.05b	4.72 ± 0.02c	5.52 ± 0.01a
	28 DAS	3.38 ± 0.05h	3.54 ± 0.02gh	3.46 ± 0.04h	4.02 ± 0.10e	3.68 ± 0.02fg	3.50 ± 0.03gh	3.84 ± 0.17f	4.72 ± 0.06d	3.74 ± 0.02f	5.23 ± 0.01b	5.02 ± 0.04c	5.76 ± 0.02a
FW (g)	7 DAS	6.72 ± 0.07f	7.18 ± 0.02e	9.26 ± 0.05c	9.76 ± 0.04a	7.26 ± 0.02e	7.18 ± 0.07e	9.40 ± 0.06bc	9.76 ± 0.07a	7.46 ± 0.07d	9.50 ± 0.04b	9.30 ± 0.06c	9.90 ± 0.03a
	14 DAS	7.50 ± 0.05j	8.44 ± 0.02h	9.62 ± 0.07f	10.48 ± 0.08e	8.64 ± 0.06g	8.58 ± 0.04gh	10.84 ± 0.09d	11.88 ± 0.09a	7.96 ± 0.04i	11.54 ± 0.04b	11.36 ± 0.04c	11.86 ± 0.05a
	21 DAS	8.56 ± 0.07h	9.08 ± 0.09fg	10.86 ± 0.14e	13.20 ± 0.05a	9.12 ± 0.11fg	8.94 ± 0.05g	11.20 ± 0.17d	13.18 ± 0.09a	9.20 ± 0.16f	11.98 ± 0.02c	11.90 ± 0.04c	12.68 ± 0.06b
	28 DAS	9.36 ± 0.07e	9.12 ± 0.06e	11.50 ± 0.19d	13.78 ± 0.20a	9.30 ± 0.15e	9.24 ± 0.13e	11.62 ± 0.15d	13.84 ± 0.22a	9.38 ± 0.15e	12.68 ± 0.04c	12.34 ± 0.12c	13.26 ± 0.08b
DW (g)	7 DAS	1.00 ± 0.03f	1.30 ± 0.08bcde	1.30 ± 0.03bcde	1.30 ± 0.09bcde	1.16 ± 0.11cdef	1.24 ± 0.05bcde	1.38 ± 0.12bc	1.10 ± 0.07ef	1.12 ± 0.04def	1.46 ± 0.02b	1.34 ± 0.06bcd	2.16 ± 0.05a
	14 DAS	1.78 ± 0.05d	1.46 ± 0.02e	1.52 ± 0.05e	1.74 ± 0.14d	1.72 ± 0.05d	1.78 ± 0.05d	1.72 ± 0.05d	1.46 ± 0.09e	1.78 ± 0.05d	2.32 ± 0.04b	2.08 ± 0.05c	2.58 ± 0.04a
	21 DAS	2.34 ± 0.05cde	2.60 ± 0.06bc	2.32 ± 0.10de	2.50 ± 0.16bcd	2.60 ± 0.05bc	2.66 ± 0.05b	2.22 ± 0.07e	2.34 ± 0.15cde	2.66 ± 0.05b	2.54 ± 0.02bcd	2.38 ± 0.04cde	3.12 ± 0.07a
	28 DAS	2.48 ± 0.04f	2.66 ± 0.14ef	2.90 ± 0.08cd	2.92 ± 0.05bcd	2.54 ± 0.05ef	2.72 ± 0.12de	2.90 ± 0.08cd	3.08 ± 0.10bc	2.66 ± 0.07ef	3.12 ± 0.04b	2.94 ± 0.05bc	3.60 ± 0.03a

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TABLE 3 (Continued)

Treatment		Control	lKPJ				fKPJ				FNF and PSB		
			0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days	KP85	KP19	KP85+ KP19
SL (cm)	45 DAS	13.8 ± 0.97e	16.4 ± 0.51de	18.0 ± 0.63d	26.0 ± 0.63c	19.2 ± 0.58d	23.6 ± 1.96c	23.4 ± 1.03c	37.2 ± 2.27a	31.0 ± 1.10b	37.4 ± 0.75a	37.8 ± 0.37a	38.4 ± 0.60a
	75 DAS	36.6 ± 0.75e	34.2 ± 0.66f	37.0 ± 1.26e	46.6 ± 1.44d	37.4 ± 1.17e	37.8 ± 0.49e	48.8 ± 1.11c	56.4 ± 1.29b	37.8 ± 0.37e	49.4 ± 1.12c	55.8 ± 1.02b	60.0 ± 0.89a
	Harvest	62.6 ± 1.21gh	64.8 ± 1.02g	62.6 ± 0.11gh	83.2 ± 1.39d	62.4 ± 1.08h	74.0 ± 2.10f	76.4 ± 2.09e	85.0 ± 1.41cd	75.6 ± 0.75ef	86.4 ± 1.50c	93.2 ± 1.77b	96.8 ± 1.16a
RL (cm)	45 DAS	7.0 ± 0.55g	10.8 ± 0.49e	11.2 ± 0.37e	13.0 ± 0.63d	10.0 ± 0.32ef	7.8 ± 0.80g	11.6 ± 0.75de	14.8 ± 1.39c	8.6 ± 0.60fg	17.8 ± 0.49b	17.6 ± 0.40b	20.0 ± 0.32a
	75 DAS	14.6 ± 0.40e	14.0 ± 0.32ef	14.2 ± 0.20ef	16.6 ± 0.40d	13.6 ± 0.24ef	11.8 ± 0.58g	15.8 ± 0.37d	18.6 ± 0.40c	13.2 ± 0.20f	20.2 ± 0.20b	20.6 ± 0.24b	22.2 ± 0.37a
	Harvest	18.4 ± 0.40de	18.4 ± 0.40de	18.0 ± 0.32e	20.2 ± 0.20c	20.6 ± 0.24c	16.6 ± 0.24f	19.0 ± 0.32d	22.6 ± 0.40b	20.6 ± 0.24c	24.2 ± 0.20a	24.6 ± 0.24a	24.2 ± 0.20a
NoT	45 DAS	3.4 ± 0.24f	4.0 ± 0.01def	4.4 ± 0.24cde	4.6 ± 0.24cd	3.8 ± 0.20ef	4.2 ± 0.20cde	4.8 ± 0.37c	5.4 ± 0.24b	4.2 ± 0.20cde	5.8 ± 0.20ab	6.0 ± 0.01ab	6.4 ± 0.24a
	75 DAS	8.2 ± 0.20f	8.4 ± 0.24ef	8.6 ± 0.24ef	10.8 ± 0.37c	8.4 ± 0.24ef	9.4 ± 0.24d	10.6 ± 0.24c	11.8 ± 0.20b	9.0 ± 0.32de	12.2 ± 0.37b	13.4 ± 0.24a	14.0 ± 0.01a
	Harvest	11.4 ± 0.40ef	10.8 ± 0.37f	12.4 ± 0.24d	13.8 ± 0.37c	12.2 ± 0.20de	12.4 ± 0.24d	14.0 ± 0.45c	15.6 ± 0.24b	12.0 ± 0.45de	14.0 ± 0.32c	15.4 ± 0.24b	16.8 ± 0.20a
SFW (g)	45 DAS	1.08 ± 0.04g	1.48 ± 0.02e	1.72 ± 0.04d	1.98 ± 0.06c	1.36 ± 0.07f	1.68 ± 0.08d	1.76 ± 0.07d	2.30 ± 0.04b	1.54 ± 0.04e	2.68 ± 0.04a	2.40 ± 0.03b	2.68 ± 0.04a
	75 DAS	4.88 ± 0.06g	5.22 ± 0.04f	5.62 ± 0.05e	6.22 ± 0.04d	5.36 ± 0.02f	5.60 ± 0.04e	6.04 ± 0.06d	6.62 ± 0.07c	5.32 ± 0.04f	7.00 ± 0.05b	7.20 ± 0.19a	7.32 ± 0.06a
	Harvest	14.88 ± 0.06g	15.22 ± 0.04f	15.62 ± 0.05e	16.22 ± 0.04d	15.36 ± 0.02f	15.60 ± 0.04e	16.04 ± 0.06d	16.62 ± 0.07c	15.32 ± 0.04f	17.00 ± 0.05b	17.60 ± 0.06a	17.38 ± 0.23a
SDW (g)	45 DAS	0.37 ± 0.01g	0.45 ± 0.01e	0.42 ± 0.01ef	0.55 ± 0.01b	0.46 ± 0.03de	0.40 ± 0.02fg	0.49 ± 0.01cd	0.61 ± 0.01a	0.52 ± 0.01bc	0.59 ± 0.01a	0.55 ± 0.02b	0.60 ± 0.01a
	75 DAS	0.93 ± 0.02h	1.29 ± 0.01g	1.33 ± 0.01ef	1.41 ± 0.01d	1.28 ± 0.01g	1.34 ± 0.01e	1.46 ± 0.01c	1.54 ± 0.01a	1.31 ± 0.01f	1.49 ± 0.01b	1.45 ± 0.01c	1.55 ± 0.01a
	Harvest	4.85 ± 0.01g	4.77 ± 0.01i	4.81 ± 0.01h	5.20 ± 0.01d	4.74 ± 0.01j	4.86 ± 0.01g	4.96 ± 0.01f	5.10 ± 0.01e	4.78 ± 0.01i	5.30 ± 0.01c	5.33 ± 0.01b	5.40 ± 0.01a
RFW (g)	45 DAS	0.31 ± 0.01f	0.40 ± 0.01ef	0.43 ± 0.01e	0.72 ± 0.01ab	0.47 ± 0.06e	0.51 ± 0.02de	0.58 ± 0.01cd	0.63 ± 0.01bc	0.46 ± 0.01e	0.71 ± 0.05ab	0.65 ± 0.07bc	0.76 ± 0.03a
	75 DAS	2.33 ± 0.02j	2.60 ± 0.01g	2.66 ± 0.01f	2.85 ± 0.01d	2.44 ± 0.01i	2.60 ± 0.01g	2.66 ± 0.01f	2.79 ± 0.01e	2.51 ± 0.01h	2.98 ± 0.01c	3.06 ± 0.05b	3.32 ± 0.01a

(Continued)

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TABLE 3 (Continued)

Treatment		Control	*lKPJ*				*fKPJ*				FNF and PSB		
			0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days	KP85	KP19	KP85+ KP19
	Harvest	4.61 ± 0.05h	4.87 ± 0.01fg	4.96 ± 0.01e	5.46 ± 0.01c	4.93 ± 0.01ef	5.10 ± 0.06d	5.17 ± 0.01d	5.39 ± 0.01c	4.84 ± 0.02g	5.77 ± 0.01b	5.85 ± 0.01b	6.18 ± 0.08a
RDW (g)	45 DAS	0.07 ± 0.01e	0.08 ± 0.01de	0.12 ± 0.01cd	0.22 ± 0.01b	0.15 ± 0.02c	0.10 ± 0.01cde	0.13 ± 0.01c	0.23 ± 0.02b	0.11 ± 0.01cde	0.25 ± 0.01ab	0.23 ± 0.03b	0.27 ± 0.01a
	75 DAS	0.80 ± 0.02j	0.90 ± 0.01h	0.96 ± 0.01fg	1.16 ± 0.01d	0.83 ± 0.01i	0.94 ± 0.01g	0.97 ± 0.01f	1.10 ± 0.01e	0.84 ± 0.01i	1.19 ± 0.01c	1.24 ± 0.02b	1.32 ± 0.01a
	Harvest	1.25 ± 0.01gh	1.21 ± 0.01i	1.32 ± 0.01f	1.46 ± 0.01e	1.27 ± 0.01g	1.24 ± 0.01h	1.31 ± 0.01f	1.55 ± 0.01d	1.21 ± 0.01i	1.66 ± 0.01c	1.74 ± 0.01b	1.88 ± 0.01a
TC ($mg\bullet g^{-1}$)	45 DAS	86.32 ± 0.94k	108.60 ± 1.23i	124.65 ± 0.63h	181.51 ± 1.16d	147.45 ± 1.02f	101.53 ± 0.60j	110.43 ± 0.15i	174.86 ± 0.66e	135.82 ± 0.22g	196.25 ± 0.91c	211.11 ± 2.05b	222.15 ± 1.30a
	75 DAS	141.97 ± 0.51i	153.74 ± 0.57h	162.10 ± 0.70f	180.83 ± 2.16d	151.75 ± 0.59h	151.65 ± 1.71h	158.71 ± 0.78g	170.17 ± 1.22e	144.39 ± 0.78i	202.51 ± 1.80c	213.51 ± 1.04b	230.49 ± 2.13a
	Harvest	153.43 ± 1.46k	176.79 ± 0.62g	183.50 ± 1.42f	196.41 ± 0.91d	161.18 ± 1.44i	168.19 ± 0.99h	177.95 ± 0.69g	192.05 ± 0.52e	157.87 ± 0.91j	206.29 ± 1.87c	219.87 ± 0.80b	243.86 ± 1.83a
TSP ($mg\bullet g^{-1}$)	45 DAS	47.64 ± 0.47h	43.07 ± 1.38i	67.50 ± 1.52e	86.01 ± 0.74c	61.97 ± 1.78f	55.12 ± 0.52g	87.10 ± 0.95c	103.60 ± 1.04a	65.32 ± 0.63ef	77.22 ± 4.18d	84.29 ± 0.63c	97.25 ± 0.50b
	75 DAS	53.84 ± 0.49h	54.37 ± 0.62h	78.40 ± 0.37e	109.59 ± 1.13a	71.86 ± 0.54f	67.60 ± 0.78g	103.41 ± 0.86b	110.19 ± 0.80a	69.43 ± 1.38g	88.30 ± 0.65d	91.46 ± 0.69c	92.97 ± 0.84c
	Harvest	71.52 ± 1.08g	69.76 ± 0.52g	84.24 ± 1.08e	113.81 ± 0.57a	84.75 ± 0.21e	80.31 ± 0.27f	108.66 ± 0.53b	113.81 ± 0.55a	78.92 ± 0.90f	100.90 ± 0.74d	106.90 ± 0.62bc	106.60 ± 0.71c
TChl ($mg\bullet g^{-1}$)	45 DAS	10.27 ± 0.02g	7.02 ± 0.14i	6.09 ± 0.24j	13.94 ± 0.18d	10.93 ± 0.19f	9.00 ± 0.09h	11.00 ± 0.05f	17.77 ± 0.53a	12.04 ± 0.11e	15.24 ± 0.29-c	16.99 ± 0.13b	17.06 ± 0.21b
	75 DAS	12.46 ± 0.04f	9.57 ± 0.06g	9.46 ± 0.04g	16.41 ± 0.04cd	13.17 ± 0.22f	15.69 ± 0.50de	17.27 ± 0.54c	20.59 ± 0.47a	14.88 ± 0.27e	17.11 ± 0.18-c	18.12 ± 0.14b	18.93 ± 0.18b
	Harvest	13.26 ± 0.12fg	12.01 ± 1.18gh	10.57 ± 0.14h	16.87 ± 1.36cde	14.73 ± 0.20ef	16.67 ± 0.29de	17.91 ± 0.43bcd	20.82 ± 0.93a	17.34 ± 1.10cd	18.23 ± 0.37bcd	19.11 ± 0.14abc	19.88 ± 0.18ab
NoET		10.4 ± 0.40de	9.6 ± 0.40e	11.4 ± 0.24d	13.2 ± 0.58bc	11.4 ± 0.24d	10.2 ± 0.49de	12.8 ± 0.37c	13.4 ± 0.40bc	11.2 ± 0.49d	13.8 ± 0.37bc	14.4 ± 0.24b	15.6 ± 0.24a
NoG		130.3 ± 0.35j	140.4 ± 0.56i	143.3 ± 0.71h	152.2 ± 0.58e	140.3 ± 0.74i	143.1 ± 0.40h	145.5 ± 0.39g	157.1 ± 0.34d	149.9 ± 0.57f	162.1 ± 0.56c	164.2 ± 0.82b	171.3 ± 0.66a
C:F		0.23 ± 0.01a	0.19 ± 0.01b	0.19 ± 0.01b	0.12 ± 0.01e	0.19 ± 0.01b	0.17 ± 0.01c	0.16 ± 0.01d	0.12 ± 0.01e	0.15 ± 0.01d	0.10 ± 0.01f	0.09 ± 0.01f	0.08 ± 0.01g
TW (g)		13.33 ± 0.12j	14.89 ± 0.16i	16.36 ± 0.27g	19.25 ± 0.09d	15.55 ± 0.03h	17.20 ± 0.21f	18.24 ± 0.09e	19.94 ± 0.16bc	15.07 ± 0.17i	20.30 ± 0.11b	19.59 ± 0.21cd	20.79 ± 0.08a
Y (g)		16.78 ± 0.23h	17.18 ± 0.15h	19.76 ± 0.11f	26.83 ± 0.19d	20.34 ± 0.22ef	18.46 ± 0.21g	20.83 ± 0.41e	26.65 ± 0.43d	19.99 ± 0.10f	29.12 ± 0.25c	30.06 ± 0.18b	30.87 ± 0.17a

Duncan's multiple range test was used to analyze the data, which are represented as the mean ± standard error of samples ($n = 5$), followed by different letters indicating that the difference was statistically significant ($p < 0.05$). The results with the same letters were not significantly different ($p > 0.05$). GP, germination percentage; DAS, date of sowing; SL, shoot length; RL. root length: FW, fresh weight per seedling; DW, dry weight per seedling; NoT, number of tillers per plant; SFW, shoot fresh weight per plant; SDW, shoot dry weight per plant; RFW, root fresh weight per plant; RDW, root dry weight per plant; TC, tot: e; TSP, total soluble protein; TChl, total chlorophyll; NoET, number of effective tillers per plant; NoG, number of grains per panicle; C:F, chaffy: filled grain ratio; TW, test weight; Y, yield per plant.

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crop technology has become widely popular in recent years (Du Jardin, 2015; Fadiji et al., 2022). In this study, we showed *Kunapajala* as a potential source of the beneficial PGPB, which includes their roles in N-fixation, P-solubilization, and IAA production, owing to their cumulative beneficial impact on the formulation to crop growth (Figures 1, 2; Table 3). We also report the molecular identification of the best-performing bacterial isolates from the *Kunapajala* source. One of the best-performing isolates, namely *Pseudomonas chlororaphis* subsp. *aurantiaca* is known to inhibit the *in-planta* growth of several important bacterial and fungal phytopathogens (Jain and Pandey, 2016). In addition, these two bacterial species with multiple plant benefits are also non-pathogenic to crop plants and humans (Anderson and Kim, 2018; Su et al., 2020). In this work, we have further established their fitness, alone or in combination, as a biostimulant on rice seedlings. We observed better performance of these bacterial isolates individually or in combination to improve plant performance on yield and other physio-biochemical traits in rice. It seems enigmatic compared to the whole microbial consortium, such as the *Kunapajala* formulation (Table 3). In the microscopic community, the bacterial species often interact with other microbes and form a complex microbial network that adjoins all possible metabolic interactions among microbes, including antagonisms and symbioses (Han and Yoshikuni, 2022). This interactive microbial metabolic network ultimately deciphers various niche-specific ecological functions, including multiple plant-related benefits to crops. The beneficial microbial consortia are known to improve the performance of agricultural crops while maintaining a healthy agroecological niche and the micro-environment. In recent years, the beneficial PGPB have been considered a promising biotechnological tool to improve crop productivity and enhance crop quality traits irrelevant to its nutrient content (Oleńska et al., 2020). Studies also revealed that the active root zone is the site of abundant microbial diversity based on its composition and function (Ray et al., 2020). Therefore, plant-associated microbiome engineering as bio-fertilizers and bio-pesticides has been a sustainable approach, with added benefits in boosting soil biological activity and fertility (Chojnacka et al., 2020; Mitter et al., 2021). These data, together, establish the earlier recommendation of *Kunapajala* as a soil drenching. When applied to soils, microbial communities of plant benefits, in essence, build a mutual relation with niche crops and act cooperatively as a cohort of "healthy-peaceful-societies" to circumvent detrimental fluctuations in crop farming, specifically under challenging conditions.

On the other hand, the microbial consortium often shows a lesser effect, as observed in this study, which may indicate the prevalence of antagonistic interactions over the best-performing single or a few beneficial bacterial isolates. Therefore, the combination of effective strains with better stability of similar genera and additive effects on plant growth promotion often termed synthetic microbial communities (e.g., SynComs), becomes more robust and is increasingly recognized in modern agriculture (Yin et al., 2022). In this line of evidence, our results strongly encourage the development of an effective bio-fertilizer derived from these two reported bacteria and a few more similarly effective isolates in the near future. Overall, *Kunapajala* technology can integrate plants, microbes, and nutrient relationships to maintain ecological harmony and promote natural resource-based sustainable farming.

4.3. Future perspectives on the scaling-up potential of the *Kunapajala* technology

Kunapajala, as an example, represents an ancient indigenous innovation of India. However, the question remains poised about the practices of such traditional knowledge for adaptation, overall impacts, and vulnerability to the modern economic world. The *Kunapajala* technology provides two main advantages: first, it requires low-cost investment for infrastructural development and encourages the circular bio-economy of agricultural farms; and second, an animal-based agroecosystem may facilitate its sustainable integration to promote inclusive agriculture and rural socio-economic transformation. This study nevertheless also discusses its pivotal role in animal waste recycling. In recent years, the *Swachh Bharat* (Clean India; https://swachhbharat.mygov.in/) Mission has been gearing up to generate public awareness and policies about the waste disposal and segregation mechanisms followed by their management into potential applications like energy, fertilizer, animal feed, chemical, and leather industries. The Government of India also launched a flagship initiative, Startup India (https://www.startupindia.gov.in/), in order to foster innovative ideas and passion for building up an inclusive ecosystem among entrepreneurs. This industry-academia knowledge exchange platform may aid in planning ideas according to local needs, industrial viability, and national policy plans. The *Kunapajala* technology, in this context, would be more appropriate and socially viable to add up in animal waste management, particularly in the Indian context. In addition, the technological operation of *Kunapajala*, starting with animal waste management to broader applications in agriculture, would also create unique linkages for its strategic implementation, especially in agriculture-based nations.

In India and other parts of the world, organic farming has been growing stupendously as an alternate model of crop cultivation aiming to achieve greater economic viability and environmental sustainability (Meemken and Qaim, 2018; Das et al., 2020). The principle of organic farming practices often emphasizes the safe and efficient recycling of natural resources, including on-farm residues, to counterbalance the antagonistic effects of agrochemicals, maintain ecological harmony, and conserve biodiversity. In order to substitute the chemical inputs, which are essential resources in conventional agriculture, organic agriculture relies on composts and manures of "true" organic standards and origin. In this study, cow dung and urine, the potential sources of organic matter and microbial consortia, have been collected from the in-house dairy farm, practicing conventional (non-organic) animal farming and therefore fails to qualify as "true" organic manure. Hence, organic animal farming, including poultry and fish, has become an integrated part of this ecological farming system (He, 2020). To monitor the "true" organic standards in complex food chains, the certification process of organic products and farms continues to evolve in the United States and other countries, including India. Therefore, stating terms such as organic manure, organic agriculture, and alike must be carefully considered and discussed. Hence, the *Kunapajala* formulation, often widely coined as organic manure in the past in India, must now qualify for the organic standards and pass through the certification process before being termed organic manure for commercial purposes and scientific agricultural practices.

India is, however, now on the verge of introducing chemical-free natural farming based on on-farm resource recycling and dairy excreta-based microbial formulations (https://www.niti.gov.in/natural-farming-niti-initiative). Surely, *Kunapajala* technology would get wider acceptance as a regenerative input under the new Indian agricultural policy. On the other hand, according to the United Nations' Sustainable Development Goals (https://sdgs.un.org/goals), our planet would experience almost 90% of global deforestation primarily due to the expansion of agricultural practices, including crop farming and livestock grazing. It consequently leads to rapid natural resource depletion, agro-pollution, biodiversity loss, unpredictable climate vulnerability, and eventually severe crop loss and yield, having short- and long-term negative impacts on the planet and human health. Therefore, sustainable intensification and ecosystem services in agriculture grab immediate global attention while strengthening the traditional culture and practices to maintain agroecosystem resilience, along with the "inclusive" strategic integration of natural resource utilization and scientific implementation of niche-specific innovations and technologies (Jhariya et al., 2021). For instance, careful animal waste utilization generated from animal farms into recycled animal manure will be far more effective for environment-friendly, sustainable agriculture (He et al., 2020). Therefore, traditional innovations, such as *Kunapajala* technology, could be revisited and assessed to promote sustainable intensification and address ecosystem services across diverse agroecological and socio-economic domains. Hopefully, in the coming years, a more holistic inter-sectoral approach to building a resilience system on the scaling-up potential of *Kunapajala* at par commercial standards would contemplate viable solutions in the socio-economic development encompassing agriculture-environment-public health nexus.

5. Conclusions

In this study, we revisited and assessed the waste recycling potential of *Kunapajala* manures prepared from livestock or fish wastes over different decomposition periods. We have further shown *Kunapajala* to be a dynamic formulation of essential plant primary nutrients and a rich source of PGPB and their metabolic network. This study also elucidates the mechanism of plant growth promotion in *Kunapajala*, including the molecular identification of two plant beneficial bacteria. Based on the 16S ribotyping method, the two best-performing bacterial isolates show strong sequence homology with *Pseudomonas chlororaphis* subsp. *aurantiaca* and *Bacillus subtilis* subsp. *subtilis*. Overall, this work provides the first mechanistic insight into the plant biostimulant potential of *Kunapajala* and its further *in-planta* validation from seed germination to sprouting shoots and roots to grain production in rice.

Data availability statement

The generated nucleotide sequences have been deposited into the NCBI database under the accession 538 numbers: OM698822 (KP19) and OM698823 (KP85).

Author contributions

SM: conceptualization, methodology, validation, investigation, resources, and writing—review and editing. AB and AC: methodology, validation, investigation, resources, and writing—review and editing. RG: methodology, formal analysis, and writing—review and editing. KR, MNA, HB, and JL: formal analysis and writing—review and editing. SS: methodology and resources. AKH and ST: methodology, resources, and writing—review and editing. ASP: writing—review and editing, project administration, and fund acquisition. NR: conceptualization, writing—review and editing, project administration, and fund acquisition. MAA: writing—review and editing and project administration. GC: conceptualization, methodology, formal analysis, investigation, supervision, writing—original draft, project administration, and fund acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1073010/full#supplementary-material

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Conservation tillage and diversified cropping enhance system productivity and eco-efficiency and reduce greenhouse gas intensity in organic farming

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Environmental pollution, resource dwindling, and soil degradation questioned the sustainability of contemporary agricultural production systems. Organic farming is advocated as a sustainable solution for ensuring food security without compromising environmental sustainability. However, poor farm productivity quizzed the sustainability of organic production systems. Hence, a field study was carried out in the Sikkim region of the Indian Himalayas to assess the efficacy of conservation-effective tilling and diversified cropping on system productivity, profitability, environmental quality, and soil nutrient balance in organic farming. Three tillage systems, namely, (i) conventional tillage (CT), (ii) reduced tillage (RT), and (iii) zero tillage (ZT), and four maize based diversified cropping systems (maize–black gram–toria, maize–black gram–buckwheat, maize–rajmash–toria, and maize–rajmash–buckwheat) were tested using a three times replicated split-plot design. The ZT system recorded 13.5 and 3.5% higher system productivity over CT and RT, respectively. Of the four diversified cropping systems, the maize–rajmash–buckwheat system recorded the maximum system productivity (13.99 Mg ha^{-1}) and net returns (3,141 US$ ha^{-1}) followed by the maize–black gram–buckwheat system. Among the tillage practices, ZT recorded the significantly high eco-efficiency index (EEI; 1.55 US$ per kg CO_2-eq emission) and the lowest greenhouse gas intensity (GHGI; 0.15 kg CO_2-eq per kg production). Of the diversified cropping systems, the maize-rajmash-buckwheat registered the lowest GHGI (0.14 CO_2-eq per kg production) and the highest EEI (1.47 US$ per kg CO_2-eq emission). Concerning soil nutrient balance, after three cropping cycles, the soil under ZT recorded significantly higher available N (340.0 kg ha^{-1}), P (16.6 kg ha^{-1}), and K (337.3 kg ha^{-1}) over the CT system at 0–10 cm soil depth. Similarly, the soil under the maize–black gram–buckwheat system had the maximum bio-available NPK. Thus, the study suggests that the cultivation of the maize–black gram/rajmash–buckwheat systems under ZT and/or RT would increase farm productivity, profitability, and soil fertility with minimum GHGI in organic farming under the Eastern Himalayan region of India.

KEYWORDS

buckwheat, crop productivity, economic returns, Himalayas, soil nutrients, sustainability

1. Introduction

Environmental crises, poor economic returns, declining factor productivity, and resource degradation quizzed the sustainability of contemporary agricultural practices (Yadav et al., 2021b; Ansari et al., 2022). Although the modern/contemporary agricultural production system enhances food production by many folds, but simultaneously creates tremendous pressure on natural resources (Babu et al., 2022). Hence, sustainable food and nutritional security without compromising environmental quality is an indispensable for the planet and population health. After air and water, food security is a basic human need. Achieving sustainable national and household-level food and nutritional security is a complicated and complex target affected by multiple factors like human competency, policies, infrastructure, technological invention, and dissemination (Yadav et al., 2021a; Babu et al., 2022). Under the current scenario of natural resource depletion, climate change further amplified the food and nutritional insecurity challenges (Panwar et al., 2022). The Conference of the Parties of the United Nations Framework Convention on Climate Change (UNFCCC) (COP 27) meeting held at Sharm El-Sheikh, Egypt in November 2022 also emphasized safeguarding the food production system from climate change vulnerability.

Monocropping, poor residue returns, and intensive tillage are the leading causes of soil quality degradation and poor economic returns (Yadav et al., 2021b). Repeated tillage adversely impacts soil porosity, water movement, and soil compactness, which result in poor crop growth and productivity (Yadav et al., 2020). The global cultivated land reached the terrestrial frontier (Henry et al., 2018), and adverse environmental outcomes are expected if traversed by this planetary line (Molotoks et al., 2018). Hence, the main question is how the existing land should be utilized intelligently to enhance farm productivity without jeopardizing environmental quality (Avasthe et al., 2020). This calls for designing and developing improved cropping systems with sustainable management practices. Sustainable intensification can be used to potentially increase food production without bringing additional land under cultivation. However, the selection of an efficient, economically feasible system for a particular site requires robust planning (Avasthe et al., 2020). Pulses, a rich source of dietary protein, capture and fix atmospheric nitrogen into the soil system. Hence, embedding pulses in diversified cropping can improve food, nutrition, and environmental security (Babu et al., 2020b).

Organic farming is widely advocated as a possible solution for achieving sustainable food and environmental security (Singh et al., 2021). However, poor crop productivity under organic agricultural systems as compared to conventional chemical farming has been reported by several researchers (de Ponti et al., 2012; Yadav et al., 2014; Knapp and van der Heijden, 2018). Organically managed farms had ∼19% less crop productivity than conventionally managed fields (de Ponti et al., 2012). However, the magnitude of yield reduction varies with soil and crop types and climatic conditions. Organic production systems may be crucial for mitigating negative environmental outcomes but poor crop productivity forces researchers to develop the appropriate location-specific agronomic management practices to boost overall farm productivity and profitability of organic production systems. Adopting farming practices that conserve soil and water through minimal soil disturbances and residue cover is one of the best management practices and is being advocated globally for improving the nutrient status in degraded soils under conventional chemical-based farming (Yadav et al., 2020).

The Indian Himalayan Region (IHR) occupies a 53.7 Mha area and a habitat of ∼50 million people. The IHR covers 16.4% of the geographical area of the country and is spread over 13 Indian states. Agriculture in the IHR is organic by default, and the productivity of most of the field crops remains low as compared to the irrigated plain lands of India (Babu et al., 2016; Das et al., 2019). In hilly ecosystems, especially under organic management conditions, the impact of tillage practices and cropping diversity on farm production efficiency and soil fertility is not widely evaluated. Therefore, it is imperative to evaluate the impact of diverse conservation tillage practices and diversified cropping on farm productivity, profitability, environmental outcomes, and soil fertility to formulate the appropriate management policies for the long-term sustainability of Indian hill agriculture. The IHR has vast scopes to increase food production and restore the risk-prone soils of the region through the adoption of conservation agricultural (CAs) practices. Farmers of the IHR, especially the Eastern Himalayan region, generally grow rainy-season crops with minimal organic inputs and keep their land fallow during the winter season due to moisture scarcity. The existing cropping scenarios are the main cause of poor farm productivity in the mountain ecosystem of India (Avasthe et al., 2020). Hence, the diversification of the prevailing cropping systems is urgently required with crops that can potentially improve farm productivity and profitability. Furthermore, the cultivation of more crops in a year as against monocrops would extend the soil covering period, which will help to protect the soil from erosion during heavy rainfall in sloping lands (Babu et al., 2020b). Hence, there is an emerging curiosity among researchers to apply the principle of conservation tillage under organic management to conserve natural resources and sustain farm productivity. The

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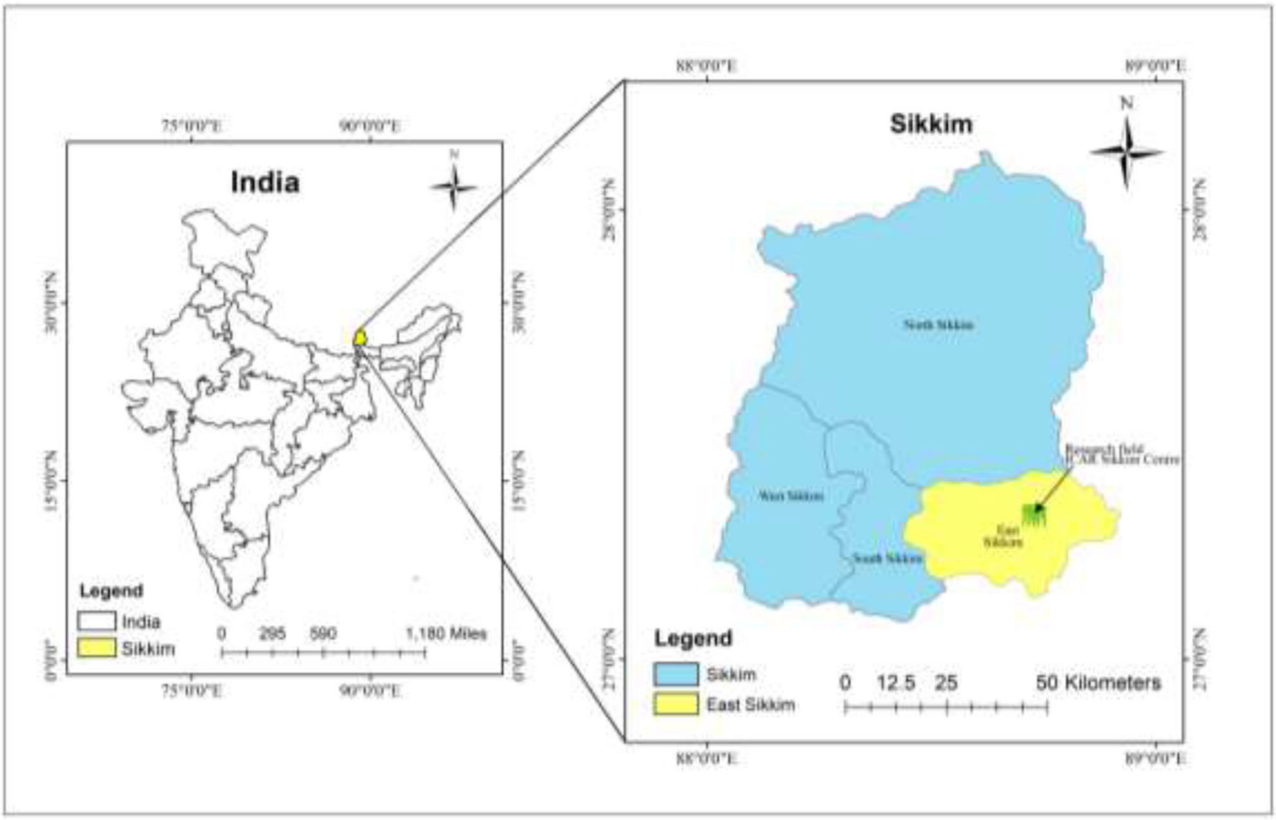

FIGURE 1
A map showing the study site in the Indian Himalayan region (IHR).

comparative effect of diversified cropping and conservation tillage on system productivity, economic returns, eco-efficiency, and soil nutrient balance is not adequately addressed under organic management, especially for Himalayan ecosystems. Hence, it was hypothesized that the cultivation of diversified cropping along with conservation tillage practices can potentially improve farm productivity, profitability, and soil fertility with minimum negative environmental outcomes under organic farming. To achieve the above hypothesis, a field study was conducted during 2015–2018 with the following objectives (i) to assess the effect of tillage and diversified cropping on system productivity and economic returns and (ii) to assess the effect of diverse tillage and cropping systems on eco-efficiency, greenhouse gas intensity, and soil nutrient availability under organic farming. The findings of the current study will help to achieve India's commitments to food security and climate change mitigation and the related sustainable development goals (SDGs).

2. Materials and methods

2.1. The study site

A 3-year (2015–2018) study was carried out at the research farm, ICAR Research Complex in the North Eastern Hill region, Sikkim Center. The research farm was located in the Tadong area of Gangtok, the capital city of the first certified organic state of India, and lies between 27°32′ N latitude and 88°60′ E longitude with an altitude of 1,350 m above the mean sea level (Figure 1). The Eastern Himalayan region (EHR) is an inimitable ecosystem in the world and is counted as a crisis eco-region. The EH region is extended from Central Nepal to Yunnan in China, including Bhutan, Arunachal Pradesh, Nagaland, Mizoram, Manipur, Sikkim, Meghalaya, Tripura, Manipur, and the hills of West Bengal and Assam, Myanmar, and Southeast Tibet. The soils are predominantly acidic and prone to degradation. The diverse ecology and altitudinal gradient range from 300 to 8,000 m and represent great diversity in flora and fauna (Singh et al., 2021). The Haplumbrept soil of the study area is sandy loam. The mean annual temperature of the region lies between 4 and 22°C. The average rainfall is about 3,000 mm annually, of which 75%—80% is received mainly from June to September.

2.2. Treatment details and crop management

Soil sampling and analysis were carried out before setting the experiment and after three cropping cycles. The experimental soil was high in carbon (17.8 g kg^{-1}), medium in available nitrogen

(312 kg ha^{-1}), and phosphorus (15.6 kg ha^{-1}), and high in plant-available potassium (320.2 kg ha^{-1}). Three tillage practices, i.e., conventional tillage (CT), reduced tillage (RT), and zero tillage (ZT) were practiced in the main plots and four diversified cropping systems, i.e., maize (*Zea mays* L.)–black gram (*Vigna mungo* var. viridis)–toria (*Brassica campestris*), maize–black gram–buckwheat (*Fagopyrum* sp), maize–rajmash (*Phaseolus vulgaris*)–toria, and maize–rajmash–buckwheat were practiced in the subplots. All the treatments were replicated three times in a fixed pattern under a split plot design. The maize composite cv. DA-61 A (20 kg seed ha^{-1}) was sown at a 50 × 20 cm spacing during March (second fortnight) every year. Black gram and rajmash were grown as late post-rainy season crops sown in the second fortnight of August in each year of experimentation. Black gram (PD-3) and rajmash (SKR-57A) were dibbled with a seed rate of 25 and 75 kg ha^{-1} at a distance of 30 × 10 and 40 × 10 cm geometry, respectively. The winter crops, namely, toria (M-27) and buckwheat (local Meethey) were sown in November every year with a seed rate of 4 and 40 kg ha^{-1}, respectively. Both the winter season crops were seeded at 30 × 10 cm geometry. The recommended doses of nitrogen for maize, black gram, rajmash, toria, and buckwheat are 60, 20, 60, 40, and 40 kg ha^{-1}, which were supplied through well-decomposed farmyard manure (FYM); ∼28% moisture) containing 0.59% N, 0.30% P, and 0.52% K. Full quantity of FYM was applied before 1 week of sowing. Four tilling under CT and two tilling under RT (∼8–12 cm depth) were done with the help of a power tiller. Whereas in ZT, the soil was not much disturbed and the tillage operation was restricted to the opening of the furrow by using a ZT row marker. Under RT and ZT, ∼30% of the maize residues and the entire residues of the succeeding crops were retained on the soil surface. Irrespective of the tillage practices, two-hand weeding was done for 20 days after sowing (DAS) and after 40 DAS in each crop to manage the weeds. To maintain an optimum plant population, thinning and gap-filling operations were done along with the first weeding. Maize, black gram, and rajmash were grown as rainfed crops. Hence, no artificial irrigation was imposed. However, lifesaving irrigations were given as and when required to winter crops. Seed treatment was done with *Trichoderma* spp. (4 g kg^{-1}) to reduce pathogen infestation. Neem oil (5 ml per L of water) was sprayed after 20 days of sowing (2–3 times at 10-day intervals) in each crop to avoid/minimize insect and pest infestation.

2.3. Harvesting and yield measurement

At physiological maturity, maize cobs were harvested using iron sickle in the first week of August. After harvesting, the maize cobs were sun-dried for a week on the threshing floor, and thereafter, the grain was removed from the cobs by a manual maize sheller. After cob harvesting, the maize plants were cut ∼30 cm above the ground in RT and ZT but at ∼5–10 cm in CT plots. The short-duration black gram and rajmash were harvested during the second fortnight of October every year. Similarly, the winter crops (toria and buckwheat) were harvested during March (first fortnight) every year. After harvesting late-rainy and winter crops, the grains of all the crops were threshed manually by beating small bundles (biomass bundles were made plot-wise after harvesting) on an iron drum on a threshing floor. The harvested produce of all the crops was sun-dried. The economic yield of the crops (maize and buckwheat) was observed at 12% moisture content, while the black gram, rajmash, and toria economic yields were recorded at 8% moisture in seed and articulated in Mg ha^{-1}.

2.4. Analysis of system productivity and economic returns

The economic yield of all the crops was converted into the maize equivalent yield (MEY) and expressed as system productivity (SP). The SP measured the productive capacity of the different tillage and diversified systems. The dominant local market rate was deployed for calculating the SP. MEY was worked out as shown in the following equation:

$$\text{MEY} = MY + \left(Y_i \times \frac{P_i}{P_m}\right), \tag{1}$$

where MEY was Mg ha^{-1}, MY and Y_{i} were the economic yields of maize and ith crops in Mg ha^{-1}, respectively; P_{i} and P_{m} were the selling/market rate (US$) of the *i*th crop and maize, respectively.

The effect of tillage and diversified systems were also assessed on per day farm production capacity by calculating the system production efficiency (SPE) (Equation 2).

$$\text{SPE (kg ha}^{-1}\text{ day}^{-1}) = \frac{System\ productivity\ (kg\ ha^{-1})}{365}. \tag{2}$$

For economic accounting, the cultivation cost of all the tillage and diversified systems was estimated based on the diverse inputs incurred and the activities done. The gross economic return was the rate of the economic products in the market. The cost–benefit ratio is the proportional valuation of the treatment in terms of per unit investment. The monetary spending and earnings attained from all the treatments were converted into US$. The system net returns (SNR), and the benefit-to-cost (B:C) ratio were derived with the following equations.

$$\text{SNR}\left(\text{US ha}^{-1}\right) = \text{Gross returns}\left(\text{US ha}^{-1}\right) - \text{Cost of cultivation (US ha}^{-1}) \tag{3}$$

$$\text{B : C ratio} = \frac{\text{Net returns (US ha}^{-1})}{\text{Cost of cultivation (US ha}^{-1})}. \tag{4}$$

2.5. Estimation of greenhouse gas intensity and eco-efficiency index

The total extent of GHG emissions (CO_2 and N_2O) released during the entire cropping period was assessed as a CO_2 equivalent (CO_2-eq) (Yadav et al., 2017). CO_2-eq is also called a carbon footprint (CF). In the present study, all the crops were grown under well-drained upland conditions. Therefore, carbon dioxide (CO_2) and nitrous oxide (N_2O) gases were taken into account for CF estimation. Total CO_2 and N_2O released from a particular treatment were expressed through CO_2-eq. by multiplying the GWP equivalent of 1 and 265 for CO_2 and N_2O, respectively, for 100 years timeframe (Yadav et al., 2017). Standard emission coefficients were used to estimate the GHG emission from different treatments. The emission factor of 0.01 was multiplied by the total N supplied through organic sources and articulated in N_2O kg N input^{-1} to quantify the total N_2O emission (Tubiello et al., 2015).

$$N_2O \text{ emission} \left(\text{kg year}^{-1}\right) = \text{N supplied by N sources} \times 0.01 \times \frac{44}{28}$$

Global warming potential (GWP) from all the treatments was calculated by summing the total CO_2-eq released as follows:

$$\text{GWP} = \text{Total } N_2O \text{ emission} \times 265 + \text{Total } CO_2 \text{ emission.}$$

Greenhouse gas intensity (GHGI) was calculated using the following expression:

$$\text{GHGI (kg } CO_2\text{eq kg}^{-1} \text{ economic product)} = \frac{\text{Total GHG emission (kg } CO_2\text{eq ha}^{-1})}{\text{System productivity (kg ha}^{-1})}.$$

Eco-efficiency estimation is imperative to judge the environmental robustness of the designed technology. Eco-efficiency indicates the economic returns capacity of a designed technology concerning environmental destruction. In the current experimentation, the ecological impact of different tillage and diversified systems was measured in terms of total GHG emission (kg CO_2-eq per year). Eco-efficiency was calculated using the following equation:

$$\text{EEI (US per kg } CO_2\text{eq)} = \frac{\text{Net economic returns} \left(\text{US\$ ha}^{-1}\right)}{\text{Total GHG emission} \left(\text{kg } CO_2\text{eq ha}^{-1}\right)}.$$

2.6. Analysis of available soil nutrients

For the analysis of soil-available NPK, the soil samples were collected after the completion of three cropping cycles. The available P and K were estimated using Bray's P_1 (0.03 N NH_4F in 0.025 N HCl) pH 4.65 and 1N NH_4OAc extractable K at pH 7.0, while available N was evaluated using the alkaline $KMnO_4$ method (Prasad et al., 2006).

2.7. Statistical analysis

The experimental data from different tillage and diversified cropping were statistically evaluated according to the procedure described by Gomez and Gomez (1984). Analysis of variance (ANOVA) and test of significance at $p = 0.05$ were computed using the SPSS software version 27.0.

3. Results

3.1. Crop productivity

3.1.1. Productivity of maize

Tillage practices exerted a significant effect on maize productivity (Table 1). Of the diverse tillage practices, a considerably higher maize yield (4.02 Mg ha^{-1}) was recorded under RT followed by ZT (3.92 Mg ha^{-1}), respectively. However, the lowest maize grain yield (3.88 Mg ha^{-1}) was recorded under CT. Overall, RT ascribed 3.7 and 2.8% higher economic productivity of maize over CT and ZT, respectively. Similarly, the economic productivity of maize was significantly higher (4.06 Mg ha^{-1}) under the maize–black gram–buckwheat system over the maize–rajmash–toria (3.81 Mg ha^{-1}) and maize–rajmash–buckwheat (3.89 Mg ha^{-1}) systems but remained statistically on par with the maize–black gram–toria (3.99 Mg ha^{-1}) system. Similarly, maize grain yield was also higher under the maize–black gram–toria system over the maize–rajmash–toria and maize–rajmash–buckwheat systems. This indicated that the inclusion of short-duration pulses (black gram) had a beneficial effect on maize yield in the system mode. Overall, the inclusion of black gram increases maize yield by 6.6 and 4.6% over the maize–rajmash–toria and the maize–rajmash–buckwheat systems, respectively.

3.1.2. Productivity of late-rainy-season crops (black gram/rajmash)

The tillage practices significantly influenced the productivity of late-rainy-season crops (black gram/rajmash). An expressively advanced seed yield of rajmash was recorded under ZT (1.10 Mg ha^{-1}) compared to CT (0.94 Mg ha^{-1}) and RT (1.04 Mg ha^{-1}), respectively. RT also recorded a significantly higher yield than CT. Among the diversified cropping systems, the maize–rajmash–buckwheat system had a significantly higher rajmash yield compared to the other cropping systems. Similarly, under the maize–rajmash–toria system, the rajmash yield was higher as compared to the black gram yield under another cropping system. Overall, the rajmash yield was higher compared to the black gram tested in the cropping system. Concerning to interaction effect of tillage practices and diversified cropping systems (Figure 2), cultivation of maize–rajmash–buckwheat under ZT recorded significantly higher economic yields (1.60 Mg ha^{-1}) over other combinations.

3.1.3. Productivity of winter season crops (toria/buckwheat)

The productivity of winter crops had also been significantly influenced by different tillage and diversified cropping. Of various tillage practices, ZT recorded a significantly higher (0.94 Mg ha^{-1}) yield than CT (0.73 Mg ha^{-1}) and RT (0.87 Mg ha^{-1}). The increase in the output of winter crops was 29.0% and 8.2% higher under ZT compared to CT and RT, respectively. Among the diversified cropping systems, buckwheat yield was significantly higher under the maize–black gram–buckwheat system over others but endured statistically on par with the maize–rajmash–buckwheat system (Table 1). Buckwheat yield was comparatively higher than toria yield under different cropping systems. The interactive impact of tillage and diversified cropping was also found to be significant in respect of the productivity of winter crops (Figure 3). The cultivation of maize–black gram–buckwheat under ZT recorded significantly higher (1.01 Mg ha^{-1}) seed yields compared to the other combinations, while the cultivation of maize–black gram–toria under CT recorded the lowest yield (0.53 Mg ha^{-1}).

TABLE 1 Impact of different tillage practices and diversified cropping on crop productivity (mean of 3 years).

Treatment	Maize yield (Mg ha^{-1})	Black gram/rajmash yield (Mg ha^{-1})	Toria/buckwheat yield (Mg ha^{-1})	System productivity (Mg ha^{-1})	SPE (kg ha^{-1} day^{-1})
Tillage practices					
CT	3.88	0.94	0.73	11.27	30.9
RT	4.02	1.04	0.87	12.36	33.9
ZT	3.92	1.10	0.94	12.79	35.0
SEm±	0.03	0.02	0.02	0.08	0.2
LSD ($p = 0.05$)	0.08	0.05	0.05	0.21	0.6
Diversified cropping					
Maize-black gram-toria	3.99	0.78	0.75	10.49	28.7
Maize-black gram-buckwheat	4.06	0.95	0.98	12.09	33.1
Maize-rajmash-toria	3.81	1.07	0.74	11.99	32.8
Maize-rajmash-buckwheat	3.89	1.31	0.93	13.99	38.3
SEm±	0.04	0.06	0.04	0.35	1.0
LSD ($p = 0.05$)	0.08	0.12	0.09	0.71	2.0

CT, conventional tillage; RT, reduced tillage; ZT, zero tillage; SEm, standard error of mean; LSD, least significant difference; SPE, system production efficiency.

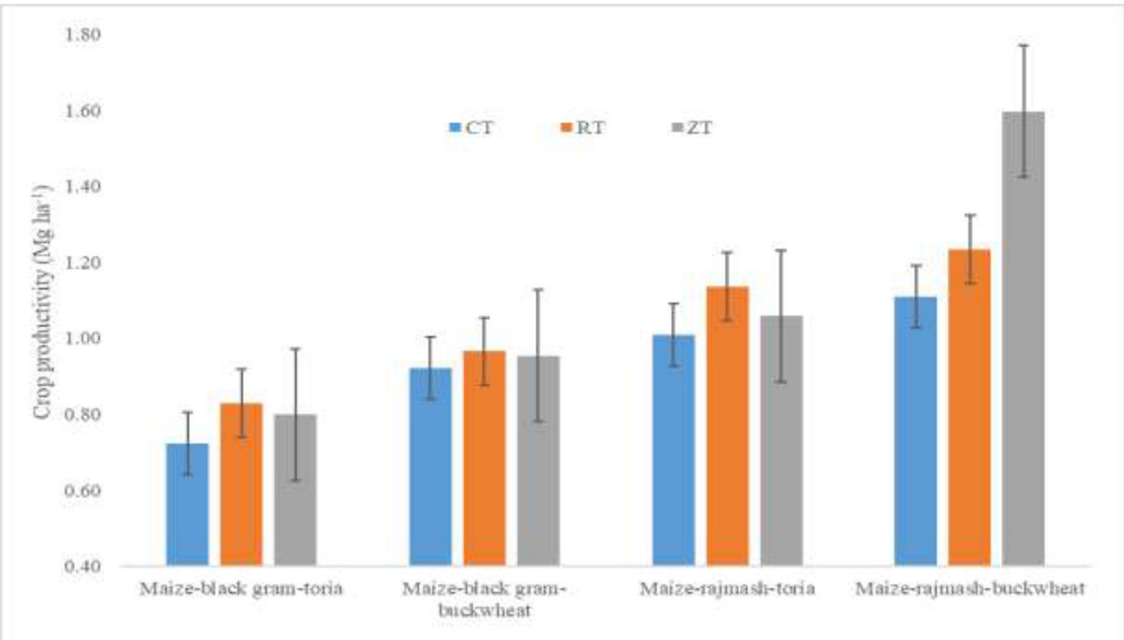

FIGURE 2
Interactive effect of tillage and diversified cropping on the productivity of late rainy season crops. CT, conventional tillage; RT, reduced tillage; ZT, zero tillage. Error bar indicates the least significant difference (LSD) values at $p = 0.05$.

3.2. System productivity and economic returns

The productive capacity of diverse tillage and diversified cropping was assessed in terms of system productivity (SP) and system production efficiency (SPE). Both SP and SPE were significantly affected by the diverse tillage and diversified cropping systems (Table 1). The SP was considerably higher under ZT (12.79 Mg ha^{-1}) than under RT (12.36 Mg ha^{-1}) and CT (11.27 Mg ha^{-1}). The lowest SP was observed under CT. Similarly, the highest SPE was recorded under ZT and the lowest was under CT. The SPE was ∼13.5 and 3.5% higher under ZT than those under CT and RT, respectively (Table 1). The production cost analysis revealed that ZT had a 4.9% lower production cost than CT (Table 2). System

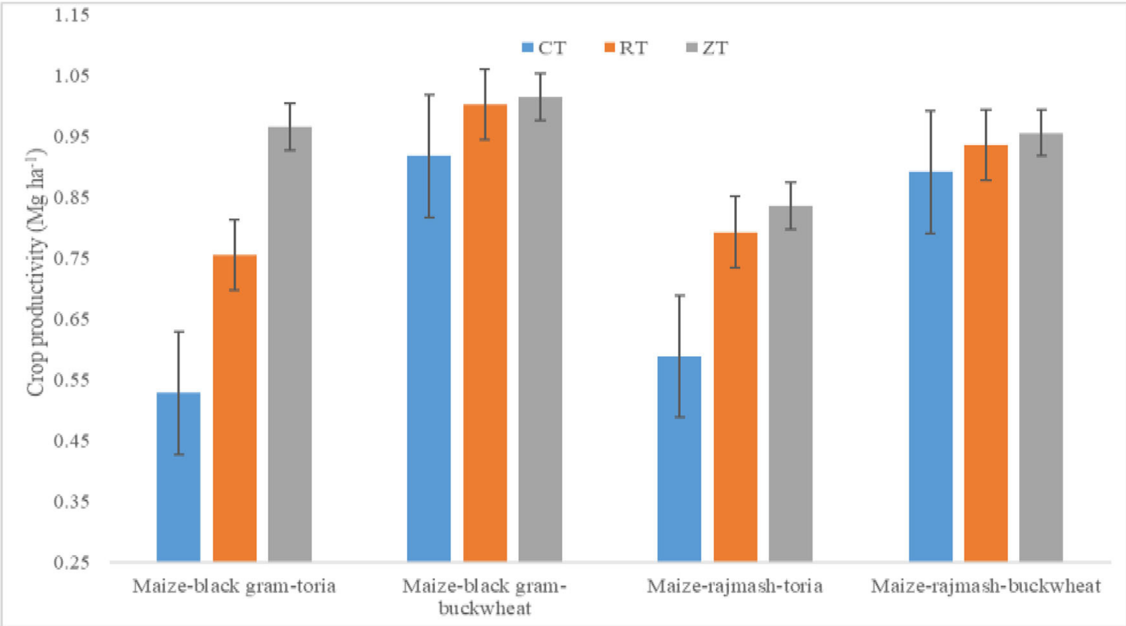

FIGURE 3
Interactive effect of tillage and diversified cropping on the productivity of winter season crops. CT, conventional tillage; RT, reduced tillage; ZT, zero tillage. Error bar indicates the least significant difference (LSD) values at $p = 0.05$.

gross returns, net returns, and the B:C ratio was significantly higher under ZT than CT and RT. The ZT system had 13.5, 24.7, and 30.3% higher system gross returns (SGR), system net returns (SNR), and B:C ratio over CT, respectively (Table 2). Similarly, RT also had a 9.6, 16.7, and 18.98% higher SGR, SNR, and B: C ratio over CT, respectively. Among the diversified cropping, the lowest production cost (1,299 US\$ ha^{-1}) was incurred upon cultivation using the maize–black gram–toria system. At the same time, the maximum cost was incurred (1,500 US\$ ha^{-1}) for production upon using the maize–rajmash–buckwheat system. Similarly, the maximum SGR was observed for the maize–rajmash–buckwheat system (4,643 US\$ ha^{-1}) followed by the maize–black gram–buckwheat system (4,014 US\$ ha^{-1}). Similarly, this particular system registered the highest SNR and B: C ratio, while the lowest was under the maize–black gram–toria system (SNR −2,181 US\$ ha^{-1} and B:C ratio −1.68).

3.3. Greenhouse gas intensity and eco-efficiency index

The GHGI is the quantity of GHG in terms of CO_2-eq released for a unit of economic production. Tillage practices have a significant impact on GHGI under an organic production system. The CT system had the highest GHGI (0.21 kg CO_2-eq per kg production), while the ZT recorded the lowest GHGI (0.15 kg CO_2-eq per kg production). ZT had 28% and 11.76% less GHGI over CT and RT, respectively (Figure 4). Concerning diversified cropping, the substitution of toria with buckwheat during the winter season and black gram with rajmash during the late rainy season had a significant impact on GHGI. Among the tested diversified cropping systems, the maize–rajmash–buckwheat system had the lowest GHGI (0.14 kg CO_2-eq per kg production), while the maize–black gram–toria system had the highest GHGI (0.20 kg CO_2-eq per kg production; Figure 5). The eco-efficiency indicates the net economic gain per unit of ecological destruction concerning GHG emission. In the current study, the eco-efficiency index (EEI) was articulated in terms of monetary gain per unit of CO_2-eq emission (US\$ per kg CO_2-eq). Among the tilling practices, CT had the lowest eco-efficiency (1.0 US\$ kg^{-1} CO_2-eq emission; Figure 4), whereas ZT had the highest EEI (1.55 US\$ per kg CO_2-eq emission). It implied that the CT in Eastern Himalayas may be replaced/substituted with conservation effective tillage for improving economic gain and environmental quality. Diversified cropping also exerted a significant impact on EEI. The maize–rajmash–buckwheat system recorded the highest EEI (1.57 US\$ per kg CO_2-eq emission) followed by the maize–black gram–buckwheat system. The maize–black gram–toria system had the least EEI (1.06 US\$ per kg CO_2-eq emission; Figure 5).

3.4. Soil NPK status

Tillage practices showed significant variations in the soil nutrient status (N, P, and K; Table 3). Soil-available N was the highest in ZT at all the soil depths (340.0, 316.5, and 295.8 kg ha^{-1} for soil depths of 0–10, 10–20, and 20–30 cm, respectively) than RT and CT. The ZT practice also recorded significantly higher soil-available P (16.6 kg ha^{-1}) than RT and CT at 0–10 cm soil depth. Whereas, at lower soil depths, 10–20 and 20–30 cm, soil-available P status was not affected due to tillage practices. Significantly higher soil-available K at the 0–10 cm soil depth was

TABLE 2 Impact of different tillage practices and diversified cropping on economic returns (mean of 3 years).

Treatment	Production cost (US $ ha^{-1})	System gross return (US $ ha^{-1})	System net return (US $ ha^{-1})	B:C ratio
Tillage practices				
CT	1,430	3,741	2,311	1.61
RT	1,404	4,101	2,697	1.92
ZT	1,364	4,245	2,881	2.10
SEm±	–	28	28	0.02
LSD ($p = 0.05$)	–	69	69	0.05
Diversified cropping				
Maize-black gram-toria	1,299	3,480	2,181	1.68
Maize-black gram-buckwheat	1,322	4,014	2,691	2.04
Maize-rajmash-toria	1,476	3,979	2,503	1.70
Maize-rajmash-buckwheat	1,500	4,643	3,143	2.10
SEm±	–	116	116	0.08
LSD ($p = 0.05$)	–	237	237	0.16

CT, conventional tillage; RT, reduced tillage; ZT, zero tillage; SEm, standard error of mean; LSD, least significant difference; SPE, system production efficiency; B:C ratio, benefit cost ratio.

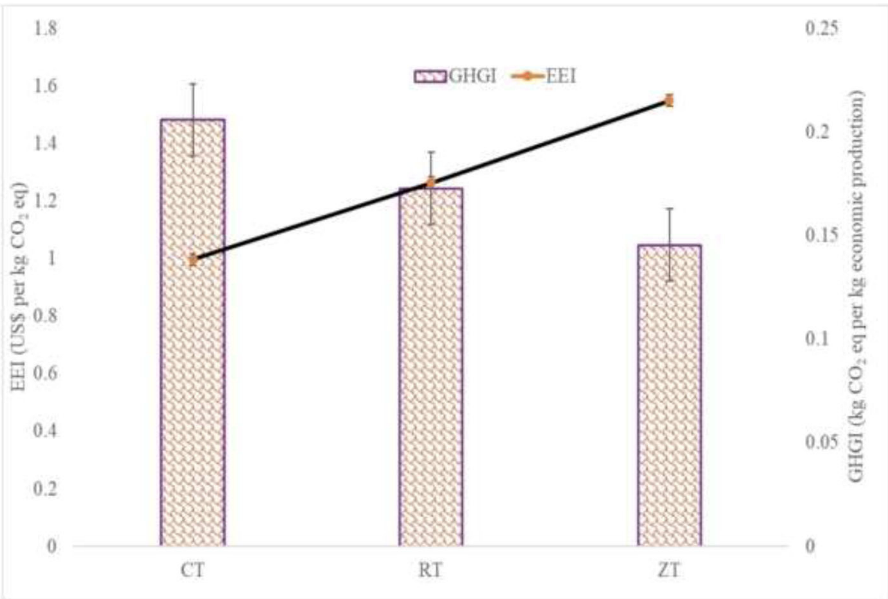

FIGURE 4
Effect of tillage practices on eco-efficiency index (EEI) and greenhouse gas intensity (GHGI). CT, conventional tillage; RT, reduced tillage; ZT, zero tillage. Error bar indicates the least significant difference (LSD) values at $p = 0.05$.

found under ZT practice (337.3 kg ha^{-1}) followed by RT. However, CT had the lowest available K (327.5 kg ha^{-1}). However, at lower soil depths (10–20 and 20–30 cm), the effect of tillage on soil-available K content was found to be non-significant. Diversified cropping also brings a significant change in plant-available N at soil depths 0–10 and 10–20, and P and K at only surface soil (0–10 cm depth). Among the diversified systems, the soil under the maize–black gram–buckwheat system had significantly higher plant-available N (336.8 kg ha^{-1} at 0–10 cm and 312.5 kg ha^{-1} at 10–20 cm). Concerning P and K, the soil under the maize–black gram–buckwheat system had higher plant-available P and K.

4. Discussion

Besides the microclimates, comprehensive crop and soil management under conservative agricultural systems regulate the productive capacity of the crops and cropping systems (Das et al.,

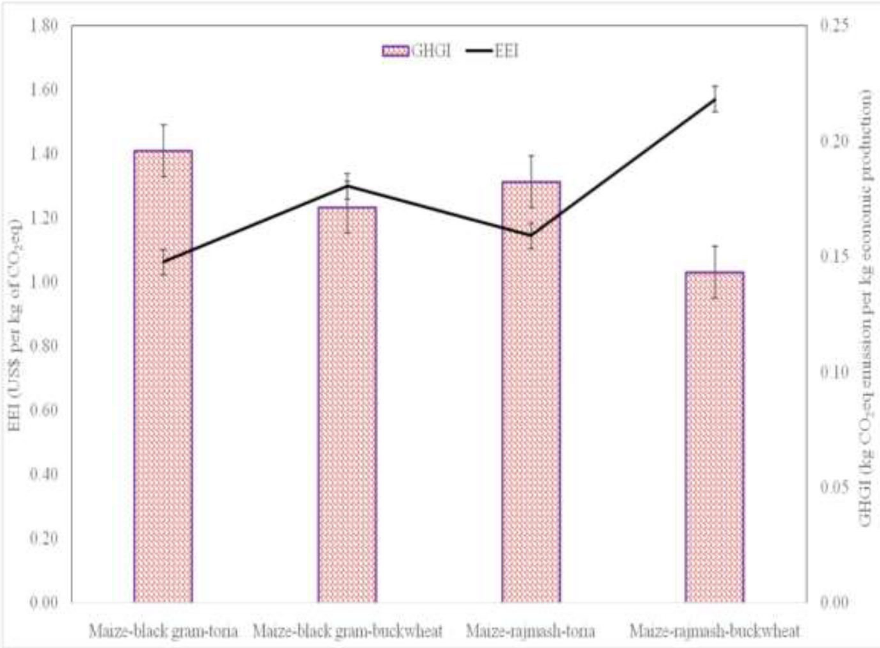

FIGURE 5
Effect of diversified cropping on eco-efficiency index (EEI) and greenhouse gas intensity (GHGI). Error bar indicates the least significant difference (LSD) values at $p = 0.05$.

TABLE 3 Impact of different tillage practices and diversified cropping on available N, P, and K after three cropping cycles.

	Available N (kg ha^{-1})			Available P (kg ha^{-1})			Available K (kg ha^{-1})		
	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20-30 cm
Tilling practices									
CT	313.8	296.6	281.5	16.0	14.8	13.9	327.5	313.6	301.8
RT	322.3	306.6	289.8	16.2	14.5	13.7	330.9	314.3	303.8
ZT	340.0	316.5	295.8	16.6	14.8	13.6	337.3	317.1	297.8
SEm±	2.3	1.9	2.6	0.02	0.3	0.5	1.9	2.7	1.8
LSD ($p = 0.05$)	5.5	4.7	6.4	0.04	NS	NS	4.7	NS	NS
Diversified cropping									
Maize-black gram-toria	319.9	299.4	289.0	16.2	14.2	13.5	329.1	311.3	297.8
Maize-black gram-buckwheat	336.8	312.5	295.0	16.8	15.4	14.3	339.3	320.4	304.4
Maize-rajmash-toria	318.0	310.6	287.2	16.0	14.5	13.7	332.0	317.4	305.5
Maize-rajmash-buckwheat	326.7	303.8	284.9	16.1	14.6	13.3	327.2	311.1	296.8
SEm±	2.5	2.2	4.8	0.2	0.5	0.4	2.1	3.8	4.7
LSD ($p = 0.05$)	5.2	4.6	NS	0.5	NS	NS	4.2	NS	NS

CT, conventional tillage; RT, reduced tillage; ZT, zero tillage; SEm, standard error of mean; LSD, least significant difference; NS, non-significant.

2020; Raj et al., 2022). The adoption of ZT/RT along with legumes as a component in diversified cropping is an economically feasible and environmentally sustainable production option in many agro-ecoregions (Das et al., 2020; Sayed et al., 2020; Kumar et al., 2021). A positive effect of ZT and RT on crop productivity was reported in the current investigation. The tillage effect was more pronounced in late-rainy season and winter-season crops. ZT increased the productivity of the late-rainy season and winter season crops by 9.6 and 22.3% over the CT system, respectively. The superiority of RT over the ZT and CT systems in terms of maize yield gain might be due to the higher accumulation of SOC and plant-available nutrients in the soil which favored maize growth (Zhang et al., 2015). Under CT, repeated tillage exposed the soil, which may accelerate soil and nutrient erosion during the splendid crop growth stage and result in poor crop productivity (Lal, 2015; Raj et al., 2022). The poor yield of maize under ZT may also be attributed to poor crop establishment during the early-growth stage as compared to RT (Yadav et al., 2018). Furthermore, RT provides a congenial microclimate for the stand establishment of maize crops, which may result in a higher maize yield. Approximately 7%−12% higher maize productivity in coarse loamy soil under RT over ZT and CT was also reported by Das et al. (2020) and Fiorini et al. (2020). Conservation tillage practices like RT and ZT enhance soil microflora and faunal diversity, SOC, and associated soil properties, besides minimizing soil and nutrient erosion (Das et al., 2019; Raj et al., 2022). Higher crop productivity under the ZT/RT system over CT has been reported by several investigators (Islam et al., 2015; Yadav et al., 2020, 2021b). Cropping system diversification also extruded a significant effect on the grain yield of maize and other component crops in the system mode. In the current investigation, maize yield was higher when rajmash was replaced with black gram during the late-rainy season and toria was replaced with buckwheat during the winter season. Rajmash yield was 18.3% higher under the maize–rajmash–buckwheat system as compared to the maize–rajmash–toria system. Similarly, black gram yield was 17.9% higher under the maize–black gram–buckwheat system than the maize–black gram–toria system. The winter crop yield was higher when rajmash was substituted with black gram. These findings suggested that the selection of crops plays a crucial role in determining the system productivity and yield of component crops in the system mode. Crop selection determines the system's economic productivity and yield of different crops accommodated in a year on the same piece of land (Babu et al., 2020b).

Higher crop yield under conservation tillage over the CT system considerably improves the system's production efficiency and economic returns. In the present investigation, ZT and RT saved 26 and 66 US\$ ha^{-1} over the CT system, respectively. Furthermore, the RT and ZT systems registered additional gains of 386 and 570 US\$ ha^{-1} over the CT system, respectively. Similarly, ZT registered a 1.3 times higher B:C ratio over the CT system. This type of trend was due to the higher economic yield of almost all the crops and lesser investment under conservation-effective tillage practices over the CT system. Higher economic returns from different crops under conservation tillage over conventional tillage were also testified by Yadav et al. (2020) and Yadav et al. (2021a). Hence, it can be inferred that the tillage elimination under organic management can be an economically feasible agronomic option. Crop diversification also had a significant impact on overall farm productivity and economic returns. Despite higher production costs, the replacement of toria with buckwheat under the maize–black gram and maize–rajmash systems during the winter season recorded considerably more system yield and economic returns. The replacement of toria with buckwheat under the maize–rajmash system registered 20.36% and 58.8% higher net returns and B:C ratio. Similarly, the replacement of toria with buckwheat had a considerable effect on the crop yield and economic returns of the maize–black gram system. A 10% yield gain under the diversified system over the existing cropping system was also reported by Bennett et al. (2012). Hence, our finding suggested that the inclusion of buckwheat is a more promising option under an intensified maize-based system in the place of toria during the winter season under organic farming in the Eastern Himalayan region of India.

In the current study, the environmental performance of diverse tillage and cropping systems were estimated in terms of GHGI and EEI. The current study inferred that the CT system had 40 and 23.5% higher GHGI over the ZT and RT systems, respectively. The higher GHGI under the CT system may be attributed to the higher energy involvement and fossil fuel combustion during the repeated plowing operations as compared to RT and ZT. The higher GHGI of the CT system over the conservation effective method under similar ecology was also reported by Yadav et al. (2021b). Cropping diversity also had a significant effect on GHGI in the current study. The replacement of toria with buckwheat during the winter season significantly reduced the GHGI. Similarly, the replacement of black gram with rajmash during the late-rainy season under the maize–buckwheat system reduces the GHGI considerably. This inferred that cropping diversity plays a significant role in climate change mitigation. The lower GHGI under the maize–rajmash–buckwheat and maize–black gram–buckwheat systems over the maize–black gram–toria system is primarily due to higher system productivity. Hence, crops must be selected very wisely when designing cropping systems under organic farming. Therefore, low-input demanding and high-productivity crops which contribute less to GHG emissions and have high resource conversion efficiency should be selected. System productivity and GHGI have an inverse relationship (Rathore et al., 2022). The results of this study have the common view that diversified production systems have lower GHGI over the monocropping system. EEI estimation is imperative when evaluating the environmental performance of a designed system. The EEI considers both the financial and ecological dimensions of the production technology (Babu et al., 2020a). CT had the lowest EEI, which indicates that the existing tillage system under the organic farming system of the Indian Himalayan region needs to be shifted toward conservation-effective tilling practices for improving the environmental robustness of the organic farming system. Cropping diversity had a significant impact on EEI. In the present study, the maize–rajmash–buckwheat system had 48.11% higher eco-efficiency than the maize–black gram–toria system. Crops that increase eco-efficiency and reduce GHG emissions can effectively achieve environmentally robust and economically feasible production systems. Thus, it can be emphasized that the adoption of conservation effective tillage with diverse cropping can increase the environmental and economic robustness of organic production systems.

Conservation-effective tillage practices favored the accumulation of SOC which may hasten nutrient accumulation and mobilization. In the present study, ZT registered 4.8%−7.7% higher plant-available N over CT in the entire soil profile up to the 30-cm depth. ZT favors the slow decomposition of organic matter and facilitates the regular supply of plant-available N (Paul et al., 2013; Das et al., 2018). Hence, ZT enhances the N availability in soil over CT and RT. Conservation-effective tillage improves the soil properties (Lal et al., 2018; Sadiq et al., 2021) and enhances soil microbial diversity and activity, thus fueling mineralization and conversion of organic N into plant-available N (López-Garrido et al., 2011; Lal et al., 2018). Furthermore, even a short period of adoption of ZT/RT improved soil health by improving soil biochemical reactions and soil structure. The combined effect of tillage and diversified cropping was also found to be significant in the present investigation at the 0–10 cm soil depth. The significant effect may be attributed due to the higher soil organic matter which increases the soil N status (Feng et al., 2013; Lal et al., 2018). On the contrary, repeated/intensive plowing under CT increases soil compaction and bulk density and reduces SOC, thereby resulting in poor soil conditions (Orzech et al., 2021; Sadiq et al., 2021).

The ZT also improved P availability in soil marginally at a soil depth of 0–10 cm over CT. However, the effect of tillage practice was not significant below the soil depth of 10 cm. An increment in soil-available P after three cropping cycles in sandy clay loam soil was also reported by Lal et al. (2018). ZT promoted SOC accumulation in the surface soil which might alter the immobilized P into the available form (López-Garrido et al., 2011; Yadav et al., 2021a). Of the different systems, higher plant-available P was noticed under the maize–black gram–buckwheat followed by the maize–rajmash–buckwheat system in surface soil (0–10 cm depth). Buckwheat can extract soil P from subsurface soil and accumulate it on the upper surface and thereby increase plant-available P in the soil surface. Higher soil-available P after buckwheat harvest was also recorded by Babu et al. (2018). Furthermore, the constant supply of organic amendments accelerates microbial functions, which reduces occluded P and increases mineralized P (Wang et al., 2012; Das et al., 2018).

In the present study, ZT increases ∼2.9% plant-available K in soil over CT after 3 years. Comparatively, ZT increases C input and reduces soil compactness besides improving other soil properties, which in turn increases soil K status (Prasad, 2010; Das et al., 2019). Similarly, among the different cropping systems, cultivation of maize–black gram–buckwheat added more K in the soil through the addition of a different type of residue. An increment in soil K status in response to the addition of a different type of biomass was previously reported by Das et al. (2018). Legumes pumped atmospheric N which may fuel the soil microbial diversity and activities and alleviate the soil nutrients status (Latati et al., 2017). It indicated that legumes, especially black gram and pseudo-cereal-buckwheat, are more justifiable substitutes for rajmash and toria for inclusion in intensified cropping during late-rainy and winter seasons in the Sikkim region of Indian Himalayas, respectively.

5. Policy implications of the study

Agricultural land can store ∼1.2 billion tons of C which can reinforce the policy and plans to achieve the goal and targets of the Paris Agreement (Henderson et al., 2022). India announced a five-fold approach to fight climate change during CoP-26 and committed to reducing 1 billion tons of carbon by 2030 and achieving zero-emission targets by 2070. Organic production systems rely upon ecological principles of farming, and the use of synthetic fertilizers and pesticides is prohibited. Hence, it has significantly lower global warming potential than conventional farming. The production of synthetic fertilizers and pesticides are energy-intensive processes. The organic production system requires ∼45% less energy and emits ∼40% less carbon as compared to conventional farming (Zimmerman, 2020). However, some researchers have reported poor crop yield under organic farming compared to conventional production systems. Conservative agricultural practices can offset GHG emissions besides improving crop productivity and soil quality to meet the growing food demand with minimum environmental footprints (Yadav et al., 2021b; Zhang et al., 2022). Conservation tillage had 26%−31% less global warming potential (Mangalassery et al., 2014) and stored more carbon in the soil than conventional tillage (Yadav et al., 2020). Furthermore, conservational tillage practices have considerably less water footprint than conventional tillage systems (Rahman et al., 2021). The crop diversification portfolio has numerous positive outlooks over monocropping. Crop diversification has a positive impact on farm productivity, profitability, household-level livelihood and nutritional security, and ecosystem services (Mortensen and Smith, 2020). Overall crop diversification can be proposed as a potential risk-coping strategy under current and futuristic climatic scenarios. The findings of the current study suggested that ZT has the potential to reduce the cost of cultivation by 4.9% and increase net income by 24.7% over the CT system under organic management conditions. Furthermore, ZT had 28% less GHGI than CT. Crop selection also played a crucial role in mitigating GHG emissions and increasing farm productivity and profitability under the current study. Hence, we propose that conservation tillage and diversified cropping in organic farming systems can potentially increase soil carbon content, crop productivity, and profitability and curtail GHG emission, which can potentially enhance the economic and environmental benefits of organic production systems. However, the development of cost-effective and practically feasible conservation tillage methods is a great challenge in adopting the ZT and/or RT practices, especially for the organic production scenarios in hill and mountain ecosystems. Diversified cropping is a multiproduct-oriented production system that needs multi specialties and marketing. Capacity building and infrastructure development are also great challenges in practicing crop diversification. Moreover, the development of realistic organic production models is highly individualistic, and location-specific need a proper understanding of available resources. Hence, joint efforts of farmers, researchers, policy planners, and other stakeholders are needed for the wider adoption of conservation agricultural practices under organic farming.

6. Conclusion

Conventional chemical-based farming practices increase food production by many folds to feed the global population but at the same time have a negative impact on natural resources, which amplifies the environmental problems. Organic farming is globally advocated as an environmentally robust agricultural production practice to produce quality food. However, the poor crop yields of organically managed fields over the inorganically managed fields force researchers to devise technologies to improve the productivity of organic agricultural systems without compromising environmental robustness. The findings of the current study proved the hypothesis that conservation-effective tillage and pulses-based diversified cropping will increase productivity, profitability, and eco-efficiency besides improving soil fertility. The study concluded that ZT had significantly higher system net returns (2,881 US$ ha^{-1}) over the CT system besides curtailing the 28% GHGI over the CT system. Furthermore, ZT had 55% higher EEI than the CT system. Concerning crop diversity, the replacement of toria with buckwheat and rajmash with black gram in a maize-based cropping system during winter and late-rainy season in organic farming was found as an economically feasible option to improve the profitability and environmental robustness of organic farming in the Eastern Himalayan region. Furthermore, the maize–rajmash–buckwheat and the maize–black gram–buckwheat systems have higher EEI and lesser GHGI over other diversified systems. Hence, the findings recommended that the implementation of ZT/RT under diversified maize-based systems is a productive, environmentally robust, and soil-supportive practice for obtaining higher economic returns under the organic farming condition of the Indian Himalayas and other similar ecoregions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

SB and RA: visualization, experimentation data curation, supervision, and project administration. RS: conceptualization, experimentation, data analysis, writing of original and first draft, and review and editing of the final draft. SR and AD: data curation, review, and editing. VSh, JL, SK, and VSi: review and editing. OW: review and editing and referencing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MC declared a shared parent affiliation with the authors SB, RS, RA, SR, AD, JL, and VSh to the handling editor at the time of review.

Publisher's note

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Prediction of Chongqing's grain output based on support vector machine

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Scientific prediction of agricultural food production plays an essential role in stabilizing food supply. In order to improve the accuracy of grain yield prediction and reduce the error of grain yield prediction in Chongqing, this paper proposes a new method for the grain yield prediction in Chongqing by using support vector machine (SVM). In this paper, based on the support vector regression structure, the support vector regression algorithm is designed, and then the support vector machine is adopted in the replacement of the error back propagation process in BP neural network. The results of case analysis show that the method based on support vector machine can effectively reduce the error of grain yield prediction.

KEYWORDS

support vector machine, food production, to predict, neural network, weights of the particle

1. Introduction

Food is the basic material for the survival of human life. Food has been an important issue concerning the development of human society since ancient times. Drought exacerbated by global warming and rising prices of the food in the world have once again heightened concerns about food security (Bingjun and Weiming, 2019). Food supply and demand are closely related to food security. Food security is not only an economic issue, but also related to the long-term and stable development of a country.

With a population of more than 1.4 billion, China is both a major grain producer and a major grain consumer. The food shortage is likely to become a major bottleneck issue impeding China's economic development and social stability. Therefore, it is very essential to ensure the food security and strike the balance between the food supply and demand (Tian et al., 2018). With the increasing population, the decrease in arable land, accelerated urbanization and the improvement in people's livelihood, the Chinese increasingly need more and more food supply, the food supply fails to meet people's demands for food. Moreover, as a special commodity related to the national economy and people's livelihood, food is highly susceptible to non-traditional factors, such as food hegemony and biofuel development. Therefore, food security has become a long-term strategic task for China (Dai et al., 2020).

At present, remote sensing prediction model, statistical dynamic growth simulation model, meteorological yield prediction model and other technologies are applied for predicting the grain yield (Hayashi et al., 2018). Generally speaking, prediction models are the major method used to predict the future situation of food supply and demand and determine its potential impact on the world food market. For example, the United States Department of Agriculture (USDA) uses econometric methods to carry out long-term prediction of the production, consumption, and trade of major agricultural products in

China. In addition, scholars have used a partial equilibrium model, to predict grain yield for the agricultural sector. The model is a global, non-regional economic model, including 35 countries and regions, 17 products, and it is a highly comprehensive model in nature. The model predicts that China's grain production will grow at an annual rate of 1.7 to 1.8 percent, reaching 640 to 660 million tons by 2030. Meanwhile, according to the prediction, China's food demand will range from 680 million tons to 717 million tons by 2030, with a gap of between 0.4 million and 57 million tons (Chao et al., 2018). A model is OECF model. It is used by the Overseas Foundation of Japan to forecast grain production and perform trend analysis without considering the effect of price.

In addition, techniques, such as time series model, regression model, systematic integrated factor forecasting method, simulation technology forecasting method, neural network forecasting method, chaos forecasting method, and gray forecasting method to forecast grain production (Fei and Xing, 2019). For example, he investment occupancy output technique and the variable coefficient forecasting model method were used to predict China's grain production, grain imports, and self-sufficiency rate by 2030. It is predicted that by 2030, China will produce 685 million tons of grain, import 50 million tons of grain, and have a self-sufficiency rate of about 93%. In addition, grain production can be divided into economic and technical production and meteorological production by calculating the climatic productivity, and the regression prediction model can be established by using the fertilizer application, percentage of planting area and monthly average temperature.

Located in the upper reaches of the Yangtze River, Chongqing is rich in biological resources and water resources, and has a developed agricultural industry, which has made a great contribution to China's grain production. In 2017, Chongqing accounted for only 3.02% of the country's arable land and 6.5% of the country's total grain production, making it the seventh largest grain-producing province and the highest rice production in China. The grain production of Chongqing not only meets the residents' requirements for better livelihood and higher income and development, but also makes an outstanding contribution to the national grain development, making Chongqing take a pivotal position in terms of the grain production in the whole country. However, in recent years, Chongqing has experienced prominent contradictions in agricultural structure, sloppy business models, serious shrinkage of arable land, and low motivation of farmers to grow grain, resulting in a declining trend of grain production in the past 2 years. It is urgent to analyze and study the grain production in Chongqing. Therefore, it is of great theoretical and practical significance to analyze the influencing factors of grain production in Chongqing, predict the future trend of grain output, and discuss the effective countermeasures to coordinate the contradiction between man and land. Through the analysis and research on the development pattern and prediction of grain yield in Chongqing, the forward-looking evaluation can be conducted on the evolution of grain production and development trend in Chongqing, and possible unreasonable phenomena and problems in the process of grain production can be detected in advance, to prevent and timely measures to solve these problems in advance, making the food production satisfy the market economy, and improving the efficiency of the government macroeconomy.

A prediction model was (Yafei et al., 2019) proposed based on principal component analysis (PCA) and particle swarm optimization (PSO) neural network. Firstly, the correlation coefficient between each influencing factor and grain yield is calculated, the principal component analysis method is used to reduce the dimension of the influencing factor, the reduced dimension factor is used as the input of the neural network, then the BP neural network is used to establish the grain yield prediction model. The PSO algorithm is adopted to optimize the weights and thresholds of the BP neural network, and finally the trained BP neural network is used to predict the grain yield value. Also, hydraulic processes in roots and the rhizosphere pertinent to increasing yield of water-limited grain crops have been proposed by some scholars (Ahmed-mutez et al., 2018): a critical review. According to a first-order approximation, the yield of water-limited food crops depends on (1) the amount of water available to the crops, and (2) the water distribution of the crops during the growing season. The water distribution of the crops during the growing season determines the harvest index of crops, that is, the proportion of aboveground biomass of crops that are converted into grains. A preferred condition is that about 30% of the seasonal available water supply is used during flowering and grain filling. This paper has analyzed the role of roots in the amount and time of extracting water from the soil, which may lead to maximum grain yield, and the mechanisms behind. These features can be categorized into architectural and anatomical features; the biophysics of water movement from soil through roots to leaves, particularly the nature and processes at the interface between roots and soil and the role of mucilage therein; and the physiological role of the root system in influencing crop canopy growth and transpiration processes that can optimize seasonal patterns of water use.

In order to predict the grain yield of Chongqing effectively, this study is based on the theory of support vector machine. As grain output is susceptible to factors related to production inputs, the level of cultivation technology, climate, environment and other natural conditions, national policy adjustment and other factors. Therefore, it is necessary to regard the formation process of food production as a gray dynamic system with both known information and unknown information, to avoid the mutual fluctuation of many external factors behind food production. At the same time, as a machine learning algorithm, support vector machine (SVM) is based on the VC dimension theory of statistical learning theory and the principle of structural risk minimization. It can minimize the actual predicted risk by minimizing the structural risk by reducing the structural risk to the minimum (Ye et al., 2020). Therefore, the algorithm can achieve a better learning result when the sample size is limited. In the process of grain production forecasting, support vector machine forecasting model is established by processing and analyzing the original data and understanding the development pattern of grain extraction and quantitative forecasting of the future condition of grain, the uniqueness and high accuracy of the required data. One of the most important functions for the model is to optimize the performance. Therefore, support vector function can make up for the shortage of econometric modeling.

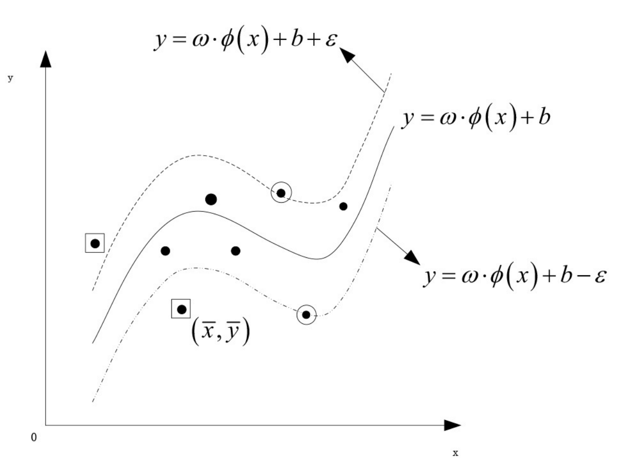

FIGURE 1
Structure diagram of support vector regression.

In this study, grain yield is taken as the behavioral characteristic quantity of support vector. An analysis and prediction model of grain yield is established using vector machine theory, and the model is optimized for prediction. On this basis, the internal change rule of grain system is studied, to make a more scientific and accurate prediction of grain production in Chongqing. A new adaptive learning support degree regression algorithm is designed to improve the prediction accuracy of grain yield. Instead of the error back propagation algorithm of BP neural network, the support vector machine algorithm is used to change the particle weights and thresholds in the BP neural network. The weights of the neural network can be obtained by determining particle parameters, and the fitness of particles can be determined through the training of the neural network. By adopting this method, BP neural network can no longer fall into the local optimum, thus effectively improving the prediction effect of grain yield.

2. Prediction method design

2.1. Design of support vector regression algorithm

In order to improve the prediction accuracy of grain yield, this study designs a new adaptive learning support quantity regression algorithm. The structure of support vector regression is shown in Figure 1.

Generally speaking, in the application of support vector regression, it is necessary to define a class of undefined target variables before the learning model is established. However, such variables can hardly be defined accurately. In fact, this type of target variable is a comparative value and only requires a relative value used for comparison rather than a very precise definition (Zhang and Xu, 2019). However, the target variable is affected by many factors. Suppose these factors are defined as n-dimensional vector $X(x_1, x_2, \cdots, x_n)$, $X \in R^n$ is mapped to $Y \in R$, and the target variable Y can be defined as $X \mapsto Y\,(R^n \mapsto R)$.

Then, the support vector regression algorithm established and applied to grain yield prediction can be expressed as the following steps:

Step 1: According to the index observation sample, construct a sample set $D = \{x_1, x_2, \cdots, x_i\} \in X$ that defines the target variable.

Step 2: The target variable $y_i \in Y = R$ is calculated based on the algorithm of the target variable.

Step 3: $x_i \in X = R^n$ and $y_i \in Y = R$ are used to form the matrix $A = (A_1 \cdots A_i \cdots)$, wherein,

$$A_i = \begin{pmatrix} x_i \\ y_i \end{pmatrix}.$$

Step 4: Calculate $\bar{A}$, wherein, $\bar{a}_i = \frac{1}{n}\sum_{i=1}^{n} a_i, \bar{a}_i \in \bar{A}$.

Step 5: Calculate cov A.

Step 6: Calculate the partial correlation coefficient.

Step 7: In D, k rows corresponding to the largest k partial correlation coefficients are retained to convert D into $D' \in R^k$.

Step 8: The target variable $y_i \in Y = R^k$ is recalculated using the target variable definition algorithm.

Step 9: On the basis of D', the attributes that do not meet the criteria for the definition of the target variable but are very important for it are added to each sample (Yaodong, 2019), the non-useful attribute is deleted from X, then $x_i \in X = R^k$, thus constituting the training sample set $T = \{(x_1, y_1), \cdots, (x_i, y_i)\} \in (X, Y)$, where,$x_i \in X = R^k, y_i \in Y = R, i = 1, 2, \cdots, n$.

Step 10: Train the support vector regression machine about X and Y with Y as the reference variable.

Step 11: The definition of the support vector regression machine of the target variable is obtained:$y(x) = \sum_{i=1}^{n}(a_i - \bar{a}_i)\,k\,(x_{i+1} - x_i) + R^k$.

It is difficult to obtain the usage in this process, but the impact on the properties of the target variable to meet the strict increase or decrease in the loosely defined target variable, while using accessible impact factors, as well as properties with high practical value, has been defined roughly as the benchmark for the target variable, support vector regression machine, the implementation of the target variable non-linear definition. As a result, the definition of the target variable and the efficiency of the application are greatly improved (Bibb et al., 2018).

Based on the algorithm of support vector regression for grain yield prediction, the SVM is used to replace the error back propagation of BP neural network and change the particle weight and threshold, to realize the grain yield prediction of Chongqing.

2.2. Prediction of grain output in Chongqing

By determining the particle parameters, the weights of the neural network can be obtained and the fitness of the particles can be determined through the training of the neural network. Therefore, BP neural network can hardly fall into the local optimum, and thus effectively the prediction effect of grain yield is improved.

The prediction process of grain yield in Chongqing is as follows:

Step 1: Pre-processing of historical data of grain yield in Chongqing.

The historical data of grain yield are analyzed by using support vector machine. The influencing factors are determined and used as initial parameters to normalize the historical data of grain yield (Weichert et al., 2017).

Step 2: Initialize the neural network and construct the neural network.

Support vector machine is one of the basic structures of neural network. Therefore, the number of nodes in the input and output layers of the neural network can be determined based on the input and output data of the neural network. In this process, there is no value algorithm that can be used directly in the selection of the number of nodes in the hidden layer, so an empirical method is adopted instead to select the number of nodes in the hidden layer, i.e., the number of nodes in the hidden layer that works best after several experiments (Tadesse et al., 2018). In addition, attention should be paid to avoid the adverse effects of the initial settings on the convergence speed and accuracy of the network.

Step 3: Set the initial parameters, including the position and speed of particles, learning factors and other parameters required by the algorithm.

Step 4: The support vector regression algorithm designed in the previous section is used to determine the optimal particle of grain yield population N in Chongqing. The particle positions and velocities are updated by successive iterations. In the whole process of iterative updating, the optimal position searched by the qth particle is the individual extreme value and the global extreme value.

Step 5: Calculate the fitness value.

Fitness can be used to determine whether the current particle position is optimal position. In the process of successive iteration and update, each particle moves through the solution space at a set speed, and keeps converging to the individual best position p_{best} and the global best position g_{best}. The fitness function of grain yield prediction is set as:

$$fitness = \frac{N}{\sum_{i=1}^{q} (e_i - t_i)^2} + b \qquad (1)$$

Where, *fitness* represents the fitness function of grain yield prediction, b is usually a constant, e_i represents the expected output value of the neural network, and t_i means the actual output value of the neural network.

The fitness value adopted in this study is a linear function of the mean square error and reciprocal between the actual value and the predicted value. The smaller the error is, the larger the corresponding fitness value is and the better its fitness is (Sundaram et al., 2018).

Step 6: Update individual extreme values and group global extreme values.

The fitness value of the calculated current fitness value of each particle is compared with the fitness value of the individual extremum. In comparison, if the current particle has a better fitness value than the individual, the current particle's position is assigned to the individual's extreme value. Then, the fitness value of the individual extreme value of each particle is compared with the fitness value of the global extreme value of the population, and the individual extreme of the particle with the better fitness value is selected and the value is assigned to the global extreme of the population.

Step 7: Determine whether the termination condition is satisfied, that is, whether the expected convergence accuracy is achieved. In this process, the global optimal solution obtained above should be applied to search the weight and threshold of BP neural network. If the termination condition is not met, repeat Step 4 to 6 until the termination condition is met.

Step 8: Achieve grain yield prediction.

The BP neural network with the optimal weight and threshold is used to predict the grain yield.

The grain yield prediction process is shown in Figure 2.

In conclusion, by introducing the structure of support vector regression, the implementation of support vector regression algorithm for grain yield prediction was designed, and the algorithm design of support vector regression was completed. On this basis, the effective prediction of grain yield in Chongqing is realized by searching the optimal particle weight and threshold of neural network.

3. Empirical analysis of grain yield prediction in Chongqing

In order to verify the effectiveness of the prediction method of grain yield in Chongqing based on support vector machine designed in this study, and to carry out effective prediction of grain yield in Chongqing, this study designs the following experimental verification and empirical analysis.

3.1. Prediction and testing of total grain output

The total grain yield in Chongqing has shown a linear trend in the past 5 years. In order to ensure the stability and similarity of the original data, the support vector machine model only needs "poor

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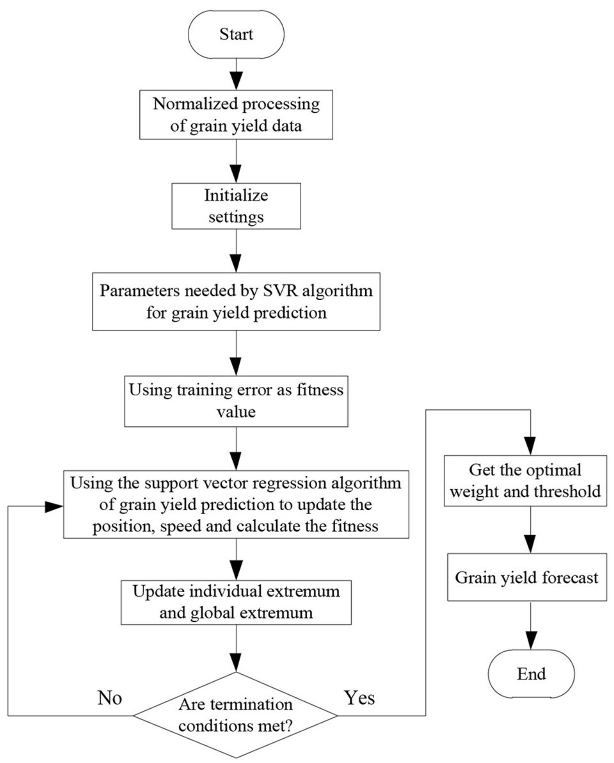

FIGURE 2
Schematic diagram of predicting the grain yield.

TABLE 1 Statistics on total grain yield of Chongqing from 2015 to 2019.

Year	Total grain output (10,000 tons)
2015	43069.5
2016	46946.9
2017	48402.2
2018	49804.2
2019	50160.3

data." The historical data of grain output in Chongqing are shown in Table 1 (Yao et al., 2020).

When the total grain production development coefficient of Chongqing a = 0.022509922 $\leq$ 0.3, the total grain production model of Chongqing can predict medium—and long-term trend.

According to the above historical data of grain yield in Chongqing, the corresponding time function can be obtained as follows:

$$\hat{x}^{(1)}(k+1)=\left[x^{0}(1)\right]e^{-k} \tag{2}$$

At $k = 0,1,2,\cdots,n$, the predicted value of the original sequence can be obtained based on $\hat{x}^{(0)}(k)=\hat{x}^{(1)}(k+1)-\hat{x}^{(1)}(k)$. As shown in Table 2, the prediction results of grain output in Chongqing in recent 5 years are obtained based on the prediction method designed in this study (Yao et al., 2020).

TABLE 2 Prediction of total grain output of Chongqing municipality.

Year	Predicted output (10,000 tons)
2015	43069.5
2016	47190.4
2017	48264.7
2018	49363.4
2019	50487.2

By comparing the results in Tables 1, 2, the prediction results of Chongqing grain yield prediction method based on support vector machine designed in this study are close to the actual results, indicating the effectiveness of the method.

According to the results in Table 2, the total grain output of Chongqing shows a trend of continuous growth. From 2015 to 2019, the grain production in Chongqing exceeds 430 million tons, 460 million tons, 480,000 tons, 49,000 tons and 50,000 tons, respectively. There is no doubt that the formation of such a sustained growth trend is closely related to the preferential agricultural policies of the Central Committee of the Communist Party of China and the State Council, thus enhancing the enthusiasm of farmers to grow grain. In addition, with the continuous scientific and technological development, the grain yield per unit area has been constantly improved, to expand the grain yield on the limited farmland.

On this basis, a support vector machine-based grain yield forecasting method is applied to examine the residuals of grain yield forecasts for 2015–2019 in Chongqing. The results are shown in Table 3.

According to the data shown in Table 3, the relative error of the prediction results of Chongqing grain yield prediction method based on support vector machine is <1%, and the average residual is 0.46%. According to the residual test criteria, the model passed the residual test. Therefore, the validity of grain yield prediction based on support vector machine in Chongqing can be further illustrated.

3.2. Yield prediction and result analysis of main grain varieties

On the basis of the preliminary verification of the effectiveness of the support vector machine based grain yield prediction method in Chongqing, the method is applied to predict the yield of rice, wheat and corn in Chongqing and is compared with the historical data so as to verify the effectiveness of the method in predicting the yield of different crops.

3.2.1. Prediction and testing of rice yield

Combined with Chongqing rice yield from 2015 to 2019, the residual test results of predicted rice yield in Chongqing are shown in Table 4 (Yao et al., 2020).

Based on Table 4, the predicted value of total rice output in Chongqing will increase steadily year by year. The projected output

TABLE 3 Residual test of forecast total grain output in Chongqing.

Year	Actual value (10,000 tons)	Predicted value (10,000 tons)	Absolute error (10,000 tons)	Relative error (%)	Residual (%)
2015	43069.5	43069.5	0	0	0
2016	46946.9	47190.4	243.4	0.5185	0.46
2017	48402.2	48264.7	137.5	0.2841	0.37
2018	49804.2	49363.4	440.8	0.8851	0.52
2019	50160.3	50487.2	326.9	0.6517	0.49
Average	–	–	–	0.5849	0.46

TABLE 4 Residual test of predicted rice yield in Chongqing from 2015 to 2019.

Year	Actual value (10,000 tons)	Predicted value (10,000 tons)	Absolute error (10,000 tons)	Relative error (%)
2015	16065.6	16065.60	0	0
2016	17908.8	17856.27	52.49	0.29
2017	18058.8	18073.96	15.12	0.08
2018	18171.8	18294.31	122.48	0.67
2019	18603.4	18517.34	86.06	0.46

TABLE 5 Residual test of wheat yield prediction in Chongqing from 2015 to 2019.

Particular year	Actual value (10,000 tons)	Predicted value (10,000 tons)	Absolute error (10,000 tons)	Relative error (%)
2015	8648.8	8648.80	0	0
2016	9195.2	9259.89	64.71	0.70
2017	9744.5	9845.56	101.04	1.04
2018	10846.6	10468.26	378.33	3.49
2019	10929.8	11130.35	200.54	1.83

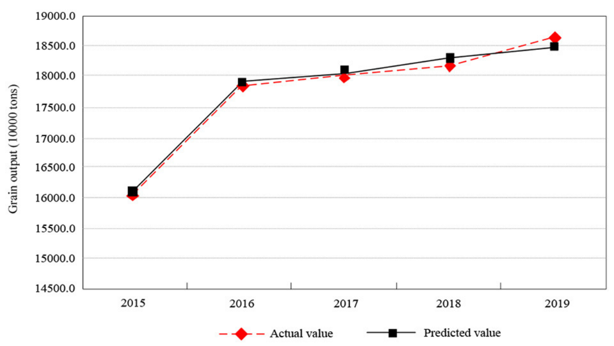

FIGURE 3
The fitting results of actual value and predicted value of rice yield forecast in Chongqing city.

from 2015 to 2019 is 16.0656 m tons, 17.85627 m tons, 18.0739 m tons, 18.29431 m tons and 18.51734 m tons, respectively. It will reach the highest level ever by 2019. The relative errors of the rice yield model in Chongqing are all <0.7%, so the model can pass the residual test. As shown in Figure 3, the fitting of the actual value and the predicted value of the predicted rice yield in Chongqing is described. The fitting degree of the actual value and the predicted value of the rice yield prediction in Chongqing is very high, and it can fully demonstrate that the prediction method of Chongqing grain yield based on support vector machine can effectively achieve accurate prediction of rice production in Chongqing.

3.2.2. Wheat yield prediction and testing

Combined with Chongqing wheat yield from 2015 to 2019, the residual test results of wheat yield prediction in Chongqing are shown in Table 5.

As it is illustrated in Table 5, the projected output from 2015 to 2019 is 86.488 million tons, 92.5989 million tons, 98.4556 million tons, 104.6826 million tons and 111.3035 million tons, respectively. The year 2019 witnessed the historical peak. The relative errors of the rice yield model in Chongqing are all <3.5%, so the model can pass the residual test. As shown in Figure 4, the fitting of actual and predicted wheat yield forecasts in Chongqing is described. The actual value and the predicted value of the wheat yield forecast in Chongqing have a high degree of fit, which can fully demonstrate that the prediction method of Chongqing grain yield based on support vector machine can effectively realize the accurate prediction of the rice yield in Chongqing.

3.2.3. Corn yield prediction and testing

Combined with Chongqing maize yield from 2015 to 2019, the residual test results of corn yield prediction in Chongqing are shown in Table 6.

Based on Table 6, the projected output from 2015 to 2019 is 115.833 million tons, 131.997 million tons, 139.2914 million tons, 147.082 million tons and 155.1525 million tons, and will reach the highest level in history by 2019. The relative errors of the maize yield model in Chongqing are all <3.1%, and the model can pass the residual test. As shown in Figure 5, the fitting of the actual value and the predicted value of corn yield prediction in Chongqing is described. The actual value and the predicted value

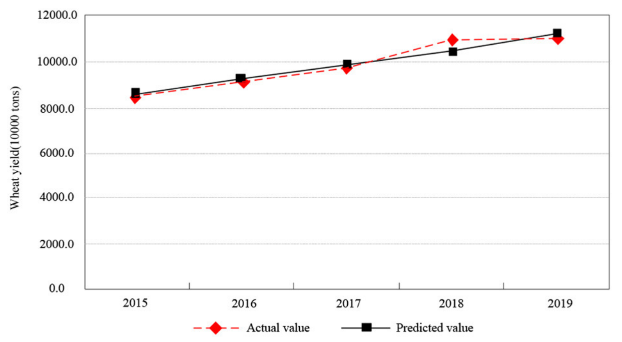

FIGURE 4
Fitting results of actual value and predicted value of wheat yield prediction in Chongqing city.

TABLE 6 Residual test of corn yield prediction in Chongqing from 2015 to 2019.

Particular year	Actual value (10,000 tons)	Predicted value (10,000 tons)	Absolute error (10,000 tons)	Relative error (%)
2015	11583.0	11583.0	0	0
2016	13028.7	13197.97	169.26	1.30
2017	13936.5	13929.14	7.40	0.05
2018	15160.3	14700.82	459.48	3.03
2019	15230.0	15515.25	285.20	1.87

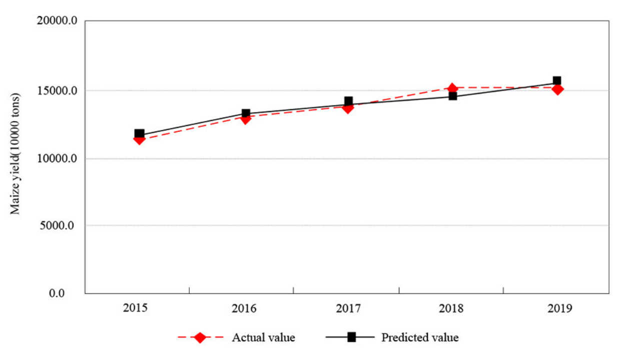

FIGURE 5
Fitting results of actual value and predicted value of corn yield forecast in Chongqing city.

of the corn yield in Chongqing are highly fit with each other and they can fully demonstrate that the method of grain yield prediction based on support vector machine can effectively realize the accurate prediction of corn yield in Chongqing.

Based on the above data, the prediction result of Chongqing grain yield prediction method based on support vector machine is highly fitting with the actual grain yield, so this method can be used for medium- and long-term prediction.

From the prediction results of support vector machine, the total grain production and the output of major grain varieties in

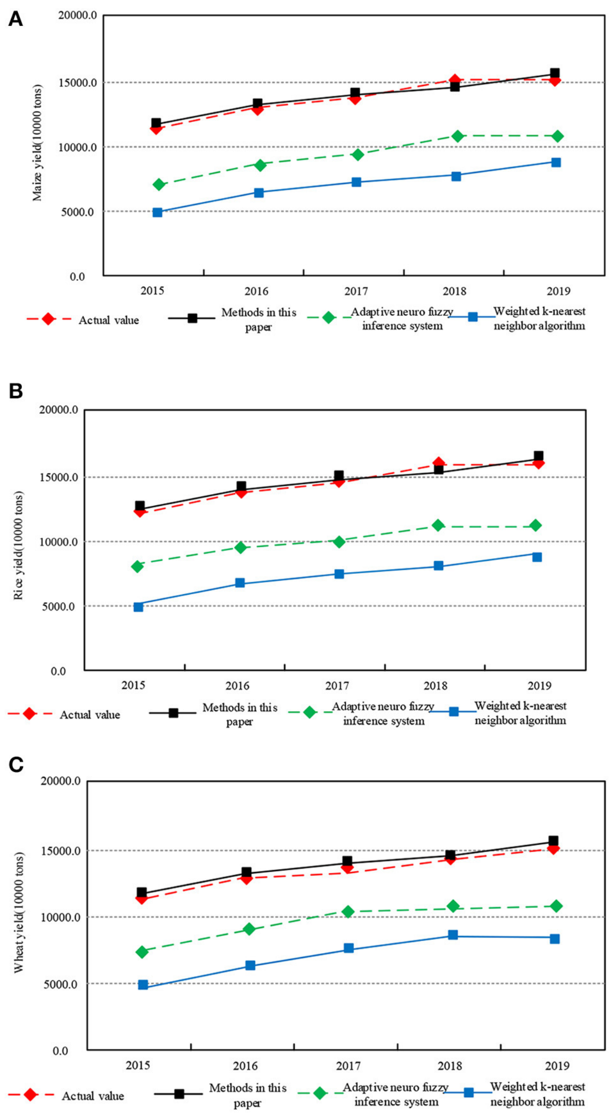

FIGURE 6
Comparison of yield prediction results of maize, rice and wheat with different methods in Chongqing. **(A)** Corn yield. **(B)** Rice yield. **(C)** Wheat yield.

Chongqing tend to grow continuously year by year. This has laid a solid foundation for ensuring food security in Chongqing in the coming years. However, as there are many factors behind the grain output, the grain production in Chongqing still faces some new challenges.

Adaptive neuro-fuzzy inference system and weighted k-nearest neighbor algorithm are compared with the proposed methods. The comparison results of different methods for corn yield prediction in Chongqing are shown in Figure 6.

Based on Figure 6, the prediction results of Chongqing grain yield prediction method based on support vector machine are highly fit with the actual output and it can be used for medium- and long-term prediction. However, the prediction results of Adaptive neuro-fuzzy inference system and weighted k-nearest neighbor algorithm have a low fit with the actual output. The results show

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that the grain yield forecasting method based on support vector machine can effectively realize the accurate forecast of maize, rice and wheat yield in Chongqing.

4. Conclusions

In this study, a new method for predicting agricultural grain production in Chongqing was designed by using support vector machine, and good results were obtained.

Support vector machine (SVM) has good performance in grain yield prediction, but there are some difficulties in the application of SVM. Therefore, in the future, the prediction method will be further optimized to provide theoretical guidance for the selection of kernel functions and parameters in the field of support vector machine (SVM) algorithm, and also to resolve the contradiction between large-scale data sets and training set size and training speed to provide more advanced grain production prediction and effective technical support.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JW has made a significant contribution to the revised draft, modified the article, added relevant literature, and made polish the article. CL contributed to the motivation, the interpretation of the methods, the data analysis and results, provided the draft versions, revised versions, and references. GT provided the data and results, the revised versions, and references. YT provided the related concepts and minor recommendations and extracted the conclusion and discussion. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Food production potential and environmental sustainability of different integrated farming system models in northwest India

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Accelerated energy use, negative environmental outcomes, and poor economic returns questioned the sustainability of contemporary agricultural production systems globally. The task is much more daunting in the northwestern part of India where the over exploitation of natural resources is a major concern for sustainable agricultural planning. An integrated farming system (IFS) encompasses various enterprises such as crops, dairy, poultry, and fisheries can offer a myriad of benefits in terms of enhanced farm productivity, profitability, and environmental sustainability. Hence, the study hypothesized that the complementary interaction between the different enterprises would improve food production and reduce negative environmental outcomes. Therefore, production potential and environmental sustainability in terms of energy efficiency, greenhouse gas emissions, and eco-efficiency of nine IFS models, namely, crop enterprise (M2); crop + dairy (M3); crop + dairy + fishery (M4); crop + dairy + fishery + poultry (M5); crop + dairy + fishery + poultry + duckery (M6); crop + dairy + fishery + poultry + duckery + apiary (M7); crop + dairy + fishery + poultry + duckery + apiary + boundary plantation (M8); crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit (M9); crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost (M10), were compared with the rice–wheat system (M1; the existing system). All the IFS models were tested between 2018 and 2021. The results revealed that the highest food production (61.5 Mg ha^{-1}) was recorded under M10 followed by M9 (59.9 Mg ha^{-1}). Concerning environmental sustainability, the combination of crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost (M10) recorded considerably higher energy output (517.6 × 10^3 MJ ha^{-1}), net energy gain (488.5 × 10^3 MJ ha^{-1}), energy ratio (17.8), and energy profitability (16.8 MJ MJ^{-1}) followed by M9. Furthermore, the M10 had the lowest greenhouse gas (GHG) intensity (0.164 kg CO_2 eq per kg food production). However, M9 had the highest eco-efficiency index (44.1 INR per kg GHG emission) followed by M10. Hence, an appropriate combination of diversified and complementary enterprises in a form of IFS model is a productive and environmentally robust approach for sustainable food production in the northwestern part of India.

KEYWORDS

energy productivity, eco-efficiency index, greenhouse gas intensity, productivity, sustainability

1. Introduction

Environmental crises, resource dwindling, and poor economic returns have jeopardized the sustainability of current agricultural production systems (Babu et al., 2020; Yadav et al., 2021). Climate change due to increased greenhouse gas concentration has emerged as a serious global environmental issue, as it may threaten global and regional food and nutritional security (Yadav et al., 2017). Currently, global food system sector accounts for ∼21–37% of total annual global greenhouse gas (GHG) emissions (Mbow et al., 2019). Therefore, there is an urgent need to reduce this emission by adopting environmentally robust agricultural production technologies. The linear economy-based agricultural production model has increased food production considerably but at the same time causes negative environmental outcomes (Babu et al., 2023).

Contemporary linear economy-based agricultural approaches such as the rice–wheat system maximize farm profitability but, at the same time, it has resulted in increased natural resource demands and pollution loads. In addition, contemporary agricultural practices have generated a huge amount of heterogeneous bio-waste (Babu et al., 2020, 2022). Open dumping of heterogeneous waste, in addition to environmental degradation, causes potential hazards to human health and livelihood security. Hence, to sustain food and nutritional security without compromising environmental quality, there is an urgent need to make agriculture more resilient to environmental degradation and climate change. Environmentally robust agricultural production technologies such as integrated farming systems (IFS), bio-intensive cropping, conservation agriculture, and organic farming offer efficient resource recycling and promote a circular economy that can potentially improve food and nutritional security without compromising environmental quality. The circular economy-based agricultural production model such as IFS relies on "take-make-waste" principle (Rathore et al., 2022; Babu et al., 2023). The IFS model focuses on minimizing external input and maximizing resource recycling, which reduces negative environmental outcomes (Babu et al., 2019). Moreover, the circular economy-led agricultural production model offers a multitude of societal, environmental, and financial benefits (Babu et al., 2023).

The best way to lower the environmental hazard of energy use is to increase energy use efficiency (Esengun et al., 2007). Hence, to maximize the efficiency of modern agricultural technology of an individual farm, the existing farming system must be characterized to capture the overall farm biodiversity (Yadav et al., 2013). It has been concluded in many studies that the yield and economic parameters increased linearly as the level of fertility increased, while the reverse trend was observed with energy use efficiency and energy productivity (Tuti et al., 2012). Furthermore, increase energy use amplified the GHG emission, as energy use and GHG emission have a positive correlation (Yadav et al., 2017). An input–output energy analysis provides farm planners and policymakers an opportunity to evaluate the economic intersection of energy use (Ozkan et al., 2004).

The IFS is a complex interrelated matrix of soil, plants, animals, implements, power, labor, capital, and other inputs controlled by farming families and influenced to varying degrees by political, economic, institutional, and other factors that operate at the farm level. It represents the integration of farm enterprises such as crops, animal husbandry, fisheries, forestry, sericulture, and poultry for optimum resource utilization (Paramesh et al., 2022). Diversified agricultural systems including livestock and crops are an ideal approach to build resilience in agricultural production systems (Sahoo et al., 2019; Babu et al., 2023). Developing climate-resilient agriculture through an integrated approach is also an ideal solution to ensure the food security of the ever-increasing global population at a time when there are twin problems of land degradation and climate change (Bhatt Sheeraz, 2016). Through diversified enterprises, IFS provides a stable and sustainable production system, and this helps in risk minimization and resilience to climate change (Behera and France, 2016). Integrating crops with livestock increases ecosystem services, minimizes environmental impact, and sustains farm profitability (Sulc and Franzluebbers, 2014). Integration of livestock with areca nut improves the ecosystem services and reduces ecological imbalance arising due to climate change scenarios in coastal agro-ecosystem (Sujatha and Bhat, 2015).

Assessment of farm productivity through energy analysis is essential to make efficient use of the naturally available resources (Soni et al., 2013). In recent times, due to the advancement of agricultural practices especially in mixed farming systems, energy consumption has increased in the form of animal feed, concentrates, minerals, fossil fuels, fertilizers, chemicals, electric power, and modern machinery causing environmental degradation (Kumar et al., 2019). Co-culturing of rice, turtle, and fish was found to be an energy and economically efficient system compared with rice monoculture (Liu et al., 2019). An integrated production system encompasses diverse enterprises and is a complex entity that needs precise estimation of energy input–output relationships and economic and environmental sustainability. Therefore, it was hypothesized that concurrent cultivation of diversified cropping along with other enterprises such as dairy, poultry, fisheries, and apiary can potentially enhance food production without compromising the environmental quality, which can mitigate the problem of food, nutritional, economic, and environmental insecurities. Hence, comparative assessments of 10 production models were undertaken in field conditions during 2018–2021 in the northwestern region of India with the following objectives: (i) to find out the effect of different enterprise's integration on food production, (ii) to quantify the effect of diverse enterprise's integration on energy dynamics and greenhouse gas emissions, and (iii) to assess the eco-efficiency index of designed IFS models over the rice–wheat system (the existing system).

2. Materials and methods

2.1. Experimental site

The field trials were executed during 2018–2021 at the ICAR-Indian Agricultural Research Institute, New Delhi, situated at 28°38′N latitude, 77°10′E longitude, and at an altitude of about 228.6 m above mean sea level. The experimental location is characterized by a sub-tropical, semi-arid climate with prominent hot dry summer and cold winter and falls under trans-Gangetic plains. New Delhi experiences hot summers (April–July) and very cold winters (December–January). The temperature ranges between 25–45°C during summer and 22–5°C during the winter season. The soil (*Inceptisol*, Mehruli series) was sandy clay loam in texture, and the baseline analysis of soil samples from 0 to 15 cm depth indicated 0.38% of soil organic carbon, 251.8 kg ha^{-1} of available nitrogen (N), 11.2 kg ha^{-1} of available phosphorus (P), 254 kg ha^{-1} of available potassium (K), and a pH of 7.6 (1:2.5 soil:water ratio).

2.2. Experimental design and management

The study objective was to assess the food production capacity, and environmental competency in terms of energy input–output relationship, greenhouse gas emissions, and eco-efficiency of designed IFS models over the conventional system. The nine IFS models, namely, M2—crop enterprise; M3—crop + dairy; M4—crop + dairy + fishery; M5—crop + dairy + fishery + poultry; M6—crop + dairy + fishery + poultry + duckery; M7—crop + dairy + fishery + poultry + duckery + apiary; M8—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation; M9—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit; and M10—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost, were designed and tested against the M1 (the existing system) in field conditions between 2018 and 2021.

The M2 model comprising of crop enterprise consisted of different cropping systems, such as baby corn–berseem–baby corn, maize–mustard–sunflower, maize–vegetable pea–okra, multi-cut sorghum–potato–onion, maize–wheat–cowpea, rice–wheat–cowpea, bottle gourd–marigold–multi-cut sorghum, red gram–wheat–baby corn, and brinjal–ratoon–cowpea (dual purpose). The crops were sown with recommended seed rates and fertilizers. The details are given in Supplementary Table 1. Farmyard manure (FYM) was applied to all the *Kharif* season crops which were available at the farm itself. The pond water was applied as irrigation to the crops to supplement rainfall. The grain yield and straw/stover yield of all the crops in the cropping systems were recorded after the harvest of each crop. The crop residues apart from those used as cattle feed were recycled in the system.

The dairy enterprise consisted of three cross-bred cows, and the cattle shed was attached to the farmhouse. The feed and fodder requirement of the cattle was met through the fodder crops included under different cropping systems. The stalks of baby corn and maize and straw of rice and wheat were given as dry fodder, whereas the vines of vegetable pea and biomass of green cowpea were fed as green fodder to the animals. Cattle feed concentrate was given as per the recommended dose. Manure output from the dairy unit was measured through regular weighing, and each cow produced ∼25 ± 3 kg of fresh cow dung per day. The cow dung and cowshed waste were recycled in the system through composting. A fishpond of 0.1 ha (50 × 20 × 2 m diameter) was constructed in the model. A total of 50 poultry birds (CARI-Devendra) (41 female and 9 male) and 32 ducks (Khaki Campbell) (female 22 and male 10) were reared above the fishpond in a low-cost house. Broken maize grains and wheat bran were fed to the birds. The number of eggs laid per annum and birds' weight were recorded. Fingerlings of freshwater fish, Rohu (*Labeo rohita*) as a column feeder (30%), Catla (*Catla catla*) as a surface feeder (30%), and Mrigal (*Cirrhinus mrigala*) as a bottom feeder (40%), were stocked in the ponds. The poultry and duck droppings acted as raw materials for the growth of plankton in the pond. As and when required, wheat bran and mustard cake (60:40) available at the farm were also fed to the fish, but no outside purchased feed was provided to the fish. In total, 10 kg of lime was applied before each stocking of fingerlings. The Fishes were harvested three times, and their live weight was recorded. The apiary unit was composed of 10 boxes consisting of 10 colonies of *Apis mellifera* which were placed at field bunds. The honey extracted from each box was weighed and recorded. The boundary plantation of flat beans as a fence crop along with 10 lemon trees, 20 kinnow trees, and 18 moringa trees was implemented on the borders of the IFS model. No fertilizers were applied to the flat bean crop, whereas the recommended dose of fertilizers was applied to lemon, kinnow, and moringa trees. Irrigation to these trees was given as and when required. The economic yield from boundary plantation crops was recorded. A biogas unit (KVIC model) of 2 m^3 capacity was established along with the cattle shed, and ∼25 kg of dung was added every day along with 25 L of water. The biogas produced was used for cooking and lighting purposes at the farmhouse, whereas the biogas slurry obtained was dried and applied to the crops in the system. Four vermicomposting units (3 × 1 × 1 m) were also established in the IFS model. In general, ∼25% of the total dung that was left after the addition to the biogas plant was used for the vermicomposting unit, whereas farmyard manure (FYM) was prepared with remaining dung and animal wastes.

2.3. Food production estimation

The food production potential of different enterprises was assessed in terms of rice equivalent yield (REY). The REY was determined by converting the economic production of different enterprises such as crops, milk, egg, meat, and fish based on their prevailing market price (INR: Indian rupees) for each product and expressed in Mg ha^{-1}.

$$\text{REY}\left(\text{Mg ha}^{-1}\right) = \text{Rice yield} + \frac{\text{Commodity yield}\left(\text{Mg ha}^{-1}\right)\text{ x Commodity price (INR Mg}^{-1})}{\text{Price of rice grain (INR Mg}^{-1})}$$

2.4. Assessment of environmental sustainability

Environmental impact of different crops and enterprises in particular models was assessed by calculating the energy dynamics, global warming potential (GWP), greenhouse gas intensity (GHGI), and eco-efficiency index (EEI).

2.4.1. Energy budgeting

Energy accounting is an imperative step for designing environmentally robust production systems. In the current study, operation-wise energy was calculated based on the input energy consumed in field preparation, sowing, fertilizer application, irrigation, intercultural operation weeding, plant protection, harvesting, threshing and other operations. Similarly, the total output from each system was converted into energy output in a particular system. Energy output and input were estimated by multiplying the particular input/output with the studded energy coefficient (Supplementary Table 3). The energy competency of different IFS models was judged by following energy indices.

$$\text{NEG } \left(\text{MJha}^{-1}\right) = \text{Total energy output } \left(\text{MJha}^{-1}\right) - \text{Total energy input } \left(\text{MJha}^{-1}\right)$$

where NEG = net energy gain,

$$\text{ER} = \frac{\text{Total energy output}\left(\text{MJha}^{-1}\right)}{\text{Total energy input}\left(\text{MJha}^{-1}\right)}$$

where ER = energy ratio,

$$\text{PE} = \frac{\text{Net energy gain } \left(\text{MJha}^{-1}\right)}{\text{Total energy input}\left(\text{MJha}^{-1}\right)}$$

where PE = energy profitability

$$\text{EP} = \frac{\text{Total food production } \left(\text{kg ha}^{-1}\right)}{\text{Total energy input}\left(\text{MJ ha}^{-1}\right)}$$

where EP = energy productivity.

2.4.2. Estimation of global warming potential (GWP) and greenhouse gas intensity (GHGI)

The total GHG emissions (CO_2, N_2O, and CH_4) during the cropping period were estimated in terms of CO_2 equivalent. The CO_2 equivalent is also known as GWP. The CO_2, N_2O, and CH_4 were converted into CO_2 equivalent by using GWP equivalent factors of 1, 265, and 28 for CO_2, N_2O, and CH_4, respectively, for the timeframe of 100 years (Intergovernmental Panel on Climate Change, 2013). The GHG emissions from farm operations (tillage, herbicide, and insecticide application, planting and fertilizer application, as well as harvest) and inputs such as fertilizer and seeds were calculated by multiplying the input with its corresponding emission coefficient (West and Marland, 2002; Lal, 2004; Yadav et al., 2017).

The CH_4, N_2O, and emissions from the rice and applied manure and biomass, respectively, were calculated by the following equation (Yadav et al., 2017):

$$CH_4 \text{ emission (kg year}^{-1}) = \text{EF X SF}_0 \text{ x (Aj + [Aj x SFj])/10}$$

where EF = 10 g m^{-2} year^{-1} for India, Aj = area under rice paddy, ha year^{-1}, $SF_0 = 1.4$ organic manures correction factor, and $SF_j = 0.7$ scaling factor for A.

To calculate the N_2O emission, the 0.01 emission factor was multiplied by the total N supplied by organic means and expressed in N_2O kg N input^{-1} (Tubiello et al., 2015) as follows:

$$\text{N2O emission } \left(\text{kg year }^{-1}\right) = \text{N contributed by N sources x 0.01 x 1.57.}$$

GWP from all crops in the cropping system except rice was estimated with the following formula:

$$\text{GWP} = \text{Total } N_2O \text{ emission x 265} + \text{Total } CO_2 \text{ emission.}$$

However, GWP from the rice was calculated as follows:

$$\text{GWP} = \text{Total } CH_4 \text{ emission x 28} + \text{Total } N_2O \text{ emission x 265} + \text{Total } CO_2 \text{ emission,}$$

$$\text{Total GWP from crop components} = \sum CF_1 \text{.....................................ith crops}$$

The GHG emission from dairy components was estimated using the Tier 1 method (Intergovernmental Panel on Climate Change, 2006). CO_2 emissions from livestock were not estimated because annual net CO_2 emissions were assumed to be zero—the CO_2 photosynthesized by plants is returned to the atmosphere as respired CO_2. Livestock production can result in methane (CH_4) emissions from enteric fermentation and both CH_4 and nitrous oxide (N_2O) emissions from livestock manure management systems. The GHG emissions from the poultry and duckery plantations were mainly CH_4 and N_2O emissions from manure management. The CH_4 and N_2O emission from manure management was calculated based on the Tier 1 method outlined in Intergovernmental Panel on Climate Change (2006) guidelines. The emission intensity for fish feeds (wheat bran and mustard oil cake) was obtained from the data available for India (Food Agriculture Organization, 2017) and was expressed as kg CO_2 eq kg DM^{-1}. The rates of electricity used per Mg of live weight (LW) were multiplied by the emission factor (EFs) to determine the emission intensity in fishery components. Regional EFs were used for grid electricity (BEIS (Department for Business Energy Industrial Strategy)., 2016). The N_2O emissions from the water body on the fish farm arise from microbial nitrification and denitrification, the same as in terrestrial or other aquatic ecosystems (Hu et al., 2012). The amount of N_2O per species group was determined by multiplying the production by the N_2O emission factor per kg of production (Hu et al., 2012), i.e., 1.69 g N_2O-N per kg of production, or 0.791 kg CO_2 eq. per kg LW production. The functional unit taken for the calculation of carbon footprint was 1 kg LW fish and expressed as kg CO_2 eq. year^{-1} kg^{-1} LW fish.

The total global warming potential from different enterprises was calculated with the following formula:

$$\text{Total GWP} = \sum GF_{1+} \text{....................ith enterprises}$$

GHGI was estimated by dividing the total GWP by total food production in terms of REY and expressed as kg CO_2 eq. kg food production.

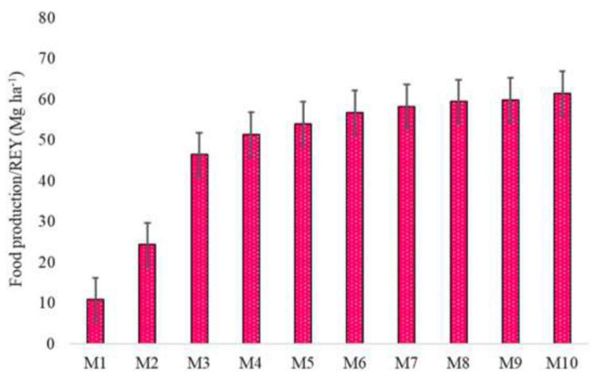

FIGURE 1
Food production potential of different IFS models. The error bars indicate the standard error between the treatment. M1—rice–wheat system; M2—crop enterprise; M3—crop + dairy; M4—crop + dairy + fishery; M5—crop + dairy + fishery + poultry; M6—crop + dairy + fishery + poultry + duckery; M7—crop + dairy + fishery + poultry + duckery + apiary; M8—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation; M9—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit; M10—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost.

2.4.3. Estimation of eco-efficiency

Estimation of the eco-efficiency index (EEI) is imperative while designing environmentally robust production systems. EEI measures the economic return ability of the designed system concerning environmental distraction. An environmentally sound production system always had higher EEI by reducing negative environmental outcomes and enhancing the net economic gain (Babu et al., 2020). In this study of the ecological impact of different IFS, models were measured in terms of economic gain concerning total GHG emission (kg CO_2 eq.). EEI was calculated with the following formula:

$$EEI\left(\mathrm{INR\ kg^{-1}\ CO_2e\ ha^{-1}}\right) = \frac{\text{Economic retruns}\left(\mathrm{INR\ ha^{-1}}\right)}{\text{GHG emission}\left(\mathrm{kg\ CO_2e\ ha^{-1}}\right)}.$$

2.5. Statistical analysis

The data collected on different parameters were subjected to appropriate statistical analysis following the procedure described by Gomez and Gomez (1984). Standard deviation (SD) and standard error (SE) were used to measure the degree of variability between the individual data values.

3. Results

3.1. Food production

In the present study, the food production capacity of different integrated farming system models was assessed in terms of rice equivalent yield (REY). Food production differed considerably among different IFS models (Figure 1).

All the designed IFS models outperformed over the rice–wheat system (the existing system). Designed IFS models registered ~2–6 times higher food production over the existing production system (M1) of northwest India. Among the designed IFS models, concurrent rearing of crop + dairy + fishery + poultry + duckery + apiary + boundary plantation along with biogas unit and vermicomposting (M10) resulted in the highest food production (61.5 Mg ha^{-1}) followed by M9 (60.0 Mg ha^{-1}), M7 (59.5 Mg ha^{-1}), and M6 (58.2 Mg ha^{-1}).

3.2. Energy budgeting

Energy budgeting is crucial for designing an environmentally efficient production system. The energy input and energy output were influenced by the integration of different enterprises in integrated farming system models (Table 1). It was assumed that increasing the enterprises correspondingly increases the energy input. This was the case in the current study as well, where all the designed systems had higher energy requirements as compared with the existing farming practice (rice–wheat). In the designed system, maximum energy (29.1 × 10^3 MJ ha^{-1}) was incurred under M10, while M2 had the least energy demand (25.0 × 10^3 MJ ha^{-1}).

Concerning energy output, M10 registered the highest gross energy returns (517.6 × 10^3 MJ ha^{-1}) followed by M9 (513.9 × 10^3 MJ ha^{-1}). Similarly, the net energy gain was also considerably influenced by integration of enterprises in different IFS models, and the maximum net energy gain was obtained in M10 (488.5 × 10^3 MJ ha^{-1}) followed by M9 (484.9 × 10^3 MJ ha^{-1}). The energy ratio (ER) indicates net energy gain per unit of energy investment. In the current study, the IFS model comprises more enterprises that yielded more energy per unit of energy investment. All the designed systems had a higher energy ratio than the conventional farming system (rice–wheat). M10 had ~2.5 times higher energy ratio over M1 (the existing systems). The M1 was the least energy-efficient system (Figure 2). Among the tested system, M10 registered the highest energy ratio (17.8) followed by M9 (17.7).

Energy productivity indicates the food production per unit of energy investment. In the current study, all the designed systems recorded higher energy productivity than the conventional system. The designed system had ~3–4 times higher energy productivity over M1. Among the designed systems, M10 recorded the maximum energy productivity (3.6 kg MJ^{-1}) and was closely followed by M9 (3.2 kg MJ^{-1}). A similar trend was noticed with energy profitability; all the designed systems recorded higher energy profitability over the conventional system. M10 was recorded to be the highest energy profitability (16.8 MJ MJ^{-1}) followed by M9 (16.7 MJ MJ^{-1}) (Figure 3). M10 registered ~2.7 times more energy profitability over M1, and this indicates that complementary interaction of different enterprises is confirmed to make conventional production system energy efficient in northwest India.

TABLE 1 Effect of different integrated farming system models on energy dynamics.

Treatment	Energy input (×10^3 MJ)	Energy output (×10^3 MJ)	Net energy gain (×10^3 MJ)	Energy productivity (kg MJ^{-1})
M1	25.2 ± 0.658	180.7 ± 70.4	155.5 ± 69.8	0.91 ± 0.585
M2	25.0 ± 0.721	246.8 ± 49.5	221.8 ± 48.8	2.58 ± 0.056
M3	26.0 ± 0.405	419.1 ± 4.9	393.1 ± 5.3	2.92 ± 0.052
M4	27.2 ± 0.025	423.3 ± 6.3	396.2 ± 6.3	2.83 ± 0.022
M5	27.2 ± 0.025	428.5 ± 7.9	401.3 ± 7.9	2.89 ± 0.042
M6	27.2 ± 0.025	431.9 ± 9.0	404.7 ± 9.0	2.90 ± 0.045
M7	27.7 ± 0.133	434.5 ± 9.8	406.7 ± 9.6	2.86 ± 0.032
M8	29.1 ± 0.576	438.4 ± 11.0	409.3 ± 10.5	2.75 ± 0.001
M9	29.1 ± 0.576	513.9 ± 34.9	484.9 ± 34.4	3.26 ± 0.158
M10	29.1 ± 0.576	517.6 ± 36.1	488.5 ± 35.5	3.67 ± 0.290

± indicates standard deviation between average mean; M1—rice–wheat system; M2—crop enterprise; M3—crop + dairy; M4—crop + dairy + fishery; M5—crop + dairy + fishery + poultry; M6—crop + dairy + fishery + poultry + duckery; M7—crop + dairy + fishery + poultry + duckery + apiary; M8—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation; M9—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit; M10—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost.

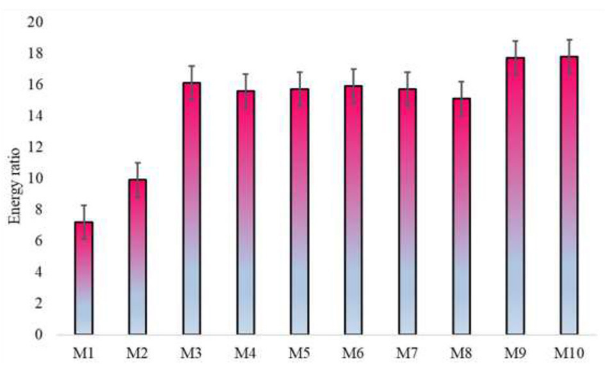

FIGURE 2
Effect of enterprise integration on energy ratio under different IFS models. The error bars indicate the standard error between the treatments. M1—rice–wheat system; M2—crop enterprise; M3—crop + dairy; M4—crop + dairy + fishery; M5—crop + dairy + fishery + poultry; M6—crop + dairy + fishery + poultry + duckery; M7—crop + dairy + fishery + poultry + duckery + apiary; M8—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation; M9—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit; M10—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost.

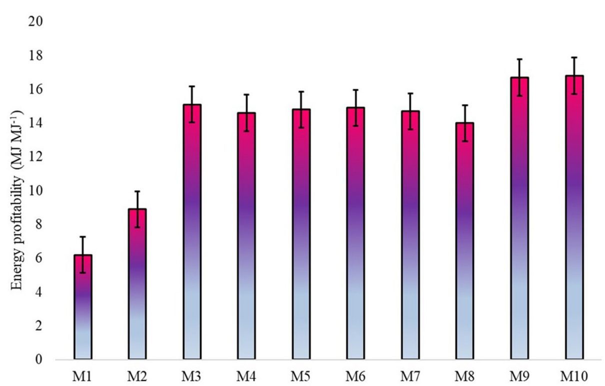

FIGURE 3
Effect of enterprise integration on energy profitability under different IFS models. The error bars indicate the standard error between the treatments. M1—rice–wheat system; M2—crop enterprise; M3—crop + dairy; M4—crop + dairy + fishery; M5—crop + dairy + fishery + poultry; M6—crop + dairy + fishery + poultry + duckery; M7—crop + dairy + fishery + poultry + duckery + apiary; M8—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation; M9—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit; M10—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost.

3.3. Global warming potential, greenhouse gas intensity, and eco-efficiency index

The global warming potential (GWP), greenhouse gas intensity (GHGI), and eco-efficiency index (EEI) were significantly influenced by enterprise integration in different IFS models (Table 2). Integration of more enterprises increased the GWP of different IFS models. M2 had the lowest GWP (7.8 Mg CO_2 eq ha^{-1}); however, M10 had the highest GWP (10.1 Mg CO_2 eq ha^{-1}), which was almost similar to M9 and M8. On the other hand, increase in the number of enterprises considerably reduced the GHGI over M1. The M10 had the lowest GHGI (0.164 kg CO_2 eq kg^{-1} food production) followed by M9 (0.169 kg CO_2 eq kg^{-1} food production). The designed system had ∼ 2–5 times less GHGI over M1 (the existing system). Concerning EEI, the lowest EEI (13.2 INR kg GHG^{-1}) was reported in M1. All the designed IFS models recorded 63–70% higher EEI over M1. Among the tested IFS models, M9 registered the maximum EEI (44.1 INR kg GHG^{-1}) closely followed by M5, M6, M7, M8, and M10.

TABLE 2 Global warming potential, greenhouse gas intensity (GHGI), and eco-efficiency index (EEI) of different IFS models.

Treatment	Global warming potential (Mg CO_2 eq. ha^{-1})	GHGI (kg CO_2 eq per kg food production)	Eco-efficiency index (INR per kg CO_2 eq)
M1	8.10 ± 0.439	0.743 ± 0.156	13.2 ± 8.16
M2	7.80 ± 0.534	0.320 ± 0.023	36.3 ± 0.85
M3	9.10 ± 0.123	0.196 ± 0.017	40.8 ± 0.57
M4	9.70 ± 0.067	0.189 ± 0.019	39.5 ± 0.16
M5	9.90 ± 0.130	0.183 ± 0.021	41.6 ± 0.82
M6	10.00 ± 0.162	0.176 ± 0.023	43.5 ± 1.42
M7	10.02 ± 0.168	0.172 ± 0.024	43.5 ± 1.42
M8	10.08 ± 0.187	0.170 ± 0.025	43.6 ± 1.45
M9	10.09 ± 0.190	0.169 ± 0.025	44.1 ± 1.61
M10	10.10 ± 0.193	0.164 ± 0.027	43.9 ± 1.55

± indicates standard deviation between average mean; M1—rice–wheat system; M2—crop enterprise; M3—crop + dairy; M4—crop + dairy + fishery; M5—crop + dairy + fishery + poultry; M6—crop + dairy + fishery + poultry + duckery; M7—crop + dairy + fishery + poultry + duckery + apiary; M8—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation; M9—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit; M10—crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost. INR—Indian rupees.

4. Discussion

4.1. Food production potential

The food production (rice equivalent yield) varied significantly among the IFS models, and it was maximum with the integration of more diverse enterprises, whereas lower food production was recorded in the rice–wheat system and crop enterprise alone. It is pertinent to mention here that even a simple integration of crop + dairy has the potential to enhance total food production as compared with sole cropping. The increased productivity may be ascribed to synergisms among the enterprises and the wastes or by-products from one enterprise used as inputs in another enterprise (Babu et al., 2023). Moreover, with the simultaneous application of recycled pond silt, poultry manure, nutrient-rich pond water for irrigation, and cow dung as FYM and vermicompost, crop residues provided a congenial situation to increase the enterprise's productivity. When a fishery unit was combined with a duckery, such as in models M6–M10, fish production improved as well because duck droppings served as a source of food for the fish. In the current study, total food production was the highest under M10, but it was statistically at par with M9, M7, and M6. Thus, it can be inferred that even with the integration of a few diverse and more productive enterprises such as crop + dairy + fishery + poultry + duckery (M6), the same level of food production can be attained as those achieved by integration of more enterprises such as crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost (M10). Gill et al. (2009) also reported statistically at par farm productivity with crops + dairy and crops + dairy + poultry production systems but significantly superior productivity over sole cropping. Enterprises such as apiary, biogas unit, and vermicompost are complementary enterprises, and their inclusion into the integrated farming systems may not achieve a significant increase in productivity though they have associated benefits such as nutrient recycling, improving the nutritional security of marginal landholders and smallholders. Farm-based crop and animal integration promote resource recycling (Soussana and Lemaire, 2014), which may improve soil fertility. Concurrent rearing of crops and livestock improves productivity and resource use efficiency (Domiciano et al., 2016; Babu et al., 2019). The complementary integration of crops with livestock and other enterprises promoted efficient resource recycling and reduces GHG emissions and follows the circular and/or green economy principles. Hence, the integration of crops with animal systems at the field level could minimize environmental pollution and increase farm production (Sartor et al., 2014; Martins et al., 2017). Integration of high-value components such as fish/poultry/duck/goat/cattle can contribute to better crop productivity (Kumar et al., 2012). Bio-intensification with complementary integration of different enterprises including crop, horticultural, livestock, fishery, poultry, and agroforestry leads to higher system productivity (Singh et al., 2007; Dhyani et al., 2016). Multiple increases in farm production under IFS over cropping alone in the lower Gangetic plains of Bihar was also reported by Kumar et al. (2012). Approximately three times higher farm productivity under field crops + fish + goat system was recorded over a crop enterprise alone in Tamil Nadu (Jayanthi et al., 2002). However, in Karnataka, India, integration of different farm enterprises resulted in ∼6-fold higher system productivity over rice–rice alone (Channabasavanna and Biradar, 2007). Hence, it can be inferred that the benefits of IFS in terms of improving productivity largely depend upon the number and kind of enterprises and their management.

4.2. Environmental sustainability

The ecological sustainability of any agricultural production system mainly depends on its carbon footprint (Dubey and Lal, 2009), soil health (Babu et al., 2020), and input use efficiency (Yadav et al., 2021). In the current study, the energy input was the highest with concurrent cultivation of crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost (M10). The highest energy consumption in M10 is due to the maximum number of enterprises, which requires higher input energy (Babu et al., 2023). Similarly, Kumar et al. (2018) found that combining poultry and mushroom with a rice–brinjal system required higher energy input, whereas sole rice cropping required the least energy. Similarly, higher energy output was recorded under M10 due to more food production. The integration of multiple enterprises with crops resulted in higher energy output indicating the necessity of an integrated farming system for efficient utilization of the scarce and costly resource (Korikanthimath and Manjunath, 2009).

The net energy gain, energy ratio, energy productivity, and energy profitability were also higher in diversified IFS models with more enterprises, which can be attributed to the fact that these models also recorded significantly higher output energy, farm productivity, and economic returns as compared with the rice–wheat system and crop enterprise. The higher energy efficiency might be due to higher energy savings in these systems. The energy efficiency was higher in M10 and other models having diverse enterprises since there was synergism among the diverse enterprises, and the output of one enterprise served as input for another (Paramesh et al., 2019; Babu et al., 2023). At the same time, chemical fertilizers were supplemented by farm-available organic manures and nutrient-rich pond water, which further reduced the input energy that otherwise would have been required for the production and transport of the chemical fertilizers (Singh et al., 2021). Hence, it is pertinent to mention that the use of more organic nutrient sources, improved irrigation technology, and precision agriculture can enhance the energy use efficiency of the IFS model (Jackson et al., 2010; Mohammadi et al., 2014). The integration of dairy with crops significantly increased the net energy gain and energy efficiency, which was mainly due to the efficient recycling of green fodder cultivated at the IFS itself and using by-products of crops such as maize, baby corn, and cowpea as animal feed. The energy efficiency further increased with the integration of a fishery, poultry, and duckery due to more food production without the purchase of feed from the market. Broken wheat and maize grains, and rice bran were fed to the ducks and poultry, whereas the integration of ducks and poultry with the fishery supplements the feed requirement of fish by poultry and duck droppings. Furthermore, the droppings of poultry and ducks enhances the nutritional quality of pond water, which was used for irrigating the crops. In addition, a duckery ensures effective aeration in the fishpond when the duckery is integrated with the fishery under IFS, which may increase fish productivity and reduce the energy requirements. Thus, it can be emphasized that the integration of complementary enterprises in IFS increases energy efficiency. Behera et al. (2014) suggested that in an IFS, energy efficiency can be achieved by exploring renewable forms of energy such as biogas to meet the energy requirements of the farm household. Higher energy efficiencies under IFS were reported by several researchers (Rahman and Sarkar, 2012; Kumar et al., 2019; Babu et al., 2023).

Concerning GWP, the models having a greater number of enterprises registered higher GWP over the rice–wheat, crop enterprise, and crop + dairy systems primarily due to higher resource consumption in their integrated fashion. There was a marked increase in the GWP of the crop + dairy system on account of higher CH_4 and N_2O emissions from the dairy component. The models that were integrated with animal components (dairy, fishery, poultry, and duckery) recorded higher GWP due to increased emissions of GHG from these components. On the other hand, the greenhouse gas intensity (GHGI) of the models integrated with a greater number of complementary enterprises was lower, suggesting that the adoption of IFS with a higher number of complementary enterprises has lower GHG emissions per unit of food production. GHG emissions and energy use had a direct relationship. GHGI and energy productivity are positively correlated (Rathore et al., 2022). GHGI was lower in these models on account of higher food production than in other models. Hence, the GHGI of the monoculture system can be decreased by good agronomic measures and sustainable intensification (Gan et al., 2014). Hence, emphasis should be given to selecting those enterprises in the IFS model that require fewer inputs and have high conversion efficiency, which contributes less to GHG emissions.

The eco-efficiency index is used to express the efficiency of a system concerning its impact on nature. Eco-efficiency encompasses both the economic and ecological dimensions of a production system (Keating et al., 2010). The models having diverse enterprises recorded higher eco-efficiency index when compared with the rice–wheat system and crop enterprise signifying that the IFS is environmentally efficient with more economic returns per unit of GHG emission. The rice–wheat system had the lowest eco-efficiency index in terms of GHG emissions, which implied that this system has more negative impact on environment than designed IFS models. Sustainable agriculture aims at increasing the eco-efficiency of a system by lowering impacts such as energy use and GHG emissions while, at the same time, increasing the economic output (Cicek et al., 2011). Thus, it can be emphasized that the adoption of the IFS model with diverse complementary enterprises can lead the way toward environmentally clean production systems.

5. Conclusion

The findings prove the hypothesis that the co-culturing of different enterprises in a complementary fashion at the farm level is an environmentally robust food production system in northwest India. The integrated farming system promotes close-loop nutrient recycling, which minimizes the external input use and enhances waste recycling thereby reducing the pollution load in the ecosystem with improved food production. However, in IFS, the enterprises should be chosen in such a way that there should be a high degree of complementarity among them and that the by-product of one enterprise should act as an input for another enterprise for enhancing resource use efficiency. Through the integration of diversified cropping systems and diverse enterprises, the IFS model can be identified as a climate–resilient system with lower GHG intensity and a higher eco-efficiency index, which makes the system environmentally friendly. The IFS model encompassing crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit + vermicompost (M10) and crop + dairy + fishery + poultry + duckery + apiary + boundary plantation + biogas unit (M9) were found to be the most productive and eco-friendly production systems. M10 and M9 enhanced food production by ~6 times while reducing the GHGI by ~78%compared to M1. Hence, M10 and M9 can be promoted as environmentally robust production systems in the northwestern part of India for ensuring the food and nutritional security of small and marginal farmers. However, enterprise selection in an IFS model is very individualistic and location-specific and, hence, needs proper planning and understanding of the interactions among the different enterprises to harness their synergies. This means that the same IFS model cannot be replicated in all agroecologies. Moreover, the IFS is a multi-product production system, which needs multi-specialty and marketing.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

AF, VS, SB, SR, RS, and PU: conceptualization, resources, supervision, and software. AF: investigation and data recording. AF and SB: writing the original draft. BK, MH, and HP: reviewing and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.959464/full#supplementary-material

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An integrated organic farming system: innovations for farm diversification, sustainability, and livelihood improvement of hill farmers

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Introduction: Organic farming is a promising solution for mitigating environmental burdens related to input-intensive agricultural practices. The major challenge in organic agriculture is the non-availability of large quantities of organic inputs required for crop nutrition and sustaining soil health, which can be resolved by efficient recycling of the available on- and off-farm resources and the integration of the components as per the specific locations.

Methods: An integrated organic farming system (IOFS) model comprising agricultural and horticultural crops, rainwater harvesting units, livestock components, and provisions for nutrient recycling was developed and disseminated in the adopted organic villages Mynsain, Pynthor, and Umden Umbathiang in the Ri-Bhoi District, Meghalaya, India, to improve the income and livelihood of farmers. Harvested rainwater in farm ponds and *Jalkunds* was used for live-saving irrigation in the winter months and diversified homestead farming activities, such as growing high-value crops and rearing cattle, pigs, and poultry.

Results: Maize, french bean, potato, ginger, tomato, carrot, and chili yields in the IOFS model increased by 20%–30%, 40%–45%, 25%–30%, 33%–40%, 45%–50%, 37%–50%, and 27%–30%, respectively, compared with traditional practices. Some farmers produced vermicompost in vermibeds (made of high-density polyethylene) and cement brick chambers, generating 0.4–1.25 tons per annum. Two individual farmers, Mr. Jrill Makroh and Mrs. Skola Kurbah obtained net returns (without premium price) of Rs. 46,695 ± 418 and Rs. 31,102 ± 501 from their respective 0.27- and 0.21-ha IOFS models, which is equivalent to Rs. 172,944 ± 1,548/ha/year and Rs. 148,105 ± 2,385/ha/year, respectively. The net returns obtained from the IOFS models were significantly higher than those obtained from the farmers' practice of maize-fallow or cultivation of maize followed by vegetable (∼30% of the areas). It is expected that, with the certification of organic products, the income and livelihood of the farmers will improve further over the years. While Mr. Jrill Makroh's model supplied 95.1%, 82.0%, and 96.0% of the total N, P_2O_5, and K_2O, respectively, needed by the system, Mrs. Skola Kurbah's model supplied 76.0%, 68.6%, and 85.5% of the total N, P_2O_5, and K_2O, respectively.

Discussion: Thus, IOFS models should be promoted among hill farmers so that they can efficiently recycle farm resources and increase their productivity, net returns, and livelihood while reducing their dependence on external farm inputs.

KEYWORDS

integrated organic farming system, nutrient balance, profitability, system productivity, water harvesting, residue recycling

1. Introduction

Organic farming emerged as a solution to the input-driven industrialization of agricultural practices and its associated environmental and social problems. Organic farming combines tradition, innovation, and science to benefit the environment and the quality of life for all involved (Pleguezuelo et al., 2018). Organic farming that relies mostly on animal manure, organic waste, crop rotation, legumes, and biological pest control methods is practiced in the majority of the areas of North-East India, especially in the hill region (Das et al., 2017a,b). In the north-eastern hill (NEH) region of the country, the application of chemical fertilizers is very low and most of them are used in the valley ecosystem (Layek et al., 2023), but the upland ecosystem is free from the use of chemical fertilizers (Layek et al., 2018). Similarly, the use of pesticides in the region is very low because the farmers practice traditional methods for controlling insect pests and diseases (Das et al., 2017a,b). As such, the farmers have shown an inclination toward organic farming, which is being harnessed for the development of the region and has ecological benefits (Layek et al., 2020). It is estimated that 18 million hectares of such land are available in the NEH region, which can be exploited for organic production (Das et al., 2018). Agriculture in North-East India, especially in Meghalaya, is characterized by the limited use of external inputs, such as fertilizers and pesticides (14.0 kg N + P + K/ha and 0.032 kg/ha, respectively), the cultivation of traditional varieties, subsistence in nature, and low productivity (Das et al., 2017a,b; Devi et al., 2017). Most areas of Meghalaya (>70%) and the north-eastern region are hilly and mountainous tracks with moderate to steep slopes, <30% of which constitutes valley areas (Layek et al., 2019; Choudhury et al., 2022). Conventional farming with monocropping of rice and maize along with the cultivation of a few vegetables in the kitchen garden with inadequate inputs leads to very low productivity (Ansari et al., 2021). Vegetables constitute an important part of the diet of the Eastern Himalayan population (Pandey, 2002). This North-Eastern Region (NER) is not only rich in vegetable diversity but also in spices and fruits, which are an integral part of the farming system there (Deka et al., 2012). Growing vegetables after *kharif* maize not only increases cropping intensity but also utilizes the land efficiently while providing employment and economic benefits to the farmers, who are mostly small and marginal in nature (Layek et al., 2020). There is a yield gap of 25%−40% for most of the vegetables, such as okra, French bean, carrot, tomato, and potato, between their farm yield and the yield obtained from the ICAR experimental organic farms (Panwar et al., 2022). However, the NER, especially Meghalaya, has a lot of potential for improving agricultural productivity. The region is one of the mega-biodiversity zones of the world (Layek et al., 2019). The region receives high rainfall (>2,450 mm). The climate varies from tropical to temperate, and as a result, most of the crops could be grown in one or other part of the region. By virtue of the lower amounts of chemical inputs imported and utilized, the state of Meghalaya has a great scope for successful organic farming (Patel D. P. et al., 2014; Das et al., 2017a,b). The soils of the region are highly degraded due to cultivation on steep slopes, negligible nutrient supplementation, and biomass burning under traditional practices (Roy et al., 2018; Ansari et al., 2022b). Organic farming is considered one of the best options for protecting/sustaining soil health and producing healthy foods. The objectives of environmental, social, and economic sustainability can be met through organic farming (Saldarriaga-Hernandez et al., 2020). It is assumed that the difference in the production gap due to the adoption of organic agriculture will be negligible in the region. There is scope for enhancing productivity with good organic management since most of the households are maintaining livestock (pig, poultry, cattle, goats, etc.) and producing enough on-farm manures, which could be efficiently used for organic agriculture (Ravisankar et al., 2021, 2022).

Most people are non-vegetarians and rear animals, especially pigs and poultry, so a good amount of animal excreta is generated, which is essential for successful organic farming (Das et al., 2017a,b). However, the major constraint to the success of 'organic farming' is the non-availability of huge quantities of organic inputs, and the application of animal excreta is not sufficient alone to meet the demand for nutrients for the crops (Das et al., 2017a,b; Layek et al., 2019). The favorable climatic conditions and high concentration of soil organic carbon (SOC) allows the huge growth of plant biomass (weeds, shrubs, residues, etc.), which can be recycled in crop production as a vital source of nutrient supply (Patel D. P. et al., 2014; Layek et al., 2023). The adoption of organic agriculture in an integrated farming system approach, viz., an integrated organic farming system (IOFS), which utilizes all the on-farm and off-farm resources judiciously by using the byproducts or output of one as the input for the other, can make organic farming sustainable and profitable (Das et al., 2019). Thus, the focus should be on integrating complementary and supplementary enterprises, such as crops, fruits, vegetables, livestock, poultry, fish, multipurpose tree species, and mushrooms, along with adequate nutrient recycling strategies (Panwar et al., 2021a,b; Ravisankar et al., 2021). One such IOFS model has been developed at the ICAR Research Complex for North Eastern Hill Region, Umiam, Meghalaya, India through the scientific integration of different enterprises, such as crops, fruits, vegetables, livestock, poultry, and fish, along with adequate nutrient recycling strategies (composting/vermicomposting) and the use of water from farm ponds (Das et al., 2017a,b; Layek et al., 2020). The net income of IOFS model was enhanced from farmer's practice I and II by 355% and 191%, respectively. The IOFS model could meet 92%, 82%, and 96% (N, P_2O_5, and K_2O, respectively) of its nutrient demand within the system.

Although the region gets substantial rainfall during the months of April to November, there is virtually no rainfall during the winter season, especially from November to March (Layek et al., 2022). The creation of water harvesting structures is essential in the hills to supply water in the winter season for livestock and crops maintained in an organic farming system. Owing to a lack of water harvesting structures/irrigation facilities in the hills, the farmers are cultivating only one crop per year, leading to low cropping intensity and limited income (Bujarbaruah, 2004; Layek et al., 2020). However, water conservation in hills is very difficult as traditional farm ponds in the hill regions are exposed to very high water loss through infiltration, percolation, and seepage loss (Lairenjam et al., 2014; Das et al., 2017a,b). The seepage and percolation from the dug-out ponds/tanks can be prevented using UV-resistant polyethylene films that have high tensile strength, are durable, and are resistant to external pressure, e.g., Silpaulin (200 GSM or more). These low-cost rainwater harvesting structures, known as "*Jalkunds*," have storage capacities of 30,000–45,000 L and can be key to the success of an IOFS model (Samuel and Satapathy, 2008; Layek et al., 2020). Major emphasis should also be placed on the management of livestock components, such as dairy, pigs or poultry, and compost preparation for the supply of year-round quality manure and income generation in an IOFS. For disseminating the IOFS technology, a model village concept in line with the Network Project on Organic Farming-Tribal Sub Plan (NPOF-TSP) was implemented in the village of Mynsain in the Ri-Bhoi District of Meghalaya with financial assistance from the ICAR-Indian Institute of Farming Systems Research, Modipuram. Several farmers in the village started practicing organic farming in an IOFS model. This practice increased crop productivity and diversified homestead farming to grow remunerable crops and rear cattle, pigs, poultry, etc.

2. Materials and methods

For disseminating organic production technologies developed by the ICAR Research Complex for NEH Region, Umiam, a model village concept for organic farming using a cluster approach in line with the Network Project on Organic Farming-Tribal Sub Plan (NPOF-TSP) was initiated between 2013 and 2014 in the village of Mynsain (25°44′21,61″ N−92°1′1,73″ E, 853–901 AMSL) in the Ri-Bhoi District of Meghalaya with financial assistance from the ICAR-Indian Institute of Farming Systems Research, Modipuram. To disseminate the IOFS technology in a cluster approach (group of neighboring farmers), areas where farmers were either not using or using a meager amount of synthetic fertilizers and pesticides were identified. A sensitization meeting with the villagers, including the village head (Headman), members of self-help groups, and the Department of Agriculture (Gram Sabha), was organized before the work was initiated, and subsequently, a group of farmers visited the ICAR, Umiam to obtain first-hand experience of the various technologies that would be used in the program. A participatory rural appraisal (PRA) was undertaken at the project site at Mynsain village to obtain information about the local agrosystem, resources, farming practices, and social structure and identify problems within the farming community. The village has 132 households with a population of 600 people. Most people in the village are Christians. The main occupation in the village is agriculture. Paddy (*Oryza sativa*), maize (*Zea mays*), and ginger (*Zingiber officinale*) are the main crops that are cultivated. Ginger is the cash crop and is the most profitable as it is a non-perishable crop, and it has become a major source of income. Paddy is mostly cultivated for self-consumption. There are other crops and vegetables that the villagers grow, such as sweet potatoes, potato, pumpkin, yam, corn, tomato, beans, and chili. There are also few households that rear livestock, including cows, pigs, and hens. The prevailing soil in the lowland and upland regions of the village were sampled at a depth of 0–15 and 15–30 cm, and the average nitrogen (N) content, phosphorus (P) content, soil organic content (SOC), and pH content were analyzed using standard procedures. The available N, P, SOC, and pH of the soil in the lowland region was 210.1 ± 27.8 kg/ha, 9.1 ± 6.4 kg/ha, 16.0 ± 3.2 mg/kg, and 4.97 ± 0.62, respectively, for a depth of 0–15 cm. Similarly, the available N, P, SOC, and pH of the soil in the upland region was 201.9 ± 35.7 kg/ha, 21.2 ± 12.5 kg/ha, 13.1 ± 3.9 mg/kg, and 4.81 ± 0.67 pH, respectively, for a depth of 0–15 cm.

Organic farming using the cluster approach was implemented in three villages over a total area of 110 ha comprising 315 farmers, 100% of whom belonged to tribal communities. In these three villages, IOFS technology had been adopted by 35 farmers. A georeferenced characterization of the All India-Network Programme on Organic Farming (AI-NPOF) adopted villages revealed that most of the farmers grew crops such as rice, maize, ginger, and turmeric only in the rainy season and produced a very low acreage of winter crops, especially vegetables, due to a lack of irrigation water. Additionally, crop productivity was much lower in the villages due to a lack of improved varieties, limited availability of manure or animal excreta, and the absence of synthetic fertilizers and pesticides. However, most of the farmers from the villages maintained farm animals (poultry, pigs, goats, or cattle) in an isolated way but paid very little attention to the production of quality manure or vermicompost. The rationale was that the adoption of an IOFS would not reduce productivity but rather would enhance it due to the fact that farmers in the villages were previously using a significant amount of manure or fertilizers and pesticides for crop production. Multidisciplinary programs covering agriculture, horticulture, fishery, livestock, food-feed crops, rainwater harvesting, composting/vermicomposting, green manuring, etc., were integrated into the IOFS model to consider the problems that farmers face and the resources available to them. Emphasis was placed on local demand, socio-economic issues, ecology, and the effective recycling of on-farm resources to minimize dependency on external resources and generate continuous income and employment while supplying nutritious food to the farmers' families.

Within the program, seeds of improved varieties of crops and vegetables, planting materials, lime, rock phosphate, neem

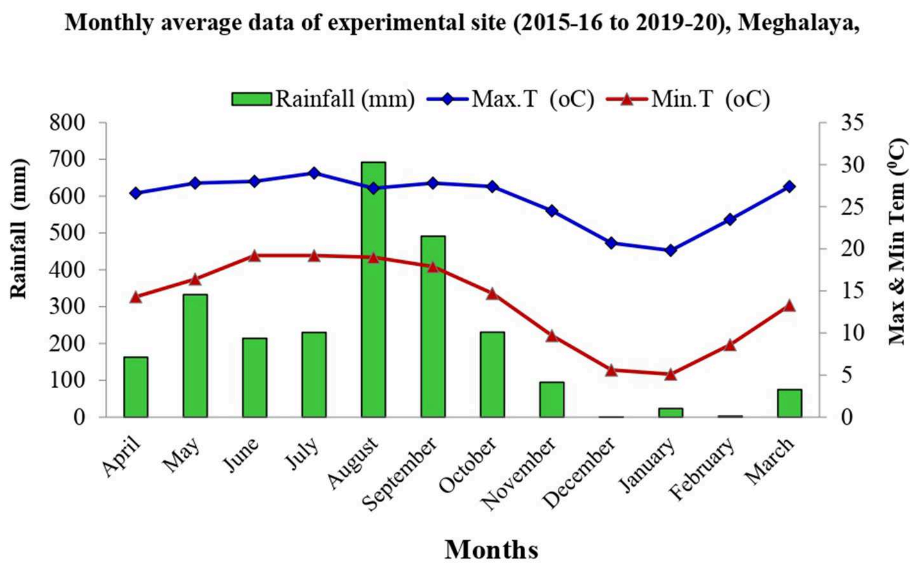

FIGURE 1
Monthly average rainfall and maximum and minimum temperatures during crop growing seasons in Umiam (average of 2015–16, 2016–17, 2017–18, 2018–19, and 2019–20).

cake, and other organic inputs were provided to the adopted farmers. Effective soil fertility management through the application of well-decomposed organic manures, such as farmyard manure, green leaf manure, and composts, was promoted. For pest and disease management, the use of neem oil, *Trichoderma*, derisome (bio-insecticide prepared from extract of *Pongamia glabra*/*Pongamia pinnata*), and indigenous technical knowledge (ITK) was emphasized. In terms of ITK, farmers mixed cow dung with water in rice fields to control rice hispa (*Dicladispa armigera*), smoked pumpkin fields to control fruit flies (*Bactocera cucurbitae*), placed red tree ant (*Oecophylla smoragdina*) nests on citrus plants to control citrus trunk borers (*Anoplophora verstegii*), and placed dried *Artemisia vulgaris* leaves and/or branches in and around granaries to control stored insects and rats (Deka et al., 2006). For promoting small-scale mechanization, implements and tools, such as paddy threshers, rice mills, sprayers, tulu pumps, and cono weeders, were provided to the village and a custom hiring center was established. Additionally, farmers were trained in various aspects of organic farming and the conservation of natural resources and residue recycling.

2.1. Weather parameters of the experimental area

The meteorological data of the villages (2014–2016) is graphically presented in Figure 1. The temperature was moderate for the most of the year except for a few months in winter. The daily minimum temperatures tended to rise from January and this trend was maintained the until June, decreasing from July onwards. For most of the year the maximum relative humidity was more than 75%. The mean annual evaporation was ∼850 mm. Although the area received an annual average rainfall of 2,450 mm, most of the rainfall occurred between April and November, and there was little or no rainfall between December and March.

2.2. Integrated organic farming system model development in the village using a cluster approach

Several IOFS models were developed from 2014 onwards in the village according to the situation and crop and livestock preferences. A flowchart showing the developmental steps of the IOFS model in the fields of farmers is presented in Figure 2. They integrated crops, viz., maize (*Zea mays* L.), vegetables, viz., tomato (*Solanum lycopersicum*), French bean (*Phaseolus vulgaris*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), broccoli (*Brassica oleracea* var. *italica*), potato (*Solanum tuberosum*), lettuce (*Lactuca sativa*), and carrot (*Daucus carota*), spice crops, viz., ginger (*Zingiber rubens*), turmeric (*Curcuma longa*), chili (*Capsicum annuum*), fruit trees, viz., Assam lemon (*Citrus limon* L. *Burmf*), papaya (*Carica papaya*), banana (*Musa paradisiaca*), guava (*Psidium guajava*), etc., livestock (dairy, pigs, and poultry), water harvesting (*Jalkund*), compost units, etc. (Figures 4A, B). Water from a micro water-harvesting structure, such as a *Jalkund*, was used for live-saving irrigation during the winter months. These structures increased crop productivity and diversified their homestead farming to growing remunerable crops and rearing cattle, pigs, poultry, etc. The IOFS model promotes crop diversification, thereby providing food security and employment for the farmers around the year. The different components in the model practiced by farmers on their farm itself are depicted in Figure 3. This approach involved the use of outputs of one enterprise component as inputs for other related enterprises wherever feasible, e.g., cattle dung mixed with crop residues

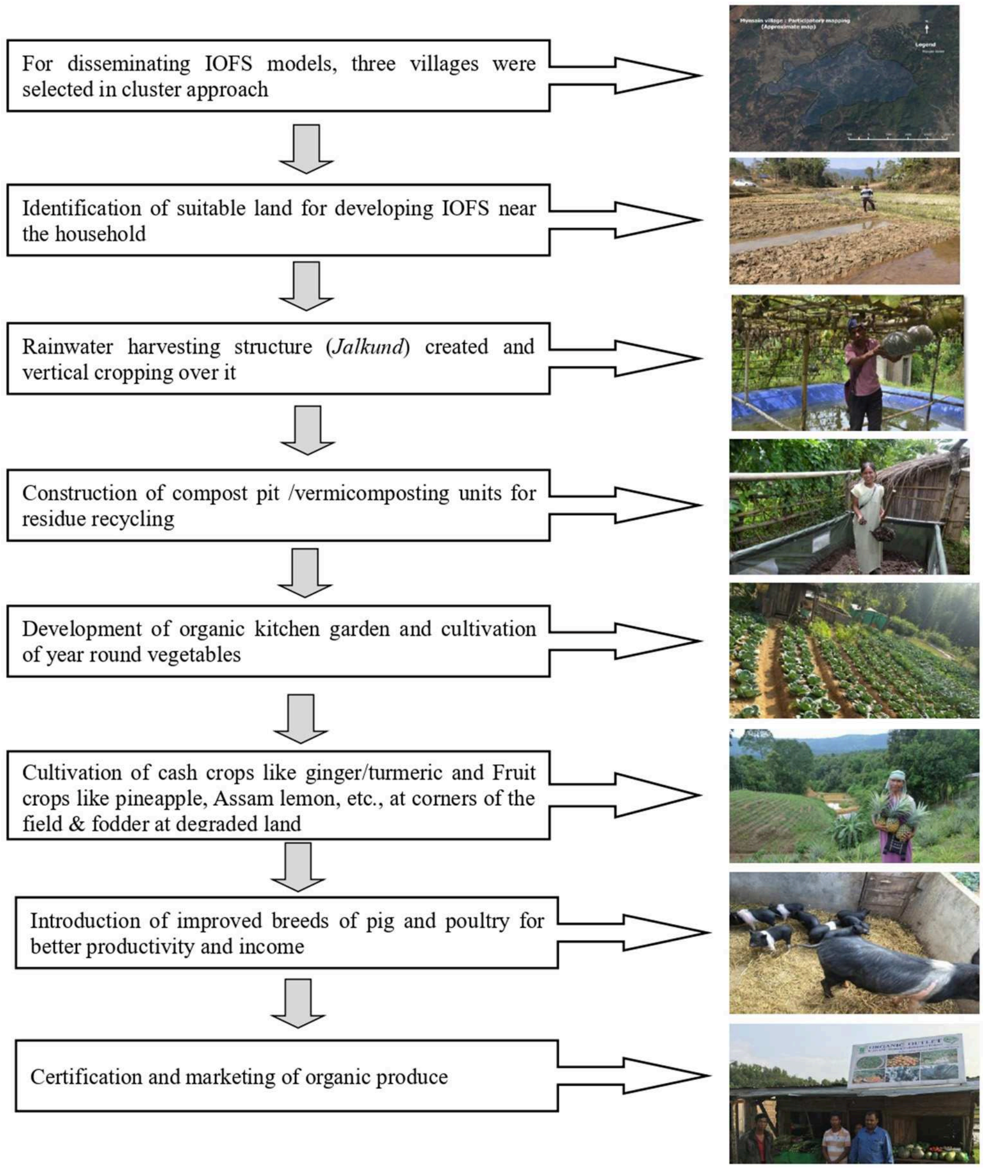

FIGURE 2
Flow chart of technology development in an IOFS model.

and farm waste was converted into nutrient-rich vermicompost. Therefore, there was less dependence on organic manure from external sources. A judicious mixture of livestock enterprises, such as dairy, poultry, fish, goat-rearing, and vermicomposting, helped to generate additional income. Climbing vegetables, such as bottle gourd, chow-chow, cucumber, and ridge gourd, were grown on a structure created above water bodies on one side of the *Jalkund* for vertical intensification. Pumpkin was raised on another side of the *Jalkund* and allowed to crawl on the ground. During the rainy season, roof water was harvested and stored in a *Jalkund*. *Jalkunds* in the model have multipurpose uses, such as irrigation, supply to the piggery and poultry, and mushroom block making. Before the distribution of improved vegetable seeds, beneficiary farmers were trained in nursery raising and the scientific methods of vegetable cultivation. A community nursery was formed in the villages for raising seedlings of cole crops, such as cabbage, broccoli, and cauliflower. This activity was crucial for obtaining strong and healthy vegetable seedlings.

New interventions, such as mushroom houses and honey boxes, were also implemented to obtain additional income and use farm resources more efficiently. Although paddy straw was used to produce organic mushrooms, honeybee plays an important role in pollination and overall crop performance. Oyster mushroom cultivation was carried out, except in July and August when the incidence of insect pests and competitor molds is very high. The PL-14-02 oyster mushroom (*Pleurotus florida*) strain was used as it has very high biological efficiency (∼106%) and therefore produces a very high yield in comparison with other strains. During the summer season, *Pleurotus pulmonarius* (*Pleurotus sajor-caju*) was grown on paddy straw. Mushroom cultivation not only provides

FIGURE 3
Different components of the IOFS model developed in the farmers' fields in Meghalaya, India.

additional income but is also a source of nutrient security in rural areas because of its high protein content, and in addition, it also possesses many nutraceutical properties.

2.3. Construction of the *Jalkund* water harvesting structure and farm ponds

To promote efficient water conservation and its multiple uses, several farm ponds were constructed and existing farm ponds were renovated in the adopted villages. Initiatives were also undertaken to popularize the low-cost rainwater harvesting structures known as *Jalkunds* (5 × 4 × 1.5 m), which were constructed using 250 GSM Silpaulin sheets, had storage capacities of 30,000 L, and were used to harvest rainwater in the IOFS fields of farmers. These structures were constructed to enable the farmers to harvest rainwater during the rainy season and subsequently use the water during dry periods to irrigate high-value winter crops. Farmers diversified their farming activities throughout the year and cultivated high-value crops, such as broccoli, tomato, and French bean. Climbing vegetables, such as pumpkin, bottle gourd, chow-chow, cucumber, and ridge gourd, were grown on a structure created above the water harvesting structure on one side of the pond dyke for vertical intensification. Additionally, the stored water was used in daily activities, such as cleaning and giving water to livestock; previously, the farmers had to fetch water from distant places. Some farmers also used the *Jalkunds* for rearing fish, which provided them with additional income.

2.4. Animal components

In addition to the improved technology of the housing system, the improved pig variety "Lumsniang" (with 25% genetic inheritance of Khasi local and 75% genetic inheritance of Hampshire) was also introduced to the village. These pigs attained a higher body weight at an early age, as well as a larger litter size at weaning, than the local non-descriptive pigs in the low input tribal production system. The deep litter housing system provides a better micro-environment during both summer and winter, with better physiological adaptation. Approximately 1,000–1,500 kg of well-decomposed manure/year was produced by replacing the bedding material in the pigsty. In Meghalaya, backyard poultry farming has emerged as an important alternative livelihood option for farm women, providing income and household nutritional security. Most of the backyard poultry production involves the rearing of indigenous birds with poor production performance. Dual-purpose backyard poultry birds (the Vanaraja and Gramapriya varieties) with high production potential were introduced into the IOFS models developed in the village. Feed for the poultry and pigs was the major constraint. Emphasis was placed on the production of maize grain for feed purposes and the cultivation of fodder (hybrid bajra napier, congo-signal, broom grass, etc.) and the multipurpose tree Colocasia for cattle and pigs.

2.5. Pisciculture

For composite fish culture, fingerlings consisting of catla (30%), grass carp (30%), and common carp (40%) were released according to the size of the pond and *Jalkund*. Lime (500 kg/ha) and well-decomposed FYM (10 t/ha) were applied after the pond was constructed to enhance soil fertility. Sun-dried cow dung was used to manure the pond (100 kg/ha per month in weekly splits).

2.6. Compost preparation

Vermicomposting units were constructed to recycle on-farm biomass to increase the fertility of the soil. Vermibeds are the latest unique technology for earthworm farming and are 12′ × 4′ × 2′ and each can produce ∼500–1,000 kg of vermicompost in a year. They are very portable, low cost, easy to handle and install, and allow the collection of vermiwash. Vermibeds can be used on a small scale by farmers with household organic waste. Crop residues and agricultural waste were collected by the farmers and used to fill in the vermibeds for decomposition processes. Even bio-enriched compost or enriched compost was used in these areas as it increases nutrient availability and suppresses diseases. The *Trichoderma*-based formulation was used for preparing bio-enriched compost. *Trichoderma* formulation (1 kg) was mixed with 100 kg of well-decomposed FYM and kept for 10 days under the polythene cover. The mixture was turned every 3 days. For the management of bacterial wilt in chili and brinjal, *Pseudomonas fluorescence*-based formulations were used for soil drenching. Other seeds were also treated with *Trichoderma*-based formulations, which help to enhance germination, provide protection from damping off and other soil-borne diseases, and increase seedling vigor. The slurry method was used for seed treatment; 5 g of *Trichoderma* formulation was prepared in 10 ml of water and this slurry was sufficient for 1 kg of seed. The compost was dried in the shade after treatment.

2.7. Organic management of insect pests and diseases

Pest management for various crops in an IOFS involved proper sanitation, clean cultivation, and the manual collection and destruction of egg masses and larvae of lepidopteron pests at the initial stage of incidence. Therefore, insect pest infestation was avoided in very severe conditions on most of the crops. However, the infestation of fruit borers in tomatoes, cabbage butterflies and aphids in cole crops, lepidopteron borers in maize, and aphids in beans was as a major problem. For keeping the pest population below the economic damage level, neem oil 0.03% (5 ml/L) and *Bacillus thuringinesis* (2 g/L) were sprayed alternatively at 10-day intervals to manage lepidopteron pests in cabbage, tomato, maize, and other crops, whereas neem oil and *Lecanicillium lecanii* (5 g/L) were sprayed to manage aphids and other sucking pests. In addition, a mixture of vermiwash (1 L) and 10–15-day-old cow urine (1 L) in 10 L of water was used as a biopesticide and liquid manure spray on vegetable crops. Diseases were managed within the system using *Trichoderma*- and *P. fluorescence*-based formulations. Seed/rhizome treatment was carried out in many cases, e.g., for ginger and French bean. For ginger, rhizome treatment was performed by preparing a suspension of *Trichoderma* formulation (3 g/L), and 10 L of this suspension was used for treating 10 kg of seed rhizomes. Rhizomes were kept in the suspension for 45 min and then shade-dried for 24 h before sowing. During July and August, the incidence and severity of *Pythium* soft rot and bacterial wilt caused by *Ralstonia solanacearum* is high; therefore, the affected spots and nearby healthy clumps were soil drenched with *Trichoderma* and *P. fluorescence*-based formulations (4 g/L).

2.8. Nutrient budgeting

While compost pits were dug in Mrs. Skola Kurbah's field, vermicompost was prepared in Mr. Jrill Makroh's field. Product or waste generation from one enterprise was judiciously used as input for the others. The requirement of nutrients, such as nitrogen (N), phosphorous (P_2O_5), and potassium (K_2O), for crops cultivated in the model was calculated, and nutrients recycled within the system through manure, compost, and vermicompost were determined. The economic and byproduct samples of IOFS models were collected and their total N concentrations were determined using the micro-Kjeldahl method (Bremner and Mulvaney, 1982), while total P and K concentrations were measured using a di-acid mixture (HNO_3:$HClO_4$ at a 3:1 ratio) (Tandon, 1995). Soil pH was measured in a 1:2.5 soil:water suspension (Jackson, 1973) and the SOC was estimated using the Walkley–Black method (Walkley and Black, 1934). While available soil N concentrations were measured using the alkaline potassium permanganate method (Subbiah and Asija, 1956), available P and K were measured using Bray's method (Bray and Kurtz, 1945) and the ammonium acetate method (Knudsen et al., 1982), respectively. While the farmyard manure (FYM) contained 0.73 ± 0.04% N, 0.24 ± 0.03% P_2O_5, and 0.98 ± 0.05% K_2O, the vermicompost prepared in the model contained 1.74 ± 0.07% N, 0.69 ± 0.05% P_2O_5, and 1.03 ± 0.06% K_2O. Nutrient balance was calculated by subtracting the amount of nutrients recycled from the nutrient requirement within the IOFS model (Das et al., 2019).

2.9. Statistical analysis

We undertook descriptive statistical analysis of the data in the IOFS models, and the year was considered as replication. Standard error of the mean is shown in Tables 2–6. Similarly, descriptive statistical analysis was also conducted for the figures and the vertical bars represent the standard error (SE), with $p < 0.05$ considered significant (Figure 5).

3. Results

3.1. System productivity, profitability, and water harvesting in a participatory IOFS model

The IOFS models have achieved success by providing diversified food, year-round employment, and improving the income of farmers. As the farmers were traditionally growing crops for decades without using any synthetic fertilizer, yield levels increased significantly after the adoption of organic practices in a systematic manner. Maize-French bean and rice-pea cropping systems were found to be popular among the farming communities as they promote crop diversification and provided additional income. As the farmers were given training on improved crop production techniques, which including field visits to ICAR Research Complex farms, farmers become confident in applying organic farming methods in their field. The IOFS model promotes crop diversification, thereby providing food security and employment for the farmers all year round. The IOFS provides better means for year-round employment in these sections of rural mass through the use of different crops in a sequence mode, livestock management, mushroom rearing, compost preparation, and pisciculture. Maize, French bean, potato, ginger, tomato, carrot, and chili yields in the IOFS were increased by approximately 20–30%, 40–45%, 25–30%, 33–40%, 45–50%, 37–50%, and 27–30%, respectively, compared with conventional practice. Additionally, the average productivity of the fruit trees pineapple, Assam lemon, and guava increased in the organic system by 35–40%, 27–30%, and 30–35%, respectively, compared with the conventional system. A small shop was constructed near the highway so that the farmers could sell organic produce (vegetables, fruits, and spices) from the village/Institute at a relatively higher price. The organic certification (PGS mode) process for the farmers of the adopted villages was also initiated (provisional registration number given) so that they could demand the premium price for organic produce, thus increasing their income further. Emphasis was placed on producing seeds in the farm itself to reduce the dependency on external seeds and reduce the cost of production. Certified organic or chemically untreated seeds of some crops were also not available all the time. Successful IOFS models can generate 75–80% of its total requirement for seeds (rice, maize, ginger, turmeric, soybean, pea, lentil, French bean, pumpkin, bottle gourd, squash, leafy mustard, coriander, spinach, brinjal, chili, etc.) and 20–25% of seeds (tomato, cauliflower, cabbage, broccoli, carrot, beetroot, radish, etc.) are purchased from the market. Improved pig breeds (75% Hampshire and 25% local) and local breeds were integrated with improved husbandry practices. A deep litter model of pig housing was introduced to increase productivity and use resources more efficiently. The pig attained a higher body weight of 90–100 kg at 12 months of age and produced a larger litter size at weaning than local non-descriptive pigs in the low-input tribal production system. The deep litter housing system provides a better micro-environment during both summer and winter and better physiological adaptation. In this housing system, little or no liquid effluent is produced, and odor is greatly reduced. Approximately 1,000–1,500 kg of well-decomposed manure/year was produced by replacing the bedding material in the pigsty. The adult upgraded pigs were sold by the farmers for Rs. 10,000–12,000 per pig, whereas the local adult pigs were sold for Rs. 6,000–7,000 per pig. The breeding farmers harvested two or three extra piglets per farrowing, compared with the earlier system, and sold each piglet for Rs. 2,500–3,000. After 1 year of stocking 2,000 fingerlings in her pond of Mrs. Pretywon of Rynghang harvested 200 kg of fish, which she sold at Rs 180 per kilogram and generated an income of Rs. 36,000. Mr. Lamare, one of the beneficiaries, said that he was able to harvest ∼7 kg of fish from his 20-m^2 *Jalkund*. He sold them at Rs. 180 per kilogram and generated an income of Rs. 1,260. Additionally, he used the azolla generated from the *Jalkund* as fish feed and a source of manure for raising his crops. Nutrient recycling through vermicomposting using animal excreta, weed biomass, kitchen waste, and the leaves of peripheral trees, along with FYM application, fulfilled most of the nutritional requirement of all the crops in the organic farming system model and sustained the overall productivity of the farm. The farmers produced vermicompost in vermibeds and cement brick chambers and generated 0.4 to 1.25 tons per annum. The data from eight IOFS models from the villages are shown in Table 1. They integrated cereal crops (rice and maize), vegetables (tomato, French bean, potato, lettuce, and carrot), livestock (dairy, pigs, and poultry), and a water harvesting structure (*Jalkund*) into the system. The average yearly income of the farmers with IOFS models of 0.18–0.35 ha was recorded within the range of Rs. 18,750–46,695 per annum without any premium price. However, the farmers could get a 20% premium price for their organic produce, and in this instance, income was increased to a range of Rs. 22,500–56,034 (Table 1).

3.2. Case studies of livelihood assessments of two farmers

3.2.1. Livelihood assessments

Two progressive farmers, Mr. Jrill Makroh and Mrs. Skola Kurbah, were the pioneers of the IOFS model in the village and started their IOFS model in March 2014. The results/performance of the systems of both farmers was analyzed after 1 year and from

TABLE 1 Farmers who adopted the IOFS model in Meghalaya, India and their economic return (average of 5 years).

Sl. No	Farmer's name	Farming components	Water source	Area (ha)	Net return/year from model without premium price (Rs)	Net return/ha/year without premium price (Rs)	Net return/year from model with 20% premium price (Rs)	Net return/ha/year with 20% premium price (Rs)
1	Jril Makhroh	Maize + vegetables + ginger + dairy + poultry + pisciculture + mushroom	*Jalkund*	0.27	46,695	172,944	56,034	207,533
2	Lahun Lapang	Fruit trees (pineapple, Assam lemon, pomelo) + vegetables + piggery + poultry	*Jalkund*	0.20	24,500	122,500	29,400	147,000
3	Judy Wahlang	Rice + vegetables + poultry + pisciculture + bamboo	Pond	0.32	29,500	92,188	35,400	110,625
4	Pynsanlang Rynghang	Maize + vegetables + ginger + + poultry + apiculture	*Jalkund*	0.18	18,750	104,167	22,500	125,000
5	Lamphrang Rympei	Rice + vegetables + turmeric + piggery + poultry + pisciculture	Pond	0.29	35,670	123,000	42,804	147,600
6	Ban War	Fruit trees (pineapple, Assam lemon, banana) + piggery +vegetables	*Jalkund*	0.35	41,590	118,829	49,908	142,594
7	Skola Kurbah	Maize + soybean + vegetables + turmeric+ piggery + poultry + apiculture	*Jalkund*	0.21	31,102	148,105	37,322	177,726
8	Hynniew Rynghang	Sweet potato + vegetables + piggery + poultry + dairy+ turmeric	*Jalkund*	0.26	33,500	128,846	33,500	154,615

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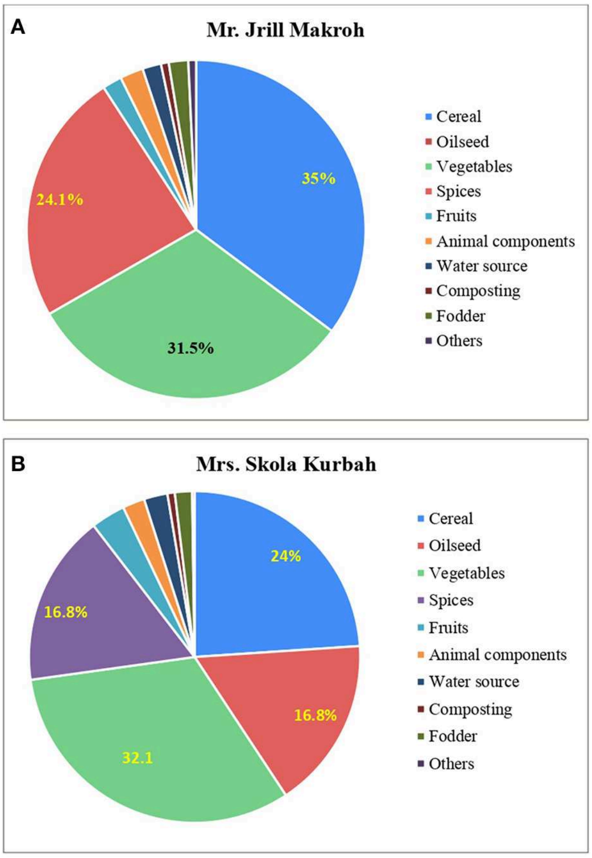

FIGURE 4
Allocation of different enterprises in the IOFS models of two farmers in the adopted villages. **(A)** Mr. Jrill Makroh and **(B)** Mrs. Skola Kurbah.

2015 onwards. The IOFS model in Mr. Jrill Makroh's field covered an area of 0.27 ha and consisted of cereals (maize), vegetables, spices (turmeric and chili), fruit crops (papaya and Assam lemon), dairy, a piggery, mushroom units, composting units, and a water harvesting unit (a *Jalkund*; Table 1). The largest area was covered by cereal (35%), followed by vegetables (31.5%) and spices (24.1%); the other components (animal, water source, composting, fodder, oilseeds, etc.) covered 9.4% of the total area (Figure 4A). Mrs. Skola Kurbah's field (0.21 ha), in addition to cereals (maize [24%]), vegetables (32%) and spices (16.8%), was used for growing oilseed in the form of soybean (16.8%), and 10.3% of the total area was used for growing fruit, composting, water sources, animals, and fodder (Figure 4B). The average cost of cultivation, gross return, and net return of five consecutive years (2014–2019) from the 0.27 ha area of Mr. Jrill Makroh's IOFS model were Rs. 71,670 ± 985, Rs. 118,365 ± 1,001, and Rs. 46,695 ± 418, respectively (Table 2). For Mrs. Skola Kurbah's 0.21-ha IOFS model, the average cost of cultivation, gross return, and net return of five consecutive years (2014–19) were Rs. 40,900 ± 973, Rs. 72,002 ± 1,159 and Rs. 31,102 ± 501, respectively (Table 3). On a 1-ha basis, the net return was assumed to be Rs. 172,944 per year (Rs. 474 per day) for Mr. Jrill Makroh and Rs. 148,105 per year (Rs. 406 per day) for Mrs. Skola Kurbah, which is a modest amount for a four- to five-member family. The two farmers, Mr. Jrill Makroh and Mrs. Skola Kurbah, could get a total net return per annum of Rs. 56,034 ± 502 and Rs. 37,322 ± 601 with a 20% premium price. The higher net return recorded in the former farmer's field compared with the latter was due to the maximum enterprises included and the greater IOFS area. The water source, composting, and fodder were the inputs for the other component in the IOFS model; therefore, a negative net return was recorded (Tables 1, 2). The net return (Rs./ha) over the years obtained from the IOFS models of the two farmers were significantly higher ($p = 0.05$) than the farmers' practice-I of maize-fallow or farmers' practice-II (cultivation of maize followed by the cultivation of vegetables in 30% of the areas; Figures 5A, B).

The system productivity (SP) of the IOFS models of the two farmers was calculated based on rice equivalent yield (REY) in terms of kg/ha. The total SP of Mr. Jrill Makroh and Mrs. Skola Kurbah was 21,919 ± 185 kg/ha and 17,143 ± 276 kg/ha, respectively (Table 4). The average highest SP for 5 consecutive years was reported for animal components in both the farmer's IOFS models (Mr. Jrill Makroh as 10,315 ± 56 kg/ha and Mrs. Skola Kurbah as 7,405 ± 83 kg/ha; Table 4), which may be due to the higher pricing of animal products and meat and the high rate of livestock. The total average production efficiency for 5 consecutive years under the IOFS model was 60.1 ± 0.51 kg/ha/day for Mr. Jrill Makroh and 47.0 ± 0.76 kg/ha/day for Mrs. Skola Kurbah (Table 4). System productivity (kg/ha) and production efficiency (kg/ha/day) was significantly higher with IOFS models than with farmers' practice-I and farmers' practice-II (Table 4). This shows that the adoption of an IOFS model in farmer's fields can achieve a premium farm income, reduce poverty, and provide better food security for the farmers.

3.2.2. Residue recycling and nutrient balance

The two IOFS models of Mr. Jrill Makroh and Mrs. Skola Kurbah had different on-farm nutrient supply balance sheets. The sheets were categorized under five modules (module I, module II, module III, module IV, and module V). Module I comprised cereals and oilseeds; module II comprised horticultural crops; module III comprised dairy; module IV comprised pigs and poultry; and module V comprised others (Tables 5, 6). All the modules of the two IOFS models generated on-farm nutrients, such as N, P_2O_5, and K_2O, through the recycling of crop residues, livestock excreta, and leftovers except module III for Mrs. Skola Kurbah. The highest on-farm nutrients recycled was recorded under module V for both the IOFS models (23.1 ± 0.11 kg N, 7.4 ± 0.32 kg P_2O_5, and 20.0 ± 0.63 kg K_2O for Mr. Jrill Makroh, and 22.9 ± 0.36 kg N, 6.9 ± 0.2 kg P_2O_5, and 20.1 ± 0.34 kg K_2O for Mrs. Skola Kurbah). The average lowest N and P_2O_5 on-farm nutrients recycled for five consecutive years was recorded under module I and module IV for K_2O for Mr. Jrill Makroh. However, the IOFS model of Mrs. Skola Kurbah had the lowest N under module I and P_2O_5 and K_2O under module II. The above results show that module V has a higher potential for supplying macronutrients (N, P, and K) than other modules in the IOFS model and macronutrient content in the residues of module I and module II were less. The more on-farm nutrients are recycled, the less off-farm nutrients will be needed; therefore, modules I and II for both farmers had a negative nutrient balance. Modules III and IV for Mr. Jrill Makroh and module IV for Mrs. Skola Kurbah

TABLE 2 Production of IOFS models of two farmers from adopted villages (average of 5 years).

IOFS components	Enterprises	Mr. Jrill Makroh		Mrs. Skola Kurbah	
		System productivity (REY kg/ha)	Production efficiency (kg/ha/day)	System productivity (REY kg/ha)	Production efficiency (kg/ha/day)
Cereals	Maize	1,278 ± 38	3.5 ± 0.10	857 ± 33	2.3 ± 0.09
Oilseed	–	0	0.0		
Vegetables	French bean, cole crops, okra, tomato, pumpkin, pea, etc.	4,963 ± 53	13.6 ± 0.15	4,262 ± 64	11.7 ± 0.18
Spices	Turmeric, chili	2,540 ± 31	7.0 ± 0.08	1,548 ± 56	4.2 ± 0.15
Fruits	Papaya, Assam lemon	361 ± 7	1.0 ± 0.02	262 ± 15	0.7 ± 0.04
Animal components	Cattle (1 cow, I calf), pig (I pig + 6 piglets)	10,315 ± 56	28.3 ± 0.15	7,405 ± 83	20.3 ± 0.23
Water source	*Jalkund*	185 ± 7	0.5 ± 0.02	238 ± 6	0.7 ± 0.02
Composting	Vermicompost and manure tank	111 ± 2	0.3 ± 0.01	0	0.0
Fodder	Napier, broom grass	0	0.0	0	0.0
Others	Mushroom cultivation	2,167 ± 35	5.9 ± 0.10	1,667 ± 26	4.6 ± 0.07
Total		21,919 ± 185	60.1 ± 0.51	17,143 ± 276	47.0 ± 0.76
Farmers' practice-I	Maize-fallow	4,059 ± 168	11.1 ± 0.46	4,304 ± 59	11.8 ± 0.16
Farmers' practice-II	Maize-vegetables (1/3rd area)	8,596 ± 302	23.6 ± 0.83	9,164 ± 388	25.1 ± 1.06

required no off-farm nutrients and they were the major source of on-farm nutrients. Furthermore, adopting the IOFS model resulted in only 1.8 ± 0.24 kg of N, 3.8 ± 0.21 kg of P_2O_5, and 0.9 ± 0.11 kg of K_2O being needed from off-farm sources to achieve a nutrient balance for Mr. Jrill Makroh, i.e., the model could supply 95.1% of N, 82.0% of P_2O_5, and 96.0 % of the total K_2O requirement of the model, and only 4.9% of the total N, 18% of P_2O_5, and 4.0% of K_2O was needed from outside sources (Table 5). This means the IOFS model is highly sustainable. For Mrs. Skola Kurbah, the IOFS could generate 76.0 % of total N, 68.6% of P_2O_5, and 85.5% of K_2O needed within the system (Table 6); 24% of N, 31.4% of P_2O_5, and 14.5% of the total K_2O requirement was supplied from external sources, such as through the purchase of FYM.

4. Discussion

4.1. Impact of the IOFS on system productivity and profitability

The IOFS integrates the management of all the production systems to achieve a sustainable result economically and environmentally (Manhoudt et al., 2002). An increase in system productivity and net returns due to the scientific integration of farming system enterprises, such as livestock, cereals, pulses, and vegetables, with the *in situ* production of compost or vermicompost by efficient recycling of farm resources was recorded by many workers (Ansari et al., 2017, 2023; Das et al., 2019; Layek et al., 2020). The sustainable rice-based integrated farming system (IFS) in an irrigated agro-ecosystem reported that the cropping system of rice-pea-okra achieved a higher rice equivalent yield (REY) (17.88 t/ha), greater system productivity, and higher employment than the conventional rice-wheat cropping system (Ansari et al., 2013; Layek et al., 2017). A crop rotation system that includes a mixture of soil fertility building leguminous crops and cash crops, such as vegetables and spices, was the main mechanism for long-term nutrient supply and reducing the pest and disease load within organic systems. Leguminous crops also have the potential for biological nitrogen fixation (BNF), which helps in supplying nitrogen and improves the organic production system (Connor, 2021). The inclusion of legumes in cropping systems or farming systems as intercrops or in sequence to prevent monocropping is very much needed to improve system productivity and soil health (Ansari et al., 2017; Layek et al., 2018). The small and marginal farmers faced under-employment due to the seasonal nature of their crop production (Ramrao et al., 2005; Ansari et al., 2014). However, IOFSs comprising crops (cereal and horticultural) and livestock (poultry, piggery, dairy, apiculture, pisciculture, and rabbit) are more economic in terms of net returns than crop production only (Das et al., 2014; Ravisankar et al., 2021). This helps to ensure that the farmers' income is above the poverty line. The concept of the IOFS is to increase the income and employment of the marginal and small land holdings by integrating various farm components (livestock, pisciculture, crop production, apiculture, vermicomposting etc.) and residue management, in which the waste of one source is the input of the other, for sustainable agriculture (Soni et al., 2014; Meena et al., 2022). For example, cattle dung mixed with crop residues and farm waste can be converted into nutrient-rich vermicompost, thereby, there is less dependence on organic manure from external sources.

There is always a chance to reduce the production cost of individual enterprises, such as livestock rearing, crop production,

TABLE 3 Detailed analysis of the IOFS model developed at Mr. Jril Makroh's farm (average of 5 years).

IOFS components	Enterprises	Total area (m^2)	Cost (Rs)	Gross returns without premium price (Rs.)	Net returns without premium price (Rs.)	Net returns without premium price (Rs./ha/year)	Net returns (Rs./component) with 20% premium price	Component wise net returns (Rs./ha) with 20% premium price in 1 ha
Cereals	Maize	950	3,600 ± 51 (5.0)	6,900 ± 206	3,300 ± 166 (7.1)	12,222 ± 613	3,960 ± 198	14,667 ± 736
Oilseed	–	–	–	–	–	–	–	–
Vegetables	French bean, cole crops, okra, tomato, pea, etc.	850	13,900 ± 117 (19.4)	26,800 ± 286	12,900 ± 200 (27.6)	47,778 ± 741	15,480 ± 240	57,333 ± 889
Spices	Turmeric, chili	650	9,200 ± 45 (12.8)	13,715 ± 166	4,515 ± 135 (9.6)	16,722 ± 499	5,418 ± 162	20,067 ± 598
Fruits	Papaya, Assam lemon	50	500 ± 20	1,950 ± 36	1,450 ± 49	5,370 ± 180	1,740 ± 58	6,444 ± 216
Animal components	Cattle (1 cow, I calf), pig (I pig + 6 piglets)	60	32,650 ± 544 (45.6)	55,700 ± 303	23,050 ± 397 (49.4)	85,370 ± 1,469	27,660 ± 476	102,444 ± 1,763
Water source	*Jalkund*	48	5,000 ± 84	1,000 ± 38	−4,000 ± 60	−14,815 ± 222	−4,800 ± 72	−17,778 ± 266
Composting	Vermicompost and manure tank	20	1,920 ± 33	600 ± 13	−1,320 ± 25	−4,889 ± 94	−1,584 ± 31	−5,867 ± 113
Fodder	Napier, broom grass	50	400 ± 14	0	−400 ± 14	−1,481 ± 53	−480 ± 17	−1,778 ± 64
Others	Mushroom cultivation	20	4,500 ± 158 (6.3)	11,700 ± 188	7,200 ± 89 (15.4)	26,667 ± 331	8,640 ± 107	32,000 ± 397
Total value of overall enterprises		**2,698**	**71,670 ± 985**	**118,365 ± 1,001**	**46,695 ± 418**	**172,944 ± 1,548**	**56,034 ± 502**	**207,533 ± 1,857**
Farmers' practice-I	Maize-fallow		10,224 ± 103	21,916 ± 908	11,692 ± 814	43,304 ± 3,013	14,030 ± 976	51,964 ± 3,615
Farmers' practice-II	Maize-vegetables (1/3rd area)		22,716 ± 488	46,420 ± 1,629	23,704 ± 1,699	87,793 ± 6,293	28,445 ± 2,039	105,351 ± 7,551

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TABLE 4 Detailed analysis of the IOFS model developed at Mrs. Skola Kurbah's farm (average of 5 years).

IOFS components	Enterprises	Total area (m^2)	Cost (Rs)	Gross returns without premium price (Rs)	Net returns without premium price (Rs)	Net returns without premium price (Rs/ha/year)	Net returns (Rs./component) with 20% premium price	Component wise net returns (Rs./ha) with 20% premium price in 1 ha of IOFS model
Cereals	Maize	500	1,850 ± 49 (4.5)	3,600 ± 139	1,750 ± 112 (5.5)	8,333 ± 533	2,100 ± 134	10,000 ± 640
Oilseed	–	350	1,500 ± 62	3,800 ± 166	2,300 ± 130	10,952 ± 618	2,760 ± 156	13,143 ± 742
Vegetables	French bean, cole crops, okra, tomato, pumpkin, pea etc.	670	9,200 ± 88 (22.5)	17,900 ± 269	8,700 ± 184 (27.3)	41,429 ± 877	10,440 ± 221	49,714 ± 1,053
Spices	Turmeric, chili	350	2,400 ± 84 (5.9)	6,500 ± 236	4,100 ± 200 (12.9)	19,524 ± 952	4,920 ± 240	23,429 ± 1,142
Fruits	Papaya, Assam Lemon	70	650 ± 40	1,100 ± 63	450 ± 37	2,143 ± 174	540 ± 44	2,571 ± 209
Animal components	Cattle (1 cow, I calf), pig (I pig + 6 piglets)	45	16,300 ± 307 (39.9)	31,100 ± 346	14,800 ± 243 (46.4)	70,476 ± 1,159	17,760 ± 292	84,571 ± 1,390
Water source	*Jalkund*	48	5,000 ± 267	1,000 ± 24	−4,000 ± 243	−19,048 ± 1,158	−4,800 ± 292	−22,857 ± 1,390
Composting	Vermicompost and manure tank	15	1,200 ± 66	0	−1,200 ± 66	−5,714 ± 313	−1,440 ± 79	−6,857 ± 376
Fodder	Napier, broom grass	35	300 ± 16	0	−300 ± 16	−1,429 ± 79	−360 ± 20	−1,714 ± 94
Others	Mushroom cultivation	5	2,500 ± 204 (6.1)	7,002 ± 107	4,500 ± 187 (14.1)	21,438 ± 892	5,402 ± 225	25,726 ± 1,071
Total value of overall enterprises		**2,088**	**40,900** ± 973	**72,002** ± 1,159	**31,102** ± 501	**148,105** ± 2,385	37,322 ± 601	177,726 ± 2,862
Farmers' practice-I	Maize-fallow		7,912 ± 91	18,077 ± 246	10,165 ± 217	48,405 ± 1,033	12,198 ± 260	58,086 ± 1,239
Farmers' practice-II	Maize-vegetables (1/3rd area)		19,283 ± 157	38,488 ± 1,629	19,205 ± 1,689	91,452 ± 8,042	23,046 ± 2,027	109,743 ± 9,651

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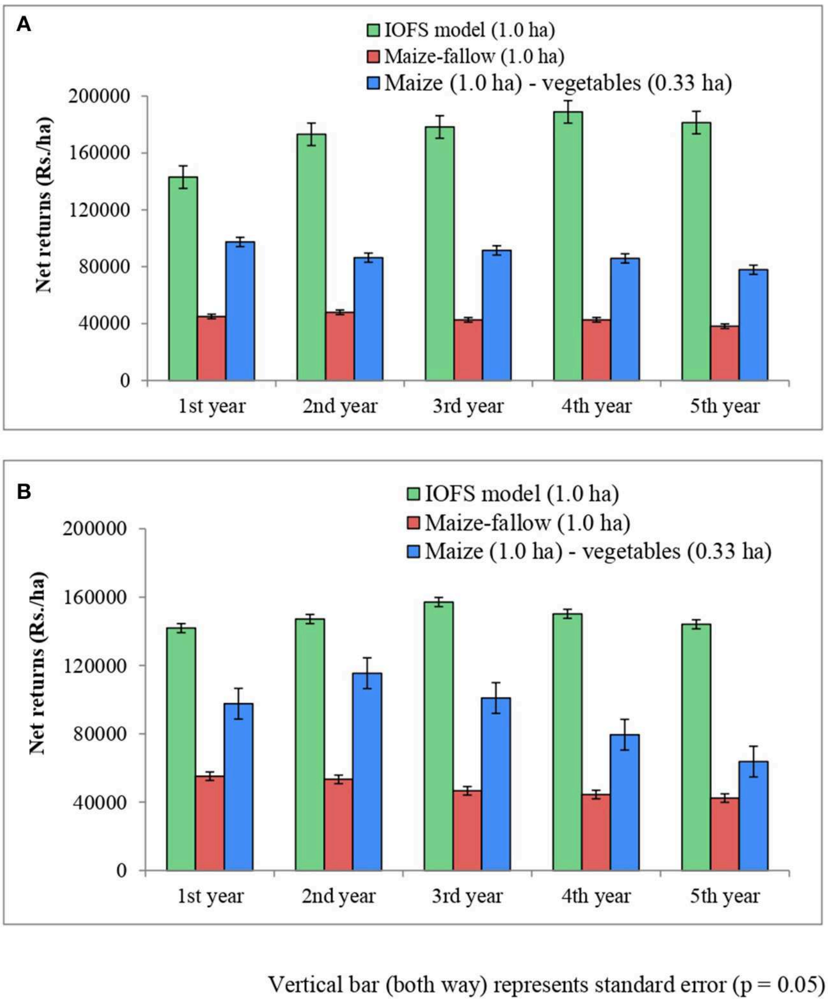

FIGURE 5
Net return/ha over 5 years from the IOFS models of Mr. Jrill Makroh **(A)** and Mrs. Skola Kurbah **(B)** compared with common farming practices.

and pisciculture, and subsequently the overall cost of a farming system (Layek et al., 2020; Das et al., 2021). Different resources generated within the farm viz., crop residues (rice straw, maize stalk, pulses biomass, etc.), weed biomass, vegetable waste, livestock dung and urine, and poultry/duck droppings can be efficiently recycled through composting or vermicomposting and subsequently can be used in an IOFS model (Das et al., 2019, 2021). The application of organic amendments, such as vermicompost, enhances the activity of soil microorganisms, thereby improving soil health long term, i.e., the physical, chemical, and biological properties of the soil (Pierre-Louis et al., 2021). The inclusion of animal components in the system has a positive link to sustainability by generating cash income, improving family nutrition, and recycling crop residues into feed. A judicious mixture of livestock enterprises, such as dairy, poultry, fish, goat-rearing, and vermicomposting, will help to generate additional income (Panwar et al., 2018). Before the initiation of the IOFS program, the pigs reared by farmers had a very high mortality rate due to diseases and poor management. The inclusion of livestock components (cattle, pig, poultry, duck, etc.) and high value vegetables (broccoli, cauliflower, tomato, brinjal, carrot, lettuce, etc.) has the potential to improve the net return of the IOFS system due to the prevailing high market demand for organic produce and price (Layek et al., 2020). The productivity of pigs and poultry was low due to low feed conversion efficiency. Farmers were also motivated by the performance of the dual-purpose improved poultry varieties as they thrived even when poorly fed and subjected to the low-intensive management practices followed by the farmers of the village. By integrating livestock units, such as cattle, pigs, or poultry/duck, with fish ponds, the input cost of fish feed, manure, fertilizers, etc., can be minimized (Layek et al., 2020; Das et al., 2021). Unlike conventional practices, organic farming practices meet the biological and ethological needs of livestock (Von Borell and Sorensen, 2004). Poultry in the organic farming system increases the renewable and local inputs for the other components (Castellini et al., 2006). According to Lepcha et al. (2018), the gross return per annum (Rs. 165,800) of backyard poultry from organic farming systems is significantly higher than that from conventional farming systems (Rs. 95,695). Among the fish species reared in ponds and *Jalkunds*, the performance of common carp was superior

TABLE 5 On-farm nutrient supply balance sheet in the IOFS model of Mr. Jrill Makroh (after 5 years).

IOFS modules	Nutrient requirement (kg)			On-farm nutrient recycled (kg)			Nutrient balance (kg)		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Module I	24.7 ± 0.30	8.8 ± 0.23	20.5 ± 0.43	6.2 ± 0.13	2.0 ± 0.14	11.3 ± 0.18	−18.6 ± 0.40	−6.8 ± 0.36	−9.3 ± 0.60
Module II	29.3 ± 0.47	10.5 ± 0.18	24.5 ± 0.30	14.7 ± 0.11	3.1 ± 0.06	10.9 ± 0.45	−14.7 ± 0.45	−7.4 ± 0.20	−13.5 ± 0.66
Module III	0.0	0.0	0.0	12.1 ± 0.14	4.5 ± 0.07	6.1 ± 0.09	12.1 ± 0.14	4.5 ± 0.07	6.1 ± 0.09
Module IV	0.0	0.0	0.0	7.5 ± 0.14	2.5 ± 0.14	5.0 ± 0.20	7.5 ± 0.14	2.5 ± 0.14	5.0 ± 0.20
Module V	11.3 ± 0.28	4.0 ± 0.29	9.3 ± 0.15	23.1 ± 0.11	7.4 ± 0.32	20.0 ± 0.63	11.8 ± 0.37	3.4 ± 0.09	10.8 ± 0.07
Total	65.4 ± 1.01	23.3 ± 0.65	55.3 ± 0.83	63.6 ± 0.14	19.4 ± 0.70	53.4 ± 1.45	−1.8 ± 0.24	−3.8 ± 0.21	−0.9 ± 0.11
Nutrient demand met from the system							95.1%	82.0	96.0%

IOFS, integrated organic farming system; Module I, cereals and oilseeds (maize and soybean); Module II, horticultural crops (vegetables, fruits, and spices); Module III, dairy; Module IV, piggery and poultry; Module V, others (green manuring crop, fodder, etc.).

TABLE 6 On-farm nutrient supply balance sheet in the IOFS model of Mrs. Skola Kurbah (after 5 years).

IOFS modules	Nutrient requirement (kg)			On-farm nutrient recycled (kg)			Nutrient balance (kg)		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Module I	28.7 ± 0.40	9.3 ± 0.43	22.3 ± 0.36	5.7 ± 0.18	3.3 ± 0.07	11.4 ± 0.39	−23 ± 0.57	−6.04 ± 0.46	−10.94 ± 0.74
Module II	29.8 ± 0.47	12.6 ± 0.22	24.8 ± 0.26	12.0 ± 0.16	3.2 ± 0.14	8.3 ± 0.08	−17.8 ± 0.58	−9.34 ± 0.34	−16.48 ± 0.30
Module III	–	–	–	–	–	–	–	–	–
Module IV	0.0	0.0	0.0	14.5 ± 0.20	4.5 ± 0.15	9.0 ± 0.16	14.48 ± 0.20	4.46 ± 0.15	8.98 ± 0.16
Module V	14.0 ± 0.19	4.3 ± 0.13	9.9 ± 0.18	22.9 ± 0.36	6.9 ± 0.20	20.1 ± 0.34	8.9 ± 0.53	2.6 ± 0.09	10.2 ± 0.52
Total	72.5 ± 0.84	26.2 ± 0.74	57.0 ± 0.72	55.1 ± 0.81	17.9 ± 0.48	48.7 ± 0.94	−17.46 ± 1.64	−8.32 ± 1.22	−8.24 ± 1.60
Nutrient demand met from the system							76.0%	68.6%	85.5%

IOFS, integrated organic farming system; Module I, cereals and oilseeds (maize and soybean); Module II, horticultural crops (vegetables, fruits, and spices); Module III, dairy; Module IV, piggery and poultry; Module V, others (green manuring crop, fodder, etc.).

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as it had a faster growth rate, high tolerance, was easy to handle, could be raised at a high density, and was associated with high production per square unit. Moreover, the *Jalkund* facilitated crop diversification (legumes, vegetables, spices, etc.) and increased net income to Rs. 43,074 per annum by increasing the yield (26.9%), with a B:C ratio of 2.1 (Lepcha et al., 2018). The rate of return from the farming system integrated with pisciculture, in which fish were reared organically by recycling the byproducts of dairy, poultry, and duckery, was Rs. 5.46/rupee invested (Behera et al., 2010). The performance of the IOFS components was 11.1%, 75%, and 16.8% better for dairy, poultry, and pigs, respectively, than that with conventional practices (Lepcha et al., 2018). The integration of poultry and livestock with a conventional farming system increases crop productivity, thereby making the enterprise more profitable and increasing farmers' income (Ali and Shivalingaiah, 2022). Apart from the increased income, farmers consume a variety of nutritional vegetables and fruits, milk, and eggs throughout the year, which leads to nutritional security.

4.2. The impact of water harvesting and the use of water in an IOFS

The availability of fresh water per person in the Himalayan area was estimated as 1,473 m^3 $year^{-1}$, 1,757 m^3 $year^{-1}$, and 18,417 m^3 $year^{-1}$ for the Ganges, Indus, and Brahmaputra basins, respectively, while for India as a whole, the average fresh water per capita is 2,214 m^3 $year^{-1}$ (Das et al., 2018). The per capita availability of water is decreasing day by day. Although the per hectare and per capita fresh water availability in the NER is the highest in India, $<$5% of the available water is being tapped for use. The success of a farming practice is dependent on the availability of irrigation water, which can be harvested in a farm pond or *Jalkund*s. These structures play a significant role in crop and vegetable production, providing drinking water for livestock, and kitchen gardening, thus increasing crop productivity, farmers' income, and employment (Chowdhury et al., 2020). Moreover, the harvested water can be used for duckery and pisciculture practices (Patel L. C. et al., 2014). The IOFS model has been shown to be climatically resilient, particularly in sustaining crops and livestock during the lean period through the development of a water harvesting unit (*Jalkund*), which operates by storing the excess water during the rainy season and then supplying the stored water during the dry season. The improved integrated farming system, including the integration of different farm enterprises in tribal population regions, can potentially be more productive, achieve greater net returns, improve nutritional value, and increase employment more than a conventional farming system in Manipur (Ansari et al., 2013). In monocropping systems, employment opportunities for farmers and laborers are limited and seasonal. In an IOFS system, labor is needed for year-round diversified farm activities (crop cultivation, livestock rearing, etc.), which has a high potential for increasing employment. One such IFS model has increased employment by 434 man days in a year for 1.0 ha over the traditional monocropping system on hills in the eastern Himalayas (Das et al., 2021). The IOFS model enables the farmer to generate income and produce from various components at various seasons of the year. This system reduces the dependence on one specific component and minimizes the overall loss of the system in case one component fails to perform (Panwar et al., 2018).

4.3. Residue recycling and nutrient balance in an IOFS

Year-round feed, fodder, labor, manure, and water are needed for a successful and sustainable IFS/IOFS model in any particular region (Das et al., 2021). Crop residue management, such as soil surface retention, has a positive impact on soil health (Mishra and Nayak, 2004; Turmel et al., 2015; Ansari et al., 2022a). The regular addition of residue as organic input maintains the soil organic matter (SOM) level for better soil health (FAO, 2011). The quantity and quality of crop residues generated within a farm and their efficient utilization influence soil fertility build-up over time and its subsequent release of nutrients to the crops that follow (Jarvis et al., 1996; Panwar et al., 2021a; Ansari et al., 2022c). The main strengths of an IOFS lie in better resource recycling as an organic farm mainly relies on internal resources and restricts or limits the input of external materials (Nemecek et al., 2011; Panwar et al., 2020). In an organic farming system, the application of crop residue based-vermicompost to the soil is biologically better than the direct application of manure or crop residue (Aynehband et al., 2017; Mukherjee et al., 2023). The integration of poultry or ducks, by virtue of creating artificial structures over farm ponds or in pond dikes, and the cultivation of year-round high value vegetables using water from *Jalkunds* or ponds can increase system productivity by up to 750% and income by 850% (Babu et al., 2019). The transfer of vermiculture technology was highly successful and widely adopted by the farmers of the village. The farmers were happy due to the growing demand for worms from other groups and they were convinced of the superiority of the farm produce due to the use of compost in their own fields. Soil fertility is degraded with the continuous use of synthetic agro-inputs, which impacts sustainable agriculture. An increase in soil organic matter content and soil microbial activity are indicators of crop and livestock productivity (Biswas et al., 2014). The adoption of IOFS also enhances soil fertility by maintaining biodiversity (Mader et al., 2002; Panwar et al., 2021b). An IOFS model includes all types of crop production and soil management systems without disturbing environmental factors and only uses organic inputs. The major fundamental differences between the management of conventional and integrated organic systems are that while conventional agriculture mostly relies on short-term solutions, such as the application of a readily available nutrient, e.g., synthetic fertilizer, IOFS mostly relies on long-term solutions at the systems level, e.g., nutrient cycling and conservation. Meta-analysis results showed that the organic farming system has a higher SOM content with a minimal loss of soil nutrients and increased soil organic and inorganic carbon sequestration (Foereid and Hogh-Jensen, 2004; Tuomisto et al., 2012). One important principle of an integrated farming system is to reduce the dependence on external inputs, especially nutrients, to sustain the model in the long run (emphasis was placed on establishing the IOFS model in the villages by increasing residue recycling and the preparation of quality compost in a compost pit or through vermicomposting) (Das et al., 2019). Livestock components, such as dairy and pigs, generate enough animal manure, and the efficient recycling of crop and weed biomass helps to generate sufficient nutrients within the system. Organic inputs in the form of FYM, compost, and vermicompost have the potential to increase the macronutrient (N, P, and K) content of the soil and act as a store house of various soil nutrients and as a soil conditioner, unlike the inorganic fertilizers, which only supply major nutrients (Mishra and Nayak, 2004). Through the efficient recycling of farm and kitchen wastes and vermicomposting, nutrient requirements can be reduced substantially in the near future. There is enough scope to increase the nutrient supply from the model by intercropping with a legume, using biofertilizers, efficiently collecting poultry manure, and adopting vermicomposting.

4.4. Integration of an IOFS model

The integration of different enterprises within the IOFS and efficient recycling of the resources may be the causes for the increase in productivity and income (Layek et al., 2019). It can be assumed that with the certification of organic products, farmers' income from the IOFS models will be increased further. Organic certification is recommended for a strict closed cycle restricting external farm inputs and achieving a standard farm production system (Reganold and Wachter, 2016). The models took some time to operate at their full potential, and once the model was established, gross and net returns increased, particularly from the second or third year (Das et al., 2019). Moreover, the introduction of a premium price for certified organic produce can increase the profitability of organic produce, i.e., 22 to 35% more profitable than the current price and a significant improvement in the B:C ratio of 20 to 24% compared with conventional farming (Crowder and Reganold, 2015). Even though crop productivity in the organic farming system was reduced by 9.2%, the farmers' net profit increased by 22.0% due to the 20–40% higher premium price for the certified organic produce (Ramesh et al., 2010).

As there is a need to employ labor on a daily basis to maintain the livestock and a need to supply costly animal feed, such as rice bran and oil cake the variable cost increased (Panwar et al., 2018). The introduction of dairy components significantly increased the gross and net income of the farmers by providing milk, with less dependence on outside feed and fodder (Panwar et al., 2018). High quality dairy production depends on feedstuff or fodder of high nutritive value, with high protein content and roughage, and that can be easily processed (Rahmann and Bohm, 2005). The cultivation of fodder in the model supplies a good amount of forage to support the cattle, especially during the lean period between November and March.

5. Constraints on the adoption of the IOFS model

Major constraints on the adoption of the IOFS models by resource-poor farmers in large areas include: (i) limited availability of quality organic pesticides; (ii) high cost of seeds of improved varieties of vegetables, such as cabbage, tomato, and cauliflower; (iii) unavailability of quality manure in sufficient quantities; and (iv) high feed cost for pigs and poultry. As the marketing for organic produce and demand in the state is still not high, farmers are not being paid premium prices for their produce. However, with the efficient management of different enterprises within the system, such as the cultivation of fodder and multi-purpose trees, efficient recycling of resources for quality manure production, and organic certification to obtain a premium price, IOFS technologies may become very popular among farming communities.

6. Conclusions

The efficient use of byproducts or waste product from one enterprise as the input of others is the major principle underpinning a farming system that significantly reduces the demand for external inputs. This study successfully demonstrated that organic agriculture in farmers' fields significantly increases the average productivity of different agricultural and horticultural crops and livestock compared with the conventional system used previously. The adoption of *Jalkund*- or pond-based IOFS with demand-based location-specific scientific integration of agricultural and horticultural crops, cattle, pigs, poultry, fish, etc., increased system productivity, production efficiency, and the net returns of farmers compared with traditional practices, such as rice monocropping or rice followed by a vegetable. On a 1-ha area basis, the net returns from the IOFS models were Rs. 172,944 per year for Mr. Jrill Makroh and Rs. 148,105 per year for Mrs. Skola Kurbah, which were significantly higher than those of their fellow farmers who only practiced crop-based farming. Efficient recycling of available farm resources by small and marginal farmers in the IOFS models through vermicomposting/composting with animal excreta, weed biomass, tree leaves, kitchen wastes, etc., fulfilled most of the nutritional requirement (76.0 to 95.1% of N, 68.6 to 82% of P, and 85.5 to 96.0% of K) and sustained the overall productivity of the farm. Extensive efforts should be made to transfer this IOFS technology to larger farm communities practicing organic agriculture to fulfill the demand for organic inputs within the farm and improve the livelihood of poor rural households.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the participant/patient(s) for the publication of this article.

Author contributions

JL: conceptualization, methodology, investigation, monitoring, data curation, and writing of the original and final draft. AD:

monitoring, data curation, and review and editing. MAA: data analysis, writing of the original and final draft, and review and editing. VM, NR, and AP: review and editing and project administration. KR, SP, PB, TR, SH, SD, MHA, and BP: review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Development of organic nutrients management system for profitable and soil-supportive French bean (*Phaseolus vulgaris* L.) farming in North Eastern Himalayas, India

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French bean (*Phaseolus vulgaris* L.) cultivation faces multipronged challenges of low farm productivity, poor economic returns, and soil health deterioration in the hilly ecosystem of India. Hence, the development of a cost-effective and soil-supportive French bean cultivation technology is highly warranted. Thus, a field experiment was conducted for two consecutive seasons in the Sikkim region of the Indian Himalayas to assess the impact of different organic nutrient sources on the production potential, profitability, and soil health of French bean. Eight organic nutrient management practices, *viz.,* farmers' practice, 100% recommended dose of nitrogen (RDN) through FYM, 100% RDN through mixed compost (MC), 100% RDN through vermicompost (VC), 50% RDN through FYM + 50% RDN through MC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through MC + 50% RDN through VC, and 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, were assigned in a three times replicated randomized complete block design. The results revealed that the supply of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC 33% recorded the highest pod yield (8.30 and 8.00 Mg ha^{-1}) and net returns (1,831 and 1,718 US$ ha^{-1}). Furthermore, the supply of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC 33% also had a positive impact on soil health. It was shown that an equal supply of RDN through FYM + MC + VC increases soil pH by 8.35%, SOC by 5.45%, available N by 6.32%, available P by 16%, available K by 9.92%, and micronutrients by 5–7% over farmers' practice. Thus, the supply of RDN through the integration of FYM + MC + VC in equal proportion is an economically robust and soil-supportive nutrients management practice for organic French bean production in the hilly ecosystem of North East India.

KEYWORDS

economic returns, organic farming, productivity, soil enzymes, vegetable

1. Introduction

Researchers and policy makers around the world are contending to achieve food and nutritional security along with environmental sustainability, particularly in hill and mountain ecoregions (Babu et al., 2020a). Soil quality deterioration contributes to an ever-widening loop of insufficient food production (Yadav et al., 2021a). To ensure food, nutritional, soil, and environmental security, contemporary production systems must move to eco-friendly production systems that combine a low ecological footprint with the production of more crops/commodities. Organic farming is a sustainable production approach that has less negative impacts on the environment, soil health, and energy consumption (Reganold and Wachter, 2016; Singh et al., 2021a). However, some researchers have observed lower crop productivity under organic farming than in the conventional farming system (De Ponti et al., 2012; Babu et al., 2023a), Yet the magnitudes of yield reduction mainly depend on the types and numbers of crops grown, agronomic management practices adopted, and soil and climatic conditions (Avasthe et al., 2020). The organic production system has good production potential to contribute to sustainable ecosystem services through better soil health (Singh et al., 2021b).

The major challenge in organic farming is the unavailability of quality organic nutrient sources for profitable crop production (Yadav et al., 2013a). Therefore, adequate nutrient supply is crucial for efficient crop production under organic farming (Babu et al., 2020b; Das et al., 2020). A satisfactory and consistent supply of nutrients to crops, from sowing to harvesting, is indispensable for better economic yield (Yadav et al., 2013b). Nutrient release and crop demand synchronization are critical in organic management conditions; thus, a complete understanding of nutrient release patterns from organic sources is critical to minimizing nutrient stresses (Babu et al., 2020b). Thus, the development and implementation of efficient organic fertilization protocols are pivotal for efficient organic crop production and to improve yield and income as well as overall soil health improvement (Saikia et al., 2018; Babu et al., 2020a).

The Indian Himalayan region is spread across the 13 Indian States/Union Territories (Jammu and Kashmir, Ladakh, Uttarakhand, Himachal Pradesh, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura, Assam and West Bengal) and harbors ∼50 million people. The northeastern hill regions (NEHR) of India cover 26.23 Mha of the total geographical area of India. Agriculture in the NEHR is subsistence in nature and organic by default (Yadav et al., 2018). However, there is a huge amount of nutrient loss through runoff and leaching during the *Kharif* season, which creates a multinutrient deficiency in the soil (Singh et al., 2021a; Yadav et al., 2021b). The region is bestowed with abundant biomass due to its congenial environmental conditions, hence making it suitable for organic farming. In realizing the potential of organic farming in the region, the Government of India has launched the Central Sector Scheme Mission Organic Value Chain Development in the North Eastern Region (MOVCDNER) as a component of the 12th plan to promote organic farming in the region. Sikkim was named the first fully certified organic state in India by the Indian Government in 2016.

French bean (*Phaseolus vulgaris* L.) is a globally important leguminous crop and a rich source of dietary protein, vitamins, and different polyphenol compounds (Datt et al., 2013). It is one of the best vegetable crop for higher altitudes to cope with climate change (Babu et al., 2020d; Kumar et al., 2020; Singh et al., 2021b). In the Himalayan region, the French bean is a highly productive crop that responds well to inputs and has a high potential for intensive cropping systems (Gudade et al., 2022). However, the productivity and profitability of French bean is quite low under current agronomic management conditions mainly due to high intensity of weeds and poor nutrient management practices. Hence, adequate nutrient management is highly warranted to increase French bean productivity and profitability without deteriorating soil health.

Weeds generate huge quantities of biomass, which cannot be used as livestock feed due to their obnoxious nature. However, this nutrient-rich weed biomass can be used to make high-value organic manure. Co-composting of weed biomass with cow dung can potentially increase the crop yield and soil health and minimize the weed pressure in cropland (Singh et al., 2021b). Supplying the entire nutrient demand through a single organic source is exceedingly difficult under organic farming conditions (Yadav et al., 2013b). Hence, applying mixed compost developed through the co-composting of two or more nutrient sources in an integrated manner may reduce the production costs, increase crop productivity and profitability, and improve soil health (Singh et al., 2016). Several researchers have reported higher crop yield and better soil health with integrated organic nutrient management under different ecologies in various crops (Partha Sarathi et al., 2011; Yadav et al., 2013a; Das et al., 2017). However, these studies cannot be replicated in Sikkim region of Indian Himalayas due to variations in soil, climate, and management practices. Very little or no work in the Sikkim region of the Indian Himalayas has been performed so far on a comparative assessment of different organic sources on productivity, profitability, and soil health in acidic soils in French bean. Hence, it was hypothesized that the supply of the recommended nitrogen dose through the different organic sources in an integrated fashion will sustainably improve French bean productivity and profitability per unit of investment and soil health buildup. Keeping this in view, the present research work was undertaken with the following objectives: (1) To evaluate the effect of the integration of organic nutrient sources on the productivity and profitability of French bean, and (2) to study the effect of the supply of the recommended dose of nitrogen through different organic sources on soil health.

2. Materials and methods

2.1. Experimental site and climate

The field experiment was conducted for two consecutive seasons (2016 and 2017) at the research farm of ICAR Research Complex Sikkim Centre, Tadong, Sikkim. The research field was located at 1,300 m amsl at latitude 27°33′N and longitude 88°62′E (Figure 1). The climate of the study site was monsoonal with three distinct seasons, marked by a total annual rainfall of 2,996.9 mm. The average maximum temperature (29.1°C) of the study site was registered in August, while the average minimum temperature (7°C) was registered in January. The maximum relative humidity (91.7%) was recorded in July and the minimum in January (34.3%).

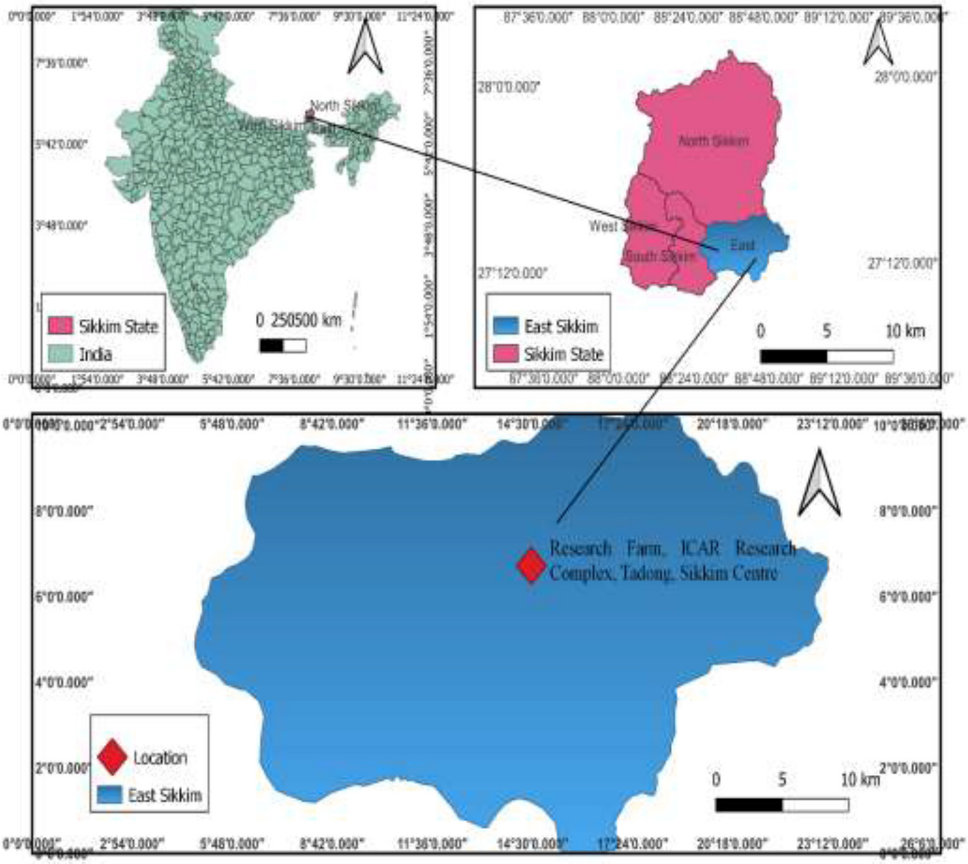

FIGURE 1
Study site in Eastern Himalayan region.

Before the commencement of the field experiment, the soil was sampled up to 0–15 cm depth for analysis of its physico-chemical and biological properties. The *Haplumbrept* soil of the study site was acidic (pH 5.82), with a high SOC content (1.71%), available N (364 kg ha^{-1}), P (17.4 kg ha^{-1}), K (223 kg ha^{-1}), Fe (6.42 mg kg^{-1}), Mn (7.96 mg kg^{-1}), Zn (1.22 mg kg^{-1}). The soil biological activities (0–15 cm depth) comprised SMBC (294 µg g^{-1} soil), DHA (20.9 µg TPF g^{-1} soil h^{-1}), acid phosphates activities (17.6 µg PNP g^{-1} soil h^{-1}), FDA (6.7 µg FDA g^{-1} soil h^{-1}), ureases (95 µg NH_4-N g^{-1}soil h^{-1}), and β-glucosidase activities (60.2 µg PNP g^{-1} soil h^{-1}).

2.2. Experimental design and crop management

Eight organic nutrient management practices, *viz.*, farmers' practice (FYM ∼50% RDN), 100% RDN through FYM, 100% RDN through mixed compost (MC), 100% RDN through vermicompost (VC), 50% RDN through FYM + 50% RDN through MC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through MC + 50% RDN through VC, and 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, were tested in a three times replicated randomized block design (RBD). French bean "SKR-57A" was sown manually at a seed rate of 100 kg ha^{-1} with a geometry of 30 cm × 10 cm during the second fortnight of September during both years. Thinning and gap-filling were performed 10 days after sowing (DAS) to maintain the optimum plant population. To provide an ideal weed-free environment for the crop, two hand weeding was performed at 20–25 and 40–45 DAS. Nutrient management was conducted as per the treatments. The recommended nitrogen (N) was @ 80 kg N ha^{-1}. The average nutrient composition (N, P and K) of the different manures are given in Supplementary Table 1. Irrespective of the fertility treatments, dolomite was applied @ 2 Mg ha^{-1} 10 days before the sowing of the crop for neutralizing the soil pH. Similarly, for control of red ant infestation, drenching with phytoneem @ 5 ml L^{-1} of water was performed. Meanwhile, the pod borer and aphids were managed by foliar application of Spinosad 45% EC @ 0.5 ml L^{-1}. Life saving irrigation was given during both years as and when required.

2.3. Preparation of mixed compost

Four predominant weed flora (*Artemisia vulgaris, Eupatorium odoratum, Ageratum conyzoides, and Galinsoga parviflora*) were collected from nearby areas. The collected weeds were heaped for a week, chopped with an electrically operated chaff cutter into 1–1.5 cm sizes, mixed thoroughly, and then stacked by spreading on the stone tiled floor. Fresh cow dung was collected from the dairy unit of the research farm. Thereafter, chopped weed biomass and cow dung were alternately placed in a 1-m-deep pit. The cow dung and weed biomass were mixed properly by adding a proper amount of water and then covered by a polythene sheet for decomposition. The heap was opened for heat release and aeration 48 h later. When the temperature and moisture became stationary below 45°C and 25%, respectively, the entire mass was kept for curing for about 20–25 days. Next, after 120–130 days, the matured co-composts were collected, processed, and stored for further chemical analysis and use in the field.

2.4. Yield measurement and financial analysis

Five plants were randomly selected and tagged from each plot to record the growth and yield-attributing parameters. The French bean green pods were ready for harvesting at 65–70 DAS. Thereafter, 2–3 pickings were performed at 8–10 days intervals. At each harvest, the green pod yield from the net plot was weighed separately and expressed as Mg ha^{-1}. Financial budgeting is the ultimate tool to judge the performance of any designed technology. The production system must be economically efficient for its wider adaptability. In the present investigation, the economic feasibility of different nutrient management systems was assessed in terms of the gross returns, net returns, benefit–cost ratio, and profitability. The cost of cultivation was computed based on the prevailing market prices of the inputs during the respective crop season. The gross returns were the monetary value of the output in terms of US dollar (US$ ha^{-1}). Meanwhile, the net returns were obtained by subtracting the cost of cultivation from the gross returns, and the return per US$ invested was obtained by dividing the gross returns by the cost of cultivation. The economics of the different treatments was calculated by using the following formulae:

$$\text{Gross return (US \$ha}^{-1}\text{)} = \text{Monetary return of pod yield (US \$ha}^{-1}\text{)} + \text{stover yield (US \$ha}^{-1}\text{)}.$$

$$\text{Net return (US \$ha}^{-1}\text{)} = \text{Gross return (US \$ha}^{-1}\text{)} - \text{Total cost of cultivation (US \$ha}^{-1}\text{)}.$$

$$\text{Return per US\$ invested} = \frac{\text{Gross returns (US\$ ha}^{-1}\text{)}}{\text{Cost of cultivation (US\$ ha}^{-1}\text{)}}.$$

$$\textit{Profitability}\ (\textit{US\$ ha}^{-1}\ \textit{day}^{-1}) = \frac{\textit{Net returns (US\$ ha}^{-1}\textit{)}}{\textit{Crop period (days)}}.$$

$$\text{Production efficiency}\left(\text{kg ha}^{-1}\ \text{day}^{-1}\right) = \frac{\text{Pod yield of French bean (kg ha}^{-1}\text{)}}{\text{Crop period (days)}}.$$

2.5. Soil sampling

After completion of the experiment, the soil was sampled with a screw augur from the plow layer (0–15 cm depth) from each plot. Samples were collected from three places in each plot, mixed, and made into the composite soil sample. The collected samples were stored in zip-top plastic bags for carrying to the laboratory. After removing the visible pieces of crop residue and roots, the soil samples were air-dried and sieved through 2 mm sieve. Part of the representative soil samples were kept at 4°C for microbial analysis.

2.6. Analysis of physical properties of soil

The bulk density (pb) of the surface (0–15 cm) soil was determined by the core sampler (Piper, 1950) from three randomly chosen points of each plot. The procedure for determining the pb was followed according to that described by Chopra and Kanwar (1991). Aggregate stability was measured using wet sieving technique (Haynes, 1993). The results were expressed as the mean weight diameter (MWD), which is the sum of the fraction of soil remaining on each sieve after sieving for the standard time multiplied by the mean weight diameter of the adjacent sieve aperture, that is, the mean weight diameter (MWD) = (fraction of sample on sieve × mean inter sieve aperture). The upper and lower limits of the mean weight diameter, in this case, covered 6 and 1.15 mm, respectively.

2.7. Analysis of chemical properties of soil

The pH was determined in the soil water (1:2.5) suspension at 25°C using a glass electrode pH meter after equilibrating for 30 mins (Jackson, 1973). Soil organic carbon (SOC) was determined by the wet oxidation method (Walkley and Black, 1934). The available N, P, and K were estimated by the methods outlined by Prasad et al. (2006) and expressed in kg ha^{-1}. The diethylene tetra-amine penta-acetic acid (DTPA) extraction method was used to determine the available Fe, Mn, and Zn (Lindsay and Norwell, 1978).

2.8. Analysis of soil biological properties

Soil microbial activity, expressed as fluorescein diacetate hydrolysis (FDA) was determined (Green et al., 2006). Acid phosphomonoesterase activity were determined by the p-nitrophenol release by use of analog substrate methods (Tabatabai, 1994). The β-glucosidase activity was determined by the method described by Eivazi and Tabatabai (1988). Soil urease activity was assayed by the method of Tabatabai (1994). Soil microbial biomass carbon (SMBC) was estimated following the chloroform fumigation–extraction method (Vance et al., 1987). Dehydrogenase activity was estimated by monitoring the rate of production of tri-phenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC), which was used as an electron acceptor (Klein et al., 1971).

2.9. Principal component analysis

To detect the effect of different organic sources of nutrients on yield attributes and soil properties, a principal component analysis (PCA) was conducted using the biplot method in Matlab R2019b version 9.7 (Math Works Inc., USA). An orthogonal set of novel orthogonal variables titled (PC) was assigned to significant information extracted from the data using PCA, which is a multivariate analytical tool that analyzes data through numerous interrelated quantitative dependent variables and shows the configuration of the relationship between the observations and the variables. The components of the PCs with high eigenvalues are best suited for identifying variation in the system, retaining only the components with eigenvalues $\geq$1.

2.10. Statistical analysis

In this study, all the data were analyzed by using the general linear model procedures within the Statistical Analysis System 9.3 software package (SAS Institute, Cary, NC). The comparison of treatment means was performed using the least significant difference (LSD) test at $p < 0.05$. The significance of the treatment effects was evaluated by using an F-test, and the significance of differences was assessed by calculating the least significant difference (LSD) using the formula given below:

$$LSD = \sqrt{2EMS \times t \text{ at a } 5\% \text{ level of significance}}/n.$$

Where MSE = mean square error, n = the number of observations of that factor for which LSD is to be calculated, and t = value of percentage point of "t" distribution for error degrees of freedom at a 5% level of significance.

3. Results

3.1. Growth parameters

Significantly higher plant height (40.9 cm and 41.3 cm) was recorded under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC as compared to farmers' practice and 100% RDN through FYM but it remained at par with other treatments. Integrated supply of the recommended nitrogen dose in equal proportion through the application of FYM + MC + VC increased the plant height by 25.6% (2-year mean) as compared to farmers' practice (Table 1). During the first year, significantly less days to 50% flowering (34 days) was observed in farmers' practice followed by 50% RDN through MC + 50% RDN through VC, 50% RDN through FYM + 50% RDN through VC, and 33% RDN through FYM + 33% RDN through MC + 50% RDN through VC, while during the second year, less days to 50% flowering (35 days) was observed in farmers' practice, which was statistically at par with 50% RDN through MC + 50% RDN through VC, 50% RDN through MC + 50% RDN through VC and 33% RDN through FYM +33% RDN through MC + 33% RDN through VC and significantly higher than other treatments, respectively. Based on two years of study, farmers' practice recorded significantly less days to 50% flowering (34.3 days) followed by 50% RDN through FYM + 50% RDN through MC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through MC + 50% RDN through VC, and 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC as compared to other treatments, respectively.

3.2. Yield attributes and crop productivity

During the first year, a significant maximum number of branches plant^{-1} (5.30) was observed with the supply of RDN through farmyard manure (FYM) + mixed compost (MC) + vermicompost (VC) in equal proportion, followed by 100% RDN through FYM (Table 1). However, during the second year, a significantly higher branches plant^{-1} (5.37) was registered under 100% RDN through MC as compared to the other treatments, except for 100% RDN through VC and 50% RDN through FYM + 50% RDN through MC. The integrated supply of RDN through FYM 33% + MC 33% + VC 33% recorded a significantly higher number of branches plant^{-1} (5.34) (2 years mean), followed by 100% RDN through VC and 50% RDN through FYM + 50% RDN through MC. A significantly higher number of pods plant^{-1} (8.90 and 9.03, respectively) was recorded under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, as compared to other treatments, but remained statistically at par with 100% RDN through VC during both the years of study. The maximum pod weight (11.5 g) was attained under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, which was statistically at par with 100% RDN through VC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through MC + 50% RDN through VC5, and 100% RDN through FYM and significantly higher than other treatments during the first year. Meanwhile, during the second year, a significantly higher pod weight (11.8 g) was noticed under 33% RDN through FYM +33% RDN through MC + 33% RDN through VC as compared to other treatments, respectively. On a 2 years mean basis, the maximum pod weight (11.7 g) was noticed under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, which was statistically at par with 100% RDN through VC and 50% RDN through FYM + 50% RDN through VC and significantly higher than other treatments, respectively. Across the study, the fresh pod yield of French bean under different organic sources of nutrients varied from 3.67 to 8.30 Mg ha^{-1}. The supply of RDN through FYM + MC + VC in equal proportion recorded a significantly higher fresh pod yield (8.30 and 8.00 Mg ha^{-1}, respectively), which was statistically at par with 100% RDN through VC and significantly higher than other treatments, respectively (Table 1). During both years of the study, the lowest fresh pod yield (3.93 and 3.67 Mg ha^{-1}, respectively) was recorded under farmers' practice.

3.3. Economic returns

The plot that received 100% RDN through VC incurred the maximum production cost as compared to the organic nutrients applied through other sources; the increase was 5.7–37.3% higher (2 years mean basis) (Figure 2). On the contrary, the plot that

TABLE 1 Effects of conjoint application of organic sources of nutrients on growth, yield attributes, and yield of French bean crop.

Treatment	Plant height at 60 DAS (cm)			Days to 50% flowering			Branches plant^{-1}			Pods plant^{-1}			Pod length (cm)			Pod weight (g)			Fresh pod yield (Mg ha^{-1})		
	2016	2017	Pooled mean	2016	2017	Pooled mean	2016	2017	Pooled mean	2016	2017	Pooled mean	2016	2017	Pooled mean	2016	2017	Pooled mean	2016	2017	Pooled mean
Farmers' practice	33.0	32.3	32.7	33.9	34.6	34.3	4.10	4.17	4.14	5.93	5.73	5.83	8.47	8.20	8.34	8.60	8.63	8.62	3.93	3.67	3.80
100% RDN through FYM	36.8	37.3	37.1	37.7	38.3	38.0	4.77	4.87	4.82	7.43	6.90	7.17	9.70	9.73	9.72	10.7	10.3	10.5	7.43	7.13	7.28
100% RDN through MC	39.0	39.8	39.4	35.3	36.3	35.8	5.20	5.23	5.22	7.97	8.27	8.12	9.30	9.27	9.29	10.6	10.5	10.6	6.53	6.27	6.40
100% RDN through VC	39.0	38.4	38.7	37.1	37.9	37.5	5.10	5.17	5.14	8.70	8.60	8.65	9.90	9.97	9.94	11.0	10.8	10.9	8.10	7.83	7.97
50% RDN through FYM + 50% RDN through MC	40.1	40.9	40.5	35.8	36.1	36.0	4.97	5.07	5.02	7.63	7.87	7.75	9.63	9.83	9.73	10.6	10.5	10.6	6.97	7.07	7.02
50% RDN through FYM + 50% RDN through VC	39.9	40.6	40.3	34.9	35.6	35.3	4.73	4.87	4.80	7.53	7.60	7.57	9.63	9.87	9.75	10.8	10.8	10.8	7.53	7.20	7.37
50% RDN through MC + 50% RDN through VC	40.5	41.2	40.9	34.1	35.0	34.6	4.70	4.77	4.74	8.00	7.83	7.92	9.37	8.93	9.15	10.7	10.5	10.6	7.03	6.90	6.97
33% RDN through FYM + 33% RDN through MC + 33% RDN through VC	40.9	41.3	41.1	36.4	37.2	36.8	5.30	5.37	5.34	8.90	9.03	8.97	10.6	10.3	10.5	11.5	11.8	11.7	8.30	8.00	8.15
SEm±	1.18	1.36	1.27	0.45	0.81	0.63	0.18	0.14	0.16	0.21	0.27	0.24	0.27	0.30	0.29	0.29	0.32	0.31	0.22	0.20	0.21
LSD ($p = 0.05$)	3.57	4.13	3.85	1.35	2.45	1.90	0.55	0.43	0.49	0.61	0.75	0.68	NS	NS	NS	0.88	0.91	0.90	0.67	0.63	0.65

FYM, farmyard manure; MC, mixed compost; VC, vermicompost; RDN, recommended dose of nitrogen.

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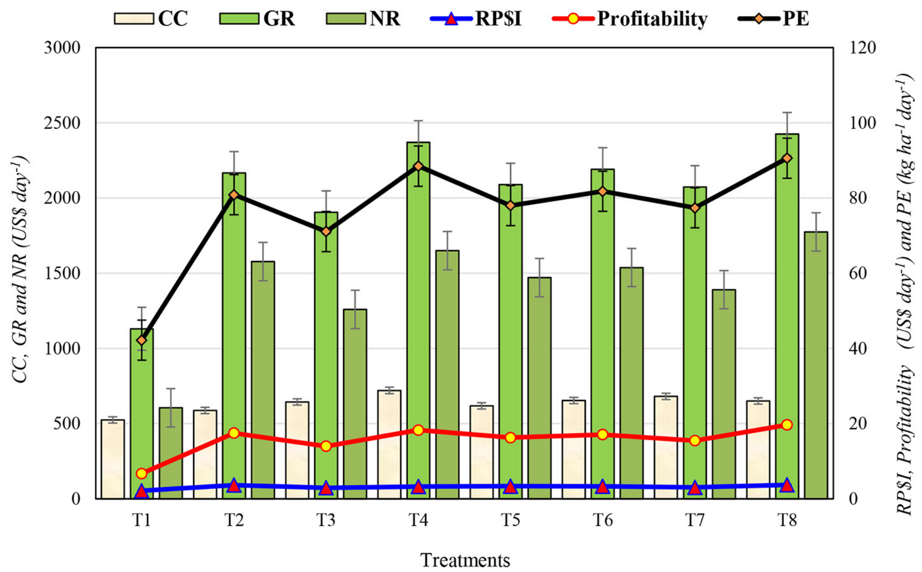

FIGURE 2
Effect of the conjoint application of different organic sources of nutrients on profitability of French bean crop (2 years pooled mean value). CC, cost of cultivation; GR, gross return; NR, net return; RP$I, return per US$ invested; PE, production efficiency; T1—farmers' practice (FYM ~50% RDN); T2—100% RDN through FYM; T3—100% RDN through mixed compost (MC); T4—100% RDN through vermicompost (VC); T5—50% RDN through FYM + 50% RDN through MC; T6—50% RDN through FYM + 50% RDN through VC; T7—50% RDN through MC + 50% RDN through VC; T8—33% RDN through FYM + 33% RDN through MC + 33% RDN through VC.

received ~50% RDN through FYM (farmer's practices) had the least cost of cultivation (528–522 US$ ha^{-1}) during both the years. Gross returns from different organic sources of nutrients varied between 1,131 and 2,426 US$ ha^{-1}. The integrated supply of RDN through FYM + MC + VC in equal proportion (33% + 33% + 33%) recorded significantly higher gross returns (2,485 US$ $ha^{-1}$and 2,366 US$ ha^{-1}), followed by 100% RDN through VC (2,426 US$ ha^{-1} and 2,316 US$ $ha^{-1)}$ and 50% RDN through FYM + 50% RDN through VC (2,255 US$ $ha^{-1}$and 2129US$ $ha^{-1)}$, respectively. Similarly, the application of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC recorded maximum net returns (1,831 US$ ha^{-1}); however, it was statistically at par with 100% RDN through VC, 50% RDN through FYM + 50% RDN through VC, and 100% RDN through FYM and remained significantly higher than other treatments in the first year. During the second year, significant maximum net returns (1,718 US$ ha^{-1}) were registered under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC. The return per US$ invested in different organic sources of nutrients varied from 2.08 to 3.80 across the study year (Figure 2). However, during the first year, a significant maximum return per US$ invested (3.80) was noticed under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC followed by 100% RDN through FYM. Meanwhile, during the second year, the highest return per US$ invested (3.65) was observed under the same application of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, which was statistically at par with 100% RDN through FYM and 50% RDN through FYM + 50% RDN through VC and significantly higher than rest of the treatments, respectively. Across the study year the conjoint application of organic sources of nutrients had a significant effect on the profitability of the French bean crop. During both the years, the application of 33% RDN through FYM + 33% RDN through MC +33% RDN through VC recorded significantly higher profitability (20.3 US$ ha^{-1} day^{-1} and 19.1 US$ ha^{-1} day^{-1}), followed by 100% RDN through VC. Concerning production efficiency, the application of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC recorded the maximum production efficiency (92.2 kg ha^{-1} day^{-1}); however, it remained statistically at par with 100% RDN through VC but significantly superior over the treatments. Similarly, during the second year, a significantly higher production efficiency of 88.9 kg ha^{-1} day^{-1} was recorded under the 33% RDN through FYM + 33% RDN through MC +33% RDN through VC, followed by 100% RDN through VC.

3.4. Soil health

The application of different organic sources of nutrients had failed to affect the soil pb significantly after two years of cropping. Soil aggregation was measured and expressed in terms of the mean weight diameter (MWD), soil aggregates (>0.25 and <0.25 mm), and total water-stable aggregates (TWSA), which were influenced significantly ($p < 0.05$) by the conjoint application of the organic sources of nutrients (Figure 3). The application of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC recorded the significantly highest amount of the percentage of >0.25 mm soil aggregates (38.6%) at 0–0.15 m soil depth, followed by 100% RDN through VC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through FYM + 50% RDN through MC, 50% RDN through MC + 50% RDN through VC, and 100% RDN through FYM. The significantly highest value of the percentage of <0.25 mm soil aggregates (49.1%) and TWSA (87.7%) at 0–0.15 m

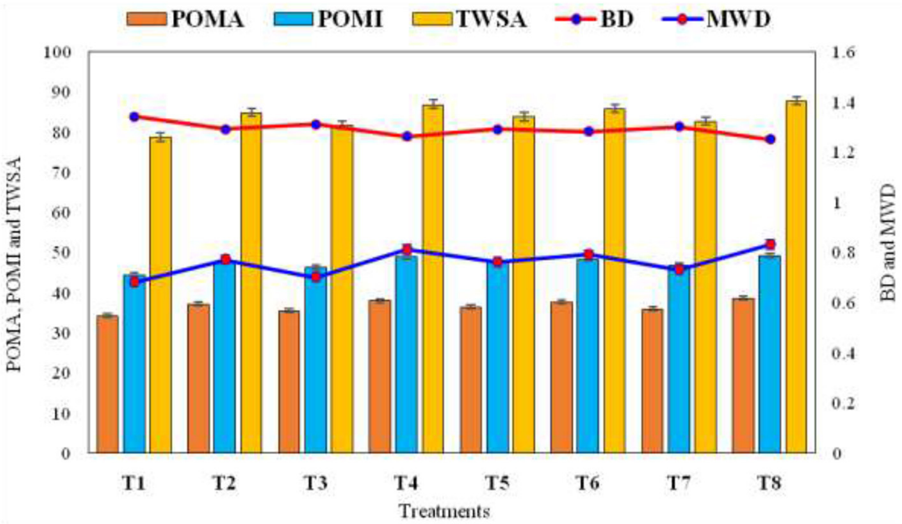

FIGURE 3
Effect of the conjoint application of different organic sources of nutrients on the physical properties of the soil after completion of the two years cropping system. POMA, percentage of macroaggregates (>0.25 mm); POMI, percentage of microaggregates (<0.25 mm); TWSA, total water-stable aggregates; BD, bulk density (Mg m^{-3}); MWD, mean weight diameter (mm); T1—farmers' practice (FYM ∼50% RDN); T2—100% RDN through FYM; T3—100% RDN through mixed compost (MC); T4—100% RDN through vermicompost (VC); T5—50% RDN through FYM + 50% RDN through MC; T6—50% RDN through FYM + 50% RDN through VC; T7—50% RDN through MC + 50% RDN through VC; T8—33% RDN through FYM + 33% RDN through MC + 33% RDN through VC.

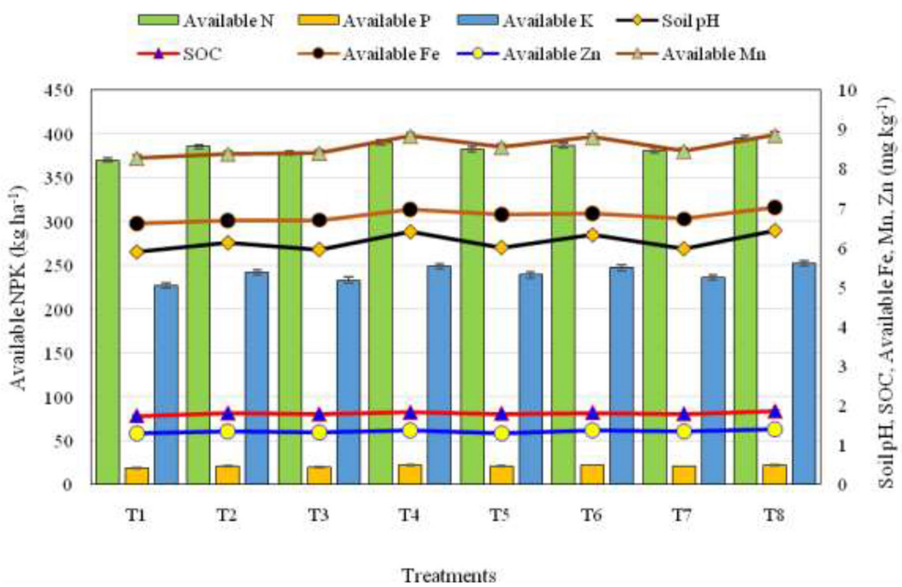

FIGURE 4
Effect of the conjoint application of different organic sources of nutrients on the chemical properties of the soil after completion of the two years cropping system. SOC, soil organic carbon (%); T1—farmers' practice (FYM ∼ 50% RDN); T2—100% RDN through FYM; T3—100% RDN through mixed compost (MC); T4—100% RDN through vermicompost (VC); T5—50% RDN through FYM + 50% RDN through MC; T6—50% RDN through FYM + 50% RDN through VC; T7—50% RDN through MC + 50% RDN through VC; T8—33% RDN through FYM + 33% RDN through MC + 33% RDN through VC.

soil depth was noticed under FYM 33% + MC 33% + VC 33% RDN, followed by 100% RDN through VC, as compared to farmers' practice. The maximum MWD (0.83 mm) was noticed under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, which was statistically at par with 100% RDN through VC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through FYM + 50% RDN through MC, and 100% RDN through FYM and significantly higher than other treatments, respectively.

Continuous application of different organic sources for two years had a significant effect on the soil pH (Figure 4). Significantly

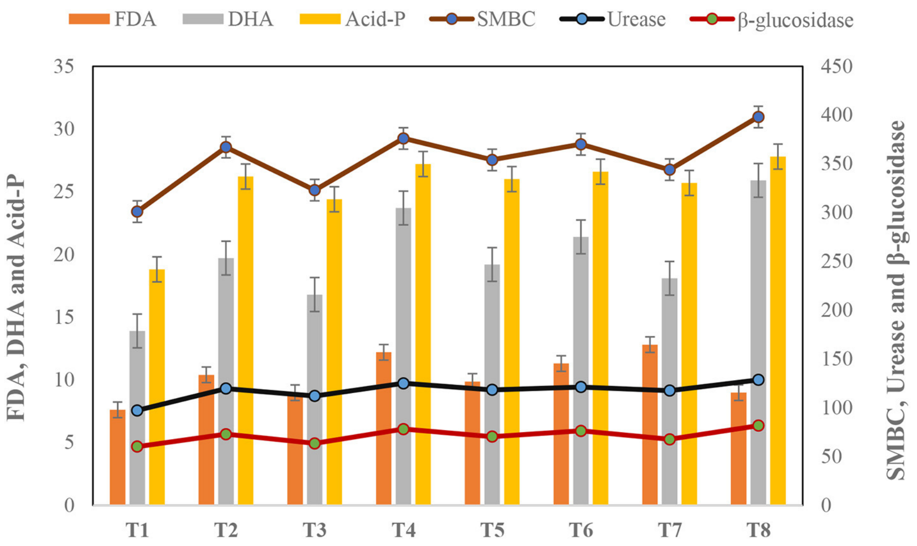

FIGURE 5
Effect of the conjoint application of different organic sources of nutrients on the biological properties of the soil after completion of the two years cropping system. SMBC, soil microbial biomass carbon (μg g^{-1} soil); FDA, mg kg^{-1} soil h^{-1}; DHA, μg TPF g^{-1} soil h^{-1}; Acid-P, μg PNP g^{-1} soil h^{-1}; Urease, μg NH_4-N g^{-1}soil h^{-1}; β-glucosidase, μg PNP g^{-1} soil h^{-1}; T1—farmers' practice (FYM ~50% RDN); T2—100% RDN through FYM; T3—100% RDN through mixed compost (MC); T4—100% RDN through vermicompost (VC); T5—50% RDN through FYM + 50% RDN through MC; T6—50% RDN through FYM + 50% RDN through VC; T7—50% RDN through MC + 50% RDN through VC; T8—33% RDN through FYM + 33% RDN through MC + 33% RDN through VC.

higher soil pH (6.43) and SOC was registered under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC. The integration of different organic manures had a significant effect on the available N, P, and K in the soil at 0–15 cm soil depth after the end of the two cropping seasons (Figure 4). Significantly higher available N (395 kg ha^{-1}) in the soil was noticed under the integrated supply of RDN through FYM + MC + VC in equal proportion, followed by 100% RDN through VC, 50% RDN through FYM + 50% RDN through VC, 50% RDN through FYM + 50% RDN through MC, and 100% RDN through FYM, respectively. Similarly, significantly higher available P (22.3 kg ha^{-1}) and available K (252 kg ha^{-1}) were also observed under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC.

The integrated use of organic manures had a significant impact on soil micronutrient availability. Significantly higher amounts of available Fe (7.03 mg kg^{-1}), available Zn (1.40 mg kg^{-1}), and available Mn (8.85 mg kg^{-1}) were recorded under 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC, followed by 100% RDN through VC and 50% RDN through FYM + 50% RDN through VC, respectively. Furthermore, the application of different organic sources of nutrients in integration had a significant effect on SMBC, FDA, DHA, acid-P, urease, and β-glucosidase in the soil at 0–15 cm soil depth after the end of two years of French bean cultivation (Figure 5). A conjoint supply of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC exhibited significantly higher SMBC (398 μg g^{-1} soil) acid-P (27.8 μg PNP g^{-1} soil h^{-1}) in the soil, followed by 100% RDN through VC and 50% RDN through FYM + 50% RDN through VC, respectively.

3.5. Correlation analysis

A Pearson's correlation analysis was conducted between the soil's physical, chemical, and biological properties to predict the relationship and dependency between the variables. The results indicated that all the soil parameters showed the existence of a highly significant ($p < 0.01$) positive correlation among them. However, the pb exceptionally exhibited a highly significant ($p < 0.01$) negative correlation with the rest of the parameters under investigation, indicating a desirable soil character required for an ideal soil physical condition (Table 2).

3.6. Principal component analysis

In our study, the PCA exercised on the tested parameters explained up to 98.4% of total variability, thereby extracting two principal components, PC1 and PC2, which explained 71.8% and 26.6% of the total variability with an eigenvalue >1. The biplot (Table 3, Figure 5) generated from principal component analysis clearly showed that the available N, SOC, K, and P had a strong loading on PC1 (Table 3, Figure 6), while PC2 exhibited comparatively greater loadings on acid-P and soil pH, respectively.

TABLE 2 Correlation matrix between soil physical, chemical, and biological properties.

	BD	POMA	POMI	TWSA	MWD	Soil pH	SOC	Available N	Available P	Available K	Available Fe	Available Zn	Available Mn	SMBC	FDA	DHA	Acid-P	Urease	β-glucosidase
BD	1.000	−0.979	−0.976	−0.983	−0.971	−0.909	−0.955	−0.933	−0.995	−0.957	−0.920	−0.974	−0.958	−0.977	−0.971	−0.991	−0.961	−0.967	−0.972
POMA	−0.979	1.000	0.979	0.995	0.987	0.888	0.911	0.932	0.986	0.963	0.935	0.961	0.954	0.973	0.957	0.977	0.959	0.959	0.979
POMI	−0.976	0.979	1.000	0.995	0.992	0.923	0.941	0.950	0.982	0.982	0.963	0.990	0.990	0.976	0.983	0.987	0.935	0.926	0.999
TWSA	−0.983	0.995	0.995	1.000	0.995	0.910	0.931	0.947	0.990	0.978	0.955	0.981	0.977	0.980	0.975	0.987	0.952	0.947	0.994
MWD	−0.971	0.987	0.992	0.995	1.000	0.895	0.902	0.956	0.974	0.962	0.947	0.985	0.983	0.971	0.970	0.973	0.949	0.939	0.994
Soil pH	−0.909	0.888	0.923	0.910	0.895	1.000	0.891	0.780	0.917	0.964	0.957	0.937	0.939	0.924	0.948	0.947	0.768	0.782	0.910
SOC	−0.955	0.911	0.941	0.931	0.902	0.891	1.000	0.883	0.962	0.943	0.903	0.929	0.910	0.945	0.949	0.965	0.900	0.901	0.933
Available N	−0.933	0.932	0.950	0.947	0.956	0.780	0.883	1.000	0.924	0.885	0.840	0.941	0.924	0.897	0.901	0.916	0.964	0.947	0.950
Available P	−0.995	0.986	0.982	0.990	0.974	0.917	0.962	0.924	1.000	0.974	0.943	0.972	0.959	0.986	0.978	0.996	0.954	0.958	0.979
Available K	−0.957	0.963	0.982	0.978	0.962	0.964	0.943	0.885	0.974	1.000	0.981	0.970	0.970	0.970	0.977	0.986	0.878	0.880	0.976
Available Fe	−0.920	0.935	0.963	0.955	0.947	0.957	0.903	0.840	0.943	0.981	1.000	0.956	0.970	0.969	0.978	0.959	0.832	0.822	0.964
Available Zn	−0.974	0.961	0.990	0.981	0.985	0.937	0.929	0.941	0.972	0.970	0.956	1.000	0.993	0.974	0.988	0.983	0.919	0.914	0.987
Available Mn	−0.958	0.954	0.990	0.977	0.983	0.939	0.910	0.924	0.959	0.970	0.970	0.993	1.000	0.967	0.983	0.973	0.895	0.883	0.989
SMBC	−0.977	0.973	0.976	0.980	0.971	0.924	0.945	0.897	0.986	0.970	0.969	0.974	0.967	1.000	0.993	0.986	0.927	0.924	0.978
FDA	−0.971	0.957	0.983	0.975	0.970	0.948	0.949	0.901	0.978	0.977	0.978	0.988	0.983	0.993	1.000	0.988	0.904	0.899	0.982
DHA	−0.991	0.977	0.987	0.987	0.973	0.947	0.965	0.916	0.996	0.986	0.959	0.983	0.973	0.986	0.988	1.000	0.929	0.933	0.982
Acid-P	−0.961	0.959	0.935	0.952	0.949	0.768	0.900	0.964	0.954	0.878	0.832	0.919	0.895	0.927	0.904	0.929	1.000	0.994	0.938
Urease	−0.967	0.959	0.926	0.947	0.939	0.782	0.901	0.947	0.958	0.880	0.822	0.914	0.883	0.924	0.899	0.933	0.994	1.000	0.925
β-glucosidase	−0.972	0.979	0.999	0.994	0.994	0.910	0.933	0.950	0.979	0.976	0.964	0.987	0.989	0.978	0.982	0.982	0.938	0.925	1.000

POMA, percentage of macroaggregates (>0.25 mm); POMI, percentage of microaggregates (<0.25 mm); TWSA, total water-stable aggregates; BD, bulk density (Mg m^{-3}); MWD, mean weight diameter (mm); SMBC, soil microbial biomass carbon (µg g^{-1} soil); FDA, mg kg^{-1} soil h^{-1}; DHA, µg TPF g^{-1} soil h^{-1}; Acid-P, µg PNP g^{-1} soil h^{-1}; Urease, µg NH_4-N g^{-1}soil h^{-1}; β-glucosidase, µg PNP g^{-1} soil h^{-1}.

TABLE 3 Principal component analysis of physical, chemical, and biological properties of soil (after completion of two years).

Principal components		PC1	PC2
Initial Eigenvalues	Total	13.64	5.07
	% of Variance	71.80	26.66
	Cumulative %	71.80	98.46
Extraction sums of squared loadings	Total	13.64	5.07
	% of Variance	71.80	26.66
	Cumulative %	71.80	98.46
Factor loadings[a]			
Eigen vectors[b]		PC1	PC2
BD		0.794	−0.607
POMA		0.698	0.178
POMI		0.683	0.170
TWSA		0.796	0.440
MWD		0.711	−0.584
Soil pH		0.864	0.603
SOC		0.983	−0.588
Available N		0.998	0.179
Available P		0.973	−0.003
Available K		0.980	0.525
Available Fe		0.806	−0.421
Available Zn		0.817	−0.576
Available Mn		0.798	−0.440
SMBC		0.979	0.108
FDA		0.813	−0.580
DHA		0.783	0.156
Acid-P		0.709	0.675
Urease		0.972	0.161
β-glucosidase		0.707	0.409

POMA, percentage of macroaggregates (>0.25 mm); POMI, percentage of microaggregates (<0.25 mm); TWSA, total water-stable aggregates; BD, bulk density (Mg m^{-3}); MWD, mean weight diameter (mm); SMBC, soil microbial biomass carbon (μg g^{-1} soil); FDA, mg kg^{-1} soil h^{-1}; DHA, μg TPF g^{-1} soil h^{-1}; Acid-P, μg PNP g^{-1} soil h^{-1}; Urease, μg NH_4-N g^{-1} soil h^{-1}; β-glucosidase, μg PNP g^{-1} soil h^{-1}.

In the Ist quadrant, the higher loading variables were found to be clustered together in a group, and these parameters were highly correlated to each other.

4. Discussion

Crop production capacity mainly depends on the nature of genotypes, climatic and agronomic management practices (Ghani et al., 2022a). French bean is a nutrient-loving short-duration winter crop that requires better nutrient management practices to explore its full potential under organic management conditions (Kumar D. et al., 2015; Singh and Chaudhary, 2016). There are significant opportunities to increase soil fertility, input-use efficiency, and crop productivity by adopting the conjoint application of organic sources of nutrients with field-specific recommendations (Babu et al., 2020c). Organic nutrient management is considered an important activity, as it helps to enhance the growth and productivity of the French bean crop and to improve the physical, chemical, and biological properties of soil (Singh et al., 2018). In French beans, instead of a single source of nutrients, applying different organic sources of nutrients in an integrated manner has been shown to increase crop productivity and improve soil health (Sharma et al., 2014; Singh et al., 2016). The constant supply of nutrients through organic sources into the active nutrients pool of soils developed a vigorous root system, resulting in the better growth and development of plants and better diversion of photosynthates from the source to sink; thus, the combined use of organic sources might be much more advantageous for healthy growth and timely flowering in crops (Aziz et al., 2019; Ghani et al., 2022b). In the present study, the conjoint application of organic sources of nutrients had a significant effect on the plant height and days to 50% flowering. The integrated supply of the recommended nitrogen dose through FYM, MC, and VC (FYM 33% + MC 33% + VC 33% RDN) increased the plant height and duration of days to 50% flowering compared to single source. The integration of vermicompost, FYM, rock phosphate, and *Rhizobium* facilitates the adequate nutrient supply to French bean, resulting in better crop growth (Sharma et al., 2014). In contrast, the significantly poor French bean growth under the suboptimal nutritional treatment may have been because the root system might not have been active for efficient nutrient uptake during the period of active crop growth and development stages, resulting in reduced plant height and the crop reaching an early flowering stage (Guo et al., 2019). Under poor nutrient management conditions, leaf senescence started earlier due to the inadequate nutrient supply to the crops (Yadav et al., 2013b).

The combined application of FYM with vermicompost has been shown to result in 23% more crop yield and better soil health (Saikia et al., 2018). In the present study, the integration of different organic nutrient sources significantly increased the number of branches per plant, pods per plant, pod weight, and fresh pod yield over farmers' practice. The integrated use of FYM + VC gave 5.87% and 22.4% higher yields over the sole application of VC and FYM, respectively (Gulati and Barik, 2011). The conjoint application of organic sources of nutrients helped to provide adequate nutrient availability in the soil for a long time, which resulted in more cell differentiation, meristematic cell division, and translocation of food materials in plants, thereby resulting in a higher production of yield attributes and ultimately more yield (Singh et al., 2018). The combined application of organic sources of nutrients might have resulted in more growth hormones released that helped in the optimum fertilization of flowers and increased pollen grain viability, thereby increasing the pods per plant (Sajid et al., 2011; Singh et al., 2021b). The increase in seed yield under adequate nutrients supply might be ascribed mainly to the combined effect of higher plant height, more dry matter accumulation at different stages, more branches per plant, pods per plant, and higher pod weight, which were the result of the better translocation of photosynthates from

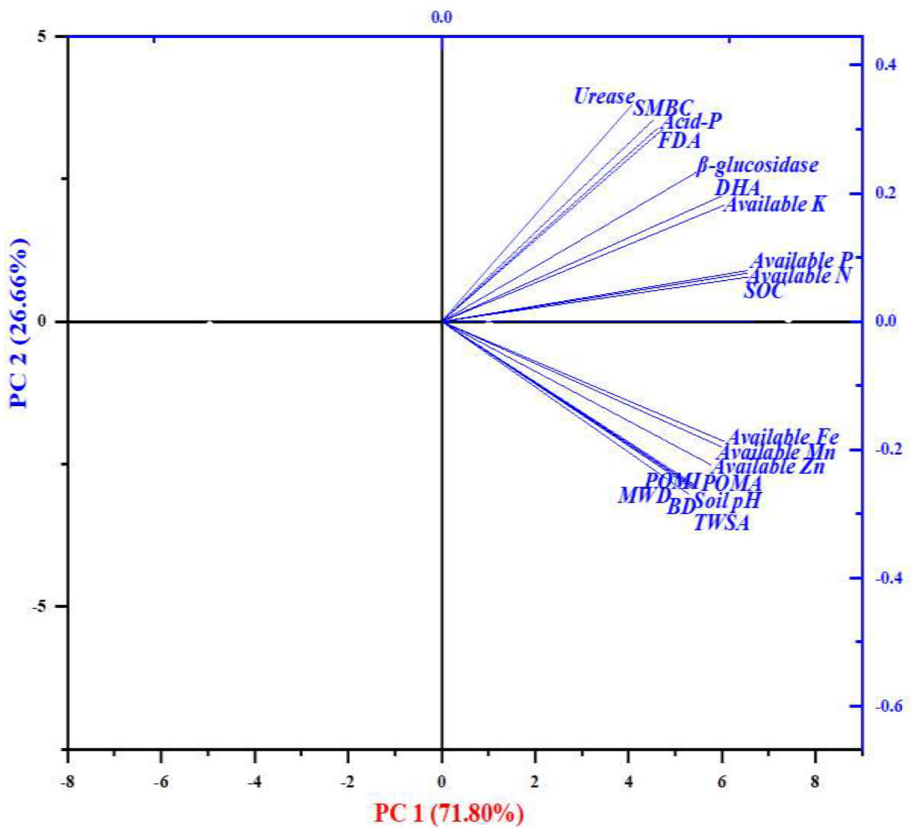

FIGURE 6
PCA of the physical, chemical, and biological properties of the soil. POMA, percentage of macroaggregates (>0.25 mm); POMI, percentage of microaggregates (<0.25 mm); TWSA, total water-stable aggregates; BD, bulk density (Mg m^{-3}); MWD, mean weight diameter (mm); SMBC, soil microbial biomass carbon (µg g^{-1} soil); FDA, mg kg^{-1} soil h^{-1}; DHA, µg TPF g^{-1} soil h^{-1}; Acid-P, µg PNP g^{-1} soil h^{-1}; Urease, µg NH_4-N g^{-1}soil h^{-1}; β-glucosidase, µg PNP g^{-1} soil h^{-1}.

the source to sink, and ultimately pod yield was increased (Singh et al., 2016, 2021a).

In the current study, the application of FYM 50% RDN resulted in the minimum cost of cultivation (525 US$ ha^{-1}) due to less input application, while the application of VC 100% RDN recorded the maximum cost of cultivation. The higher cost of cultivation due to VC was attributed to the higher cost of VC (Babu et al., 2020b). However, the integration of FYM + MC + VC in equal proportion as per the nitrogen content recorded considerably higher gross returns, net returns, and returns per US$ invested (73.5%), meaning it had greater profitability over the other nutrient management options. This may be attributed to the favorable effects of organic sources of nutrients on soil physico-chemical and biological properties, which augment the economic yield (Babu et al., 2023b). Datt et al. (2013) reported that the combined use of FYM + VC recorded 17.2% and 36.6% higher net returns over VC and FYM alone, respectively.

Integrated organic nutrient management helped to improve the soil properties, including those of physical, chemical, and biological nature (Patil et al., 2012). Among the physical properties, the soil pb and soil aggregation are very important components of soil health. In the present investigation, the conjoint application of organic sources of nutrients had a significant effect on the soil aggregation at the end of the two cropping cycles. The integrated supply of the recommended nitrogen dose through FYM 33% + MC 33% + VC 33% increased the soil aggregates >0.25 mm by 11.4%, soil aggregates <0.25 mm by 9.51%, TWSA by 10.4%, and MWD by 18%, as compared to farmers' practice. This might be due to the incorporation of mixed compost in the soil increasing the SOC and aeration that results in lower pb and better soil aggregation (Kumar R. et al., 2015). The application of integrated organic manure and straw has shown a positive effect on the stability of the aggregates in the soil (Singh et al., 2020).

Integrated use of the organic nutrient had a positive impact on the soil pH in acidic soil. The combined use of different organic sources might release several acids and bases, which may slightly modify the soil pH in acidic soil. Organic inputs are an important source of plant nutrients, especially N, and the supply of N from applied manures makes an important contribution to the nitrogen demand of growing crops (Jarvan et al., 2014). The supply of the recommended nitrogen dose through FYM 33% + MC 33% + VC 33% had a significant impact on the SOC available N, available P, available K, and micronutrients (Fe, Zn, and Mn) over farmers' practice. The organic nutrients that fertilized the plots gave better soil health, because organic sources of nutrients help to improve the water regimes, adsorption of nutrients, and soil structure (Babu et al., 2020b). Singh et al. (2018) reported that higher available N, P, and K were found in the plots where cattle dung manure was applied on a nitrogen equivalent basis.

The microbiomes and enzymes present in the soil play an important role in balancing the soil properties, ultimately helping in the overall process of decomposition in the soil system. In the present study, the soil biological properties in the surface layer (0–15 cm) were significantly affected by the conjoint application of organic sources of nutrients after the two cropping cycles. It was demonstrated that the integrated use of different organic sources may provide a constant substrate to the soil microbes, which may increase the soil enzymatic reactions. The integrated supply of nitrogen through FYM 33% + MC 33% + VC 33% considerably improved the SMBC, FDA, DHA, Acid-P, urease, and β-glucosidase over farmers' practice. The application of organic manures in the soil increases the soil's organic carbon content, which is an important source of food for soil microbiomes that results in more microbial population and enzymatic activity (Babu et al., 2020c).

Principal components analysis (PCA) is a statistical tool used to recognize patterns in data and analyze the resemblances and variances between the data (Mishra et al., 2017). In our study, the biplot (Figure 5) generated from the principal component analysis clearly showed that available N, SOC, K, and P had a strong loading on PC1, while PC2 exhibited comparatively greater loadings on acid-P and soil pH, respectively. In the Ist quadrant, the higher loading variables were found to be clustered together in a group, and these parameters were highly correlated with each other.

5. Conclusions

The findings prove the hypothesis that the integration of different organic sources of nutrients enhances French bean growth, productivity, and economic returns as well as soil healthy build-up. The combined use of various organic nutrient sources improves the plant growth, yield-attributing parameters, and fresh pod yield of French bean over farmers' practice. The integrated supply of the recommended dose of nitrogen through FYM 33% + MC 33% + VC 33% increases gross returns, net returns, and return per US$ invested compared to the single source-dependent nitrogen supply. Furthermore, the application of 33% RDN through FYM + 33% RDN through MC + 33% RDN through VC increases soil aggregation, soil pH, SOC, nutrient availability, and soil enzymatic reactions.

Thus, this study has suggested that the supply of 100% of the nitrogen demand of French bean through the integration of FYM + MC + VC in equal proportion (33% each) is economically viable and the best alternative option for profitable organic French bean production and maintaining the soil health in long run in the acidic soils of the Eastern Himalayas.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RS and AK: conceptualization, experimentation, and data analysis. SB: visualization, experimentation, data curation, writing of original and first draft, review, and editing. RA: supervision and project administration. SR and AD: data curation, review, and editing. CS, VS, and IB: review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1115521/full#supplementary-material

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Net effects of pasture-raised poultry on arthropod communities driven by top-down and bottom-up forces in a mixed-cover crop system

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As consumer demand and grower interest for pasture-raised poultry grow, more research is needed to understand the ecological consequences of the integration of pasture-raised poultry on agroecosystems. Poultry could have profound and complex net effects on arthropod communities given their high density per area, broad omnivory, and high manure deposition. Further, some studies suggest poultry may aid in the suppression of agricultural pests in integrated systems. Yet, unlike wild birds, pasture-raised poultry have received little attention in the field of agroecological net effects. Across 2 years, we examined how an absence (control- cover crop only), low- [9.51 m^2 (102.4 $ft.^2$) of pasture per broiler] and high-densities [4.76 m^2 (51.2 $ft.^2$) of pasture per broiler] of broilers impacted cover crop biomass, ground-dwelling arthropods, and plant-dwelling arthropods in a rotationally grazed mixed-cover crop system. High- and low-density poultry treatments had 7.8-fold and 3.5-fold less cover crop biomass compared to the control treatment after 1–3 days of access, respectively. Despite the depletion of cover crops, there were substantial positive effects on ground-dwelling arthropods. Most striking was the impact on house fly larvae where high-density poultry treatments had ∼1,432-fold more house fly larvae relative to the control treatments. Dung beetle, spider, and rove beetle mean relative abundances increased 47-, 2.4-, and 3.5-fold, respectively, from the control treatment to the high-density poultry treatment. In contrast, the mean relative abundances of plant-dwelling arthropod orders Coleoptera, Hemiptera, and Hymenoptera were 4-, 5-, and 3.6-fold higher, respectively, in the control treatment relative to the high-density poultry treatment. Overall, these results suggest that pasture-raised poultry may promote the abundance of ground-dwelling arthropods through bottom-up mechanisms by depositing fecal material. However, poultry decreased the abundance of plant-dwelling arthropods, likely by destroying their habitat and food resources (via consumption and trampling of cover crop) and direct consumption of arthropods. While the integration of poultry into crop rotations is thought to benefit crop yield through nutrient deposition in the form of manure, this study suggests it may also stimulate the soil and ground-foraging arthropod food webs. This study is the first to evaluate the impacts of pastured poultry to arthropod communities in a mixed-cover crop system.

KEYWORDS

pasture-raised poultry, ground-dwelling arthropods, chicken manure, crop-livestock integration, top-down, bottom-up, net effects

1. Introduction

Once common across North America, integrated crop-livestock systems have greatly declined as specialization and agricultural intensification have come to characterize the agricultural landscape over the last century (Dimitri et al., 2005; Naylor et al., 2005; Hilimire, 2011). Prior to the industrialization and specialization of agriculture, the functioning of agroecosystems heavily relied on the complexity, diversity, and synergy conferred by crop-livestock integrated systems to produce food and fiber, fertilize soil, and to power farm machinery (Russelle et al., 2007; Hilimire, 2011). Despite these benefits, crop and livestock systems became ecologically disintegrated and spatially disconnected as specialization was facilitated by the wide availability of manmade inputs such as synthetic fertilizer and the mechanization of farm equipment (Clark, 2004). Further, specialization has been historically incentivized through policies that minimized risks for specific crops, catalyzing farmers to shift from diverse integrated farms to farms consisting of homogenized crops, with the average number of commodities produced by individual farms dropping from five commodities per farm in 1990 to less than two crops per farm in 2002 (Dimitri et al., 2005; Hilimire, 2011).

Recently, however, there has been a renewed interest in integrated crop-livestock systems and the ecological benefits that they may confer to agroecosystems (Hilimire, 2011; Sossidou et al., 2011; Elkhoraibi et al., 2017). In particular, there has been an increased interest in pasture-raised poultry over the last decade, defined by the American Pastured Poultry Producers Association (APPPA) as operations in which poultry have continuous access to pasture and are moved to fresh pasture regularly (Rothrock et al., 2019; American Pastured Poultry Producers Association., 2022). While the number of pasture-raised poultry operations in the United States is not tracked and reported in the Census of Agriculture conducted by the USDA National Agriculture Statistics Service (USDA NASS, 2017), there has been an increase in small to medium-sized poultry operations in the United States within the last decade (USDA NASS, 2007, 2017). This re-emerging interest in pasture-raised poultry is driven by potential ecological benefits such as increased soil quality, control of weeds, and manure deposition as fertilizer as well as prospects of improved meat and egg quality and growing consumer interest alike (Sossidou et al., 2011; Elkhoraibi et al., 2017; Rothrock et al., 2019).

Like wild birds, it is often hypothesized that pasture-raised poultry will exert top-down forces on arthropod communities and have the potential to consume insect pests, though this has rarely been quantified. While there have been studies devoted to investigating the net effects of wild birds on arthropod communities (e.g., Mooney et al., 2010 and references therein), there has been scant attention on investigating the net effects of pasture-raised poultry on arthropod communities in agroecosystems. Studies on the impacts of wild birds in agriculture show that wild birds can impact arthropod communities primarily through two pathways. First, insect-eating birds can consume pest insects in agroecosystems, providing ecosystem services of pest suppression to a given agroecosystem. For example, wild birds provide pest suppression services in coffee by reducing coffee berry borer beetle [*Hypothenemus hampei* (Ferrari)] activity by ∼50% in Costa Rica (Karp et al., 2013). Second, wild birds can act as intraguild predators by consuming natural enemy arthropods, essentially providing an ecosystem disservice by disrupting pest control services provided by insect predators. For example, Grass et al. (2017) found that wild bird activity disrupted biological control of aphid pests in cereal crops when insect-feeding birds consumed natural enemies of aphids. When birds were excluded from the cereal crops, arthropod natural enemy abundance was greater and aphid densities were lower (Grass et al., 2017). Overall, from the literature on the net effects of wild birds on arthropod communities in agroecosystems we can glean that wild birds typically exert top-down forces on arthropod communities (Maas et al., 2013; Díaz-Siefer et al., 2021). Thus, it can be expected that chickens and other insect-eating poultry would interact with arthropod communities in a similar way. Indeed, a small study that investigated the role of free-range chickens and geese as biological control agents of insect and weed pests associated with an intercropping of apples and potatoes found, through dissections of chicken digestive crops, that chickens consumed a variety of insects including beneficial (dung beetles and ground beetles) insects and pest insects such as Japanese Beetles, which were found in 75% of dissected digestive crops (Clark and Gage, 1996). Further, geese were able to maintain weed cover to <10% across the season through consistent grazing. However, chickens or geese did not have an impact on yield (Clark and Gage, 1996).

Yet, there are key differences between wild birds and pasture-raised poultry that may yield differences on their net effects to agroecosystems, especially through bottom-up forces. Pasture-raised poultry are large birds, in high densities, and confined to small areas within agroecosystems, likely concentrating their consumptive effects of arthropods and plants and intensifying their deposition of manure. On the contrary, wild birds are highly mobile and likely to interact with agroecosystems at a landscape scale (Gonthier et al., 2014). Specifically, the prolonged persistence of pasture-raised poultry on the same patch of vegetation may mean that poultry will likely deposit high quantities of feces, and their associated nutrients, to the agroecosystem, likely at much greater densities than wild birds. A typical chicken layer, for example, is estimated to produce 58.97 kg (130 lb.) of fresh manure per year, with a flock of 1,000 hens estimated to produce 58,967 kg (65 tons) of fresh manure per year, though these estimates vary depending on the type of poultry (chickens, turkeys, etc.) and whether the poultry are broilers, layers, breeders, etc. (McCall, 1980; Chastain et al., 2001). Although the literature on the impact of pasture-raised poultry manure deposition on arthropod communities is scarce, studies focusing on how applying animal manure as fertilizer impacts arthropod communities show that such applications can in some instances reduce abundances of insect crop pests (Brown and Tworkoski, 2004) and in some instances increase activity of arthropod generalist predators (Rowen et al., 2019). Thus, in addition to top-down effects of arthropod consumption that are typical for avian predators such as arthropod-eating birds, pastured-raised poultry may exert bottom-up effects through manure deposition. Additionally, in contrast to wild birds, pasture-raised poultry may produce additional top-down effects by reducing plant biomass through vegetative consumption and trampling.

Across 2 years, we investigated how the addition of pasture-raised poultry at varying densities impacted cover crop biomass and the abundances of plant- and ground-dwelling arthropods relative to control plots in an organic mixed-cover crop system. Here we hypothesized that poultry, given their wide diet breadth and their documented role in consuming insects, would exhibit strong top-down effects on cover crop biomass and arthropod communities. We predicted that low- and high-density poultry treatments would have reduced cover crop biomass and decreased arthropod abundances relative to the control treatment. As pastured poultry operations continue to grow across the United States, it remains critical to investigate potential ecological impacts that pastured poultry activity may have on agroecosystems and to evaluate how these impacts may stray from our predictions which are largely based on net effects studies that focus on wild bird activity.

2. Materials and methods

This study was conducted under the approval and guidance of the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky under #2020-3446.

FIGURE 1
Chicken tractor. Chicken tractor with base dimensions of 2.44 m by 2.44 m (8 ft. by 8 ft.).

2.1. Experimental setup

This experiment was conducted in the fall of 2020 and 2021 during the fall poultry integration phase of a larger project that examined various aspects of integrating pasture-raised poultry (as defined by APPPA; see intro) within a vegetable crop rotation system. The basic rotation sequence for this larger experiment was as follows: spring vegetable crop, summer cover crop, fall integrated poultry treatments established on the summer cover crop, and finally winter cover crop. The three integrated rotational treatments were: (1) no poultry, (2) low-density poultry [9.51 m^2 (102.4 $ft.^2$) of pasture per broiler], or (3) high-density [4.76 m^2 (51.2 $ft.^2$) of pasture per broiler] poultry in the fall rotational segments (Supplementary Figure S1). The low-density treatment approximates Pasture Raised Certification standard densities [10.03 m^2 (108 $ft.^2$) of pasture per broiler; Certified Humane® 2014]. Given that this study focused on the short-term ecological impacts of poultry on arthropod communities in the fall of 2020 and 2021, the methodological description will focus on summer cover crop and fall poultry integration phases. For a more detailed description of field preparation throughout the rotational sequence, see the Supplementary material.

The initial experimental setup in March of 2020 utilized two experimental fields [each ~82.3 m (270 ft.) long by 15.24 m (50 ft.) wide] within the certified organic section of the University of Kentucky Horticulture Research Farm. Four blocks of three 9.75 m (32 ft.) by 9.75 m (32 ft.) plots across the two fields (n = 12 plots) were centered and separated by 3.05 m (10 ft.) between each plot. Within each block, each plot was randomly assigned to one of the three fall integrated poultry treatments. In the spring of 2020, before poultry integration began, baseline arthropod sampling was collected.

In the summer of 2020 and 2021, all plots were mowed, drip tape was removed, and fields were spaded and cultivated. On June 10th, 2020, a cover crop mixture [buckwheat 44.83 kg/ha (40 lb./acre), cowpea 44.83 kg/ha (40 lb./acre), and teff 13.45 kg/ha (12 lb./acre)] was drill seeded across all plots. On June 28th of 2021, a cover crop mixture [teff 50.44 kg/ha (45 lb./acre), crimson clover 36.99 kg/ha (33 lb./acre), annual rye grass 35.31 kg/ha (31.5 lb./acre)] was broadcast seeded across all plots. Changes in seeding methods and densities between years were made to better establish a dense cover crop within a short time period and changes to cover crop mixture between years was primarily due to the cowpea being very tall in 2020, making it challenging to move poultry pens (see Supplementary material for details). Summer cover crops were mowed prior to the integration of poultry to facilitate the movement of chicken tractor hoop pens ("chicken tractors" hereafter; Figure 1; Skelton et al., 2012).

2.2. Pastured poultry integration

Poultry were brooded to 3 weeks of age (see Supplementary material for brooder management) before being integrated into experimental plots at the following densities: no poultry (n = 0), low density poultry (n = 10 in 2020, n = 12 in 2021) and high-density poultry (n = 20 in 2020, n = 22 in 2021). The number of chickens in the poultry density treatments differed across years because in 2021 additional poultry were needed for an additional experiment by a colleague. We used the Red Ranger breed in 2020 and the Cornish Rock Cross breed in 2021. The change in breed was made to better reflect the type of pastured-poultry operations in this region in which Cornish Rock Cross are typically used. Within each experimental plot (except the no poultry treatment), poultry were housed in chicken tractors, floorless movable pens that allow chickens to interact with the vegetation that they are placed on while the structures simultaneously provide protection from predators. Chicken tractors were constructed following Skelton et al. (2012). The chicken tractors consisted of a frame base of dimensions 2.44 m x

2.44 m (8 ft. x 8 ft.) with cattle panel looping from one side of the base to the other to give the tractor a hooped structure, and welded wire to exclude predators (Figure 1). Each tractor was equipped with a door, allowing for easy entrance by chicken caretakers. Tarps were placed over each chicken tractor to provide chickens with protection from flying predators and rain. All chicken tractors were moved to the next adjacent position within experimental plots on the same day and received the same time duration within each position. However, the chicken tractors were moved to the next adjacent position every few days for the first 2 weeks of the experiment and roughly every day for the remainder of the experiment (Figure 2).

The perimeter of the entire experimental setup was bordered by an electric fence to prevent entrance from predators. For added protection, each plot containing poultry was enclosed with an additional electric fence. Two solar-powered electric fence chargers were used to power the electric fence, with one charger dedicated to powering the perimeter fence located immediately outside of the experimental field and the other charger located in the non-cover cropped corridor. The same layout was followed for both years of the experiment.

2.3. Cover crop and biomass cover

To measure the effect of pasture-raised poultry on cover crop biomass and percent vegetative cover we collected biomass samples and conducted visual assessments of percent cover of each 2.44 m by 2.44 m (8 ft. by 8 ft.) chicken tractor position. In order to avoid edge effects, we focused biomass sampling efforts to the central 1.22 m^2 (4 $ft.^2$) area within the 2.44 m^2 (8 $ft.^2$) area that had previously been occupied by a chicken tractor (i.e., the dimensions of the base of the chicken tractors). Within this 1.22 m^2 (4 $ft.^2$) area we randomly sampled a 0.305 m^2 (1 $ft.^2$) quadrat of biomass per plot. All vegetation that was rooted within the quadrat was collected, placed in paper bags, and dried in a drying oven for ∼48–72 h or until completely dry. In 2020, there were six biomass samples collected per plot (2020 total = 72 biomass samples) and in 2021 there were nine biomass samples collected per plot (2021 total = 108 biomass samples). To account for spatial and pseudo replication, we averaged biomass samples that were collected from the same plot for each year. Percent vegetative cover was assessed through visual observation and was recorded for each 2.44 m^2 (8 $ft.^2$) area that had previously been occupied by a chicken tractor.

2.4. Arthropod sampling

In order to assess the impact of poultry presence on arthropod communities, we used pitfall traps and sweep-nets to collect ground-dwelling and plant-dwelling arthropods, respectively. Pitfall traps were deployed for seven days, and sweep-net sampling took place during the afternoon on days in which pitfalls were deployed. Pitfall traps consisted of a plastic cup and a removable funnel, with the cup depth being ∼10.8 cm (4.25 in.) (https://www.carolina.com/entomology/pitfall-trap-pk-10/654131.pr). Pitfalls were placed flush with the ground, allowing for arthropods to easily walk or fall into trap. Pitfall solution was 10% NaCl and a few drops of unscented dish detergent to break surface tension, a common solution used across studies that use pitfalls (Hohbein and Conway, 2018). Plastic covers made of plastic disposable plates were secured over each pitfall with landscape staples to prevent rainwater from contaminating traps. Following the first three tractor movements, we immediately deployed three pitfalls in the center of each of the 2.44 m^2 (8 $ft.^2$) spaces that were previously occupied by a chicken tractor in each plot. We repeated this sampling strategy an additional two times per year for a total of three collecting events per year, resulting in a total of nine pitfall samples per plot each year (see Figure 2 for sampling scheme). In total, 216 pitfall traps were deployed across the 2 years. Following pitfall trap collection, the samples were transferred from the NaCl solution to 70% EtOH until samples could be sorted. Sweep-net sampling consisted of 30 sweeps per plot per collecting event using a standard 15-inch diameter sweep-net (BioQuip Products, Rancho Dominguez, CA 90220, USA) as the collector walked up and down a 2.44 m by 7.32 m (8 ft. by 24 ft.) area within the plot. This 2.44 m by 7.32 m (8 ft. by 24 ft.) area is meant to represent an area previously occupied by a chicken tractor across three movements. Sweep-net samples were stored in a standard freezer until sorting and identification could take place. In order to account for spatial and temporal pseudo replication, we aggregated arthropod abundances by averaging abundances from samples collected within the same plot for each year. Additionally, analyses were limited to common taxa found within the collected samples (at least 100 individuals across both years resulting in the exclusion of pseudoscorpions and millipedes from pitfall analysis and the exclusion of spiders, Lepidoptera, and Orthoptera from sweep-net analysis).

2.5. Functional groups

In order to determine whether the addition of poultry to a cover crop system impacted functional arthropod groups we adopted the following guild classifications: natural enemies, known crop pests, predatory hemipterans, and herbivorous hemipterans. The composition of these groups varied by collection method (pitfall vs. sweep net) as these different methods collected different insect types, but functional trait identity remained consistent for insects that were collected by both methods. For pitfall samples, the natural enemies group included spiders (Class: Arachnida; Order: Araneae), ground beetles (Coleoptera: Carabidae), minute pirate bugs (Hemiptera: Anthocoridae), big eyed bugs (Hemiptera: Geocoridae), damsel bugs (Hemiptera: Nabidae), lady beetles (Coleoptera: Coccinellidae), and wasps (including both predators and parasitoids). The sweep net natural enemies group included spiders, minute pirate bugs, big eyed bugs, damsel bugs, lady beetles, soldier beetles (Coleoptera: Cantharidae), predatory stink bugs (Coleoptera: Pentatomidae), and wasps. The known crop pest group for pitfall samples consisted of any identifiable pest of common fruit and vegetable crops, including: cucumber beetles (*Diabrotica undecimpunctata* Mannerheim and *Acalymma vittatum* (Fabricius)), bean leaf beetle (*Cerotoma trifurcata* (Forster)), flea beetles (Coleoptera: Chrysomelidae), aphids (Hemiptera: Aphidae), corn earworm (*Helicoverpa zea*

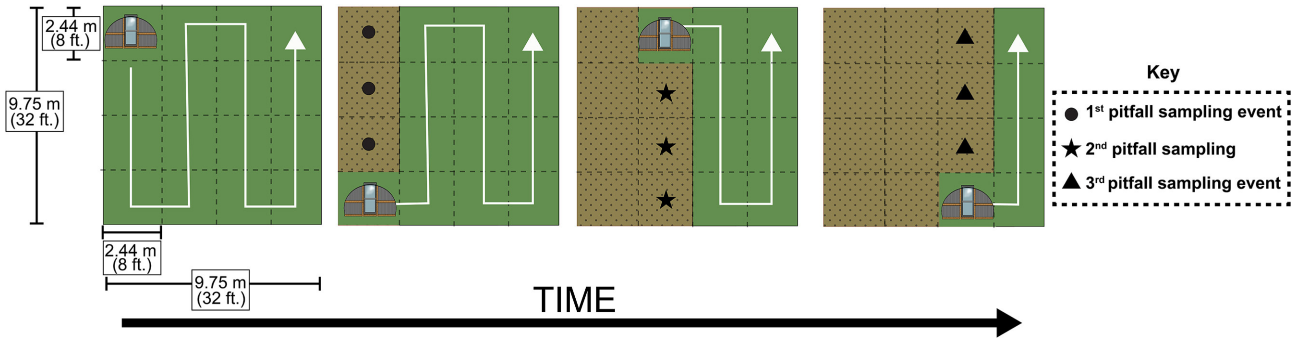

FIGURE 2
Tractor movement and pitfall sampling scheme. Tractor movement across a poultry treatment plot. This graphic depicts the movement of a chicken tractor across the duration of the experiment within a 9.75 m by 9.75 m (32 ft. by 32 ft.) plot. White arrows depict the direction in which the tractors move, with tractors moving into adjacent 2.44 m by 2.44 m (8 ft. by 8 ft.) positions across time. Green spaces depict cover crop that has yet to be reached by chicken tractors while brown stippled spaces are meant to depict spaces that have already been occupied by chicken tractors. Circle, star, and triangle shapes represent the arrangement of pitfall traps across three sampling events following chicken tractor movements.

(Boddie)), weevils (Coleoptera: Curculionidae), green June beetle (*Cotini nitida* (Linnaeus)), pigweed flea beetle (*Disonycha glabrata* (Fabricius)), squash bugs (*Anasa tristis* (De Geer)), armyworms (*Spodoptera* spp.), and the tarnished plant bug (*Lygus lineolaris* (Palisot de Beauvois)). The sweep net samples known crop pest group included cucumber beetle, bean leaf beetle, flea beetles, pigweed flea beetle, aphids, weevils, corn earworm, false chinch bugs (*Nysius* spp.), tarnished plant bug, and non-predaceous stinkbugs. The predatory hemipteran group for pitfall samples consisted of minute pirate bugs, big eyed bugs, and damsel bugs. The predatory hemipteran group for sweep net samples consisted of minute pirate bugs, big eyed bugs, damsel bugs, and predatory stinkbugs. The herbivorous hemipterans group consisted of hemipterans that were not recognized as major agricultural pests including plant hoppers (Hemiptera: Cicadellidae), tree hoppers (Hemiptera: Membracidae), milkweed bugs (Hemiptera: Lygaeidae), and other hemipterans for the pitfall samples and leaf hoppers, tree hoppers, spittle bugs (Hemiptera: Cercopidae), plant bugs (Hemiptera: Miridae), and other hemipterans for sweep net samples.

2.6. Statistical analysis

All analyses were performed in R Studio version 4.2.1 (R Core Team 2022). Linear mixed effects models (LMM) were fit to each of the response variables using the "lmer" function in the package "lme4" with block and year as random effects and poultry density treatment as a fixed effect (Bates et al., 2019). If the residuals of the model were not normal, as tested by the Shapiro-Wilk Test, we applied a log (X+1) or square root transformation to meet assumptions of Gaussian distribution. In order to account for false discovery rates associated with multiple comparisons, we adjusted *p*-values from our models using the function "p.adjust" from the package "stats" with a Holm-Bonferroni correction (R Core Team, 2021). Post hoc tests were performed using the function "emmeans" with a Tukey adjustment from the package "emmeans" to determine significant pairwise comparisons (Lenth et al., 2019). Non-metric multidimensional scaling (NMDS) was used to visualize differences in composition between poultry and control treatments of pitfall and sweep net samples at the order level using the function "metaMDS" with a Bray-Curtis dissimilarity calculation from the "vegan" package (Oksanen et al., 2020). In order to determine whether composition of pitfalls and sweep net samples differed across treatments at the order level we performed permutational multivariate analysis of variance (PERMANOVA) with the "adonis" function from the "vegan" package. Post hoc pairwise comparisons were conducted by using the function "pairwise.adonis" from the package "pairwiseAdonis" and p-values reported from this analysis are adjusted with a Holm-Bonferroni correction (Martinez Arbizu, 2020). As mentioned throughout the methods section, some changes were made between 2020 and 2021. Specifically, seeding method (drill vs. broadcast seeding), cover crop composition, and poultry breed changed across the two experimental years. Given these differences and how they might impact abundance of ground- and plant-dwelling arthropods we ran additional models in which year, treatment, and an interaction between year and treatment were included as fixed effects. Despite all these differences between years, the impact of poultry integration on the arthropod community was relatively consistent (See Supplementary Tables S7–S9 and Supplementary Figures S3–SS5 for model output and graphs).

3. Results

3.1. Cover crop biomass and percent cover

Poultry integration significantly reduced cover crop biomass (g) ($F_{2,20}$ = 51.306, p < 0.001). A Tukey post hoc test (Tukey, 1977) revealed that the high-density poultry treatment (mean ± SE; 3.42 g ± 0.825 g) had less biomass than the low-density poultry treatment (5.83 g ± 0.852 g; p=0.021), and the no poultry control treatment (37.2 g ± 8.74 g; p < 0.001). There was also significantly less biomass in the low-density poultry treatment relative to the control (p < 0.001). Percent cover of cover crops also varied by treatment ($F_{2,21}$ = 254.78, p < 0.001). A Tukey post hoc test revealed that the high-density poultry treatment (14.9 ± 2.73) had significantly lower percent cover relative to the low-density poultry treatment (25.4 ± 3.93; p=0.041), and the control (96.7 ± 0.594; $p < 0.001$). The low-density poultry treatment also had significantly less cover than the control ($p < 0.001$).

3.2. Arthropod abundance

In total, across the 2 years, 52,692 arthropods of varying life stages were captured in the pitfall traps, with the greatest abundances being from the order Diptera (22,756) of which 11,069 were house fly larvae, followed by the order Coleoptera with 12,496 individuals, and order Hymenoptera with 8,500 individuals. A total of 5,507 arthropods were collected from sweep net samples, with the greatest abundances being from the order Diptera with 4,178 individuals, followed by order Hemiptera with 805 individuals, and Hymenoptera with 229 individuals.

Further, prior to the addition of poultry to the experimental field in fall of 2020, arthropod abundances were sampled via sweep-net and pitfall samples, following the collection methods outlined above, during the spring of 2020 when broccoli was in rotation to assess the baseline arthropod community. This was to assess whether there were any pre-existing differences in arthropod abundances in the areas to be used as experimental plots. Analysis of these samples, at the order level, found no differences in relative abundance of either plant-dwelling or ground-dwelling arthropods in the areas that were to be used as experimental plots during the fall poultry rotation See Supplementary Tables S1–S4 for details on the abundances of these baseline samples.

3.3. Ground-dwelling arthropods

Poultry density treatments had a significant effect on the relative abundances of 15 of the 20 insect taxa and functional groups that we examined, with exceptions being centipedes (Class Chilopoda), pill bugs (Order: Isopoda), ground beetles, and herbivorous hemipterans (Table 1).

TABLE 1 LMM results for ground-dwelling arthropods.

	Response variable	*F*-value	*p*-value (adjusted)
(A) Class/Order	Centipede	1.44	0.893
	Coleoptera	8.36	**0.023**
	Dermaptera	10.16	**0.014**
	Diptera	6.95	**0.046**
	Hemiptera	30.18	**<0.001**
	Hymenoptera	15.03	**0.002**
	Isopod	1.64	0.893
	Lepidoptera	6.46	**0.046**
	Orthoptera	7.17	**0.046**
	Psocodea	6.96	**0.046**
	Spider	16.58	**0.002**
(B) Taxon	Ant	14.9	**0.002**
	Ground Beetle	0.26	1
	Dung Beetle	33.26	**<0.001**
	Rove Beetle	6.5	**0.046**
(C) Functional Groups	Herbivorous Hemipterans	0.57	1
	Known Pests	17.51	**0.001**
	Natural Enemies	31.63	**<0.001**
	Predatory Hemipterans	16.41	**0.002**

Linear mixed effects model results for ground-dwelling arthropods. Each row represents a model for a different arthropod (A) class/order, (B) other taxon or (C) functional group as a response variable with treatment as a predictor. Each model included year and block as random effects.
P-values are adjusted with a Holm-Bonferroni correction.
Bold p-values indicate significant effect of treatment.

3.3.1. Orders

At the order level, it was revealed that poultry density treatments had a significant effect on relative abundances of Coleoptera, earwigs (Order: Dermaptera), Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Psocodea, and Spiders (Table 1). The orders Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, and Spiders followed a similar trend in which there were greater abundances of these orders in the high-density poultry treatment relative to control treatment and greater abundance in low-density poultry treatment relative to the control treatment but there was no difference between high-and low-density treatments (Figures 3A–E, I; see Supplementary Table S5 for post hoc pairwise comparisons and associated p-values). The orders Lepidoptera and Psocodea shared the same trend in which the abundance of these orders was greater in high-density poultry treatment relative to control, but there were no differences in abundance between low-density and control treatment or between high-density and low-density treatments (Figures 3F, H and Supplementary Table S5). Orthoptera, in contrast to the other orders, had a greater abundance in the control treatment relative to high-density treatment and greater abundance in control treatment relative to low-density treatment but there was no difference between high-density and low-density treatments (Figure 3G and Supplementary Table S5).

3.3.2. Other taxa

Below the order level, we found that poultry density treatment had a significant effect on the abundance of ants, dung beetles (Coleoptera: Scarabaeidae), house fly larvae, and rove beetles (Coleoptera: Staphylinidae) (Table 1). Ant, house fly larvae, and rove beetle abundances were greater in high-density poultry treatment relative to control and greater in low-density poultry treatment relative to control, but we found no differences in abundance between high-density and low-density poultry treatments (Figures 4A, C, D and Supplementary Table S5). Ant, house fly larvae, and rove beetle abundances were 43.3-, 1432-,

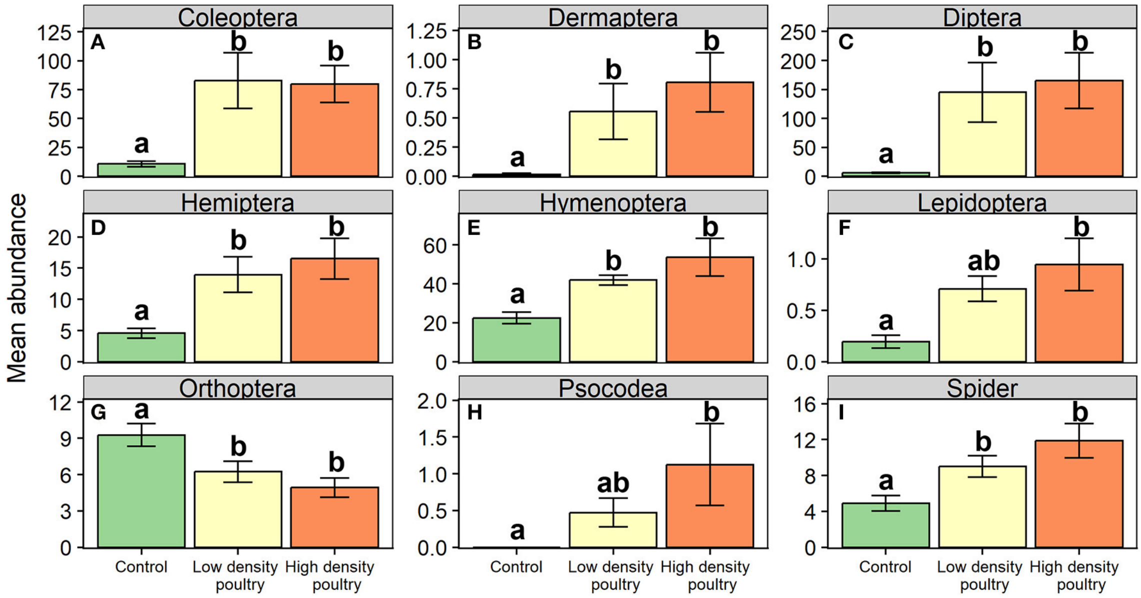

FIGURE 3
Ground-dwelling arthropod orders. Mean abundances of ground-dwelling arthropod orders **(A–I)**. Bars represent standard error bars while letters above bars indicate significant differences ($p < 0.05$) between treatments per Tukey HSD post hoc (see Supplementary Table S5 for Tukey HSD post hoc results).

and 3.5-fold greater in high-density relative to control treatments, respectively. Dung beetle abundance was 43.3 times greater in high-density poultry treatment relative to control treatment and was greater in high-density poultry relative to low-density poultry treatments, but we found no difference between low-density poultry treatment and control treatment (Figure 4B and Supplementary Table S5).

3.3.3. Functional groups

We found that poultry density treatment had a significant effect on the relative abundance of known crop pests, natural enemies, and predatory hemipterans functional groups (Table 1). Known crop pests and predatory hemipterans had greater abundances in high-density poultry relative to control treatment greater abundances in low-density poultry treatment relative and control treatment but we found no difference between high- and low-density poultry treatments (Figures 4E, G and Supplementary Table S5). We found greater abundances of natural enemies in high-density poultry treatment relative to control treatment and relative to low-density poultry treatment, and greater abundances in low-density poultry treatment relative control treatment (Figure 4F and Supplementary Table S5).

3.4. Plant-dwelling arthropods

At the ordinal level, poultry density treatment had a significant effect on the abundance of plant-dwelling Coleoptera, Hemiptera, and Hymenoptera but not Diptera (Table 2). Coleoptera and Hemiptera abundances were greater in the control treatment relative to the high-density treatment and greater in control treatment relative to low-density treatment but there was no difference between high-density and low-density treatments (i, ii in Figure 5A and Supplementary Table S6). Coleoptera and Hemiptera abundances were 4.1- and 5.1-fold greater in the control treatment relative to the high-density poultry treatment, respectively. Hymenoptera had 3.6-fold greater abundance in control treatment relative to high-density and Hymenoptera abundance was greater in control treatment relative to low-density treatment and between low- and high-density poultry treatments (iii in Figure 5A and Supplementary Table S6).

Poultry density treatments had a significant effect on plant-dwelling arthropod abundances of herbivorous hemipteran, known crop pest, natural enemy, and predatory hemipteran functional groups (Table 2). Abundances of herbivorous hemipterans, natural enemies, and predatory hemipterans were greater in control treatment relative to high-density and low-density treatments, but no differences were observed between high- and low-density poultry treatments (i, iii-iv in Figure 5B and Supplementary Table S6). Herbivorous hemipteran, natural enemy, and predatory hemipteran abundances were 3.8-, 8.6-, and 22.9-fold times greater in control treatment relative to high-density poultry treatment, respectively. Known crop pest abundance was 8.1-fold greater in control treatment relative to high-density and was greater in control treatment relative to low-density treatments (ii in Figure 5B and Supplementary Table S6). Further, known crop pest abundance was greater in the low-density poultry treatment relative to the high-density poultry treatment.

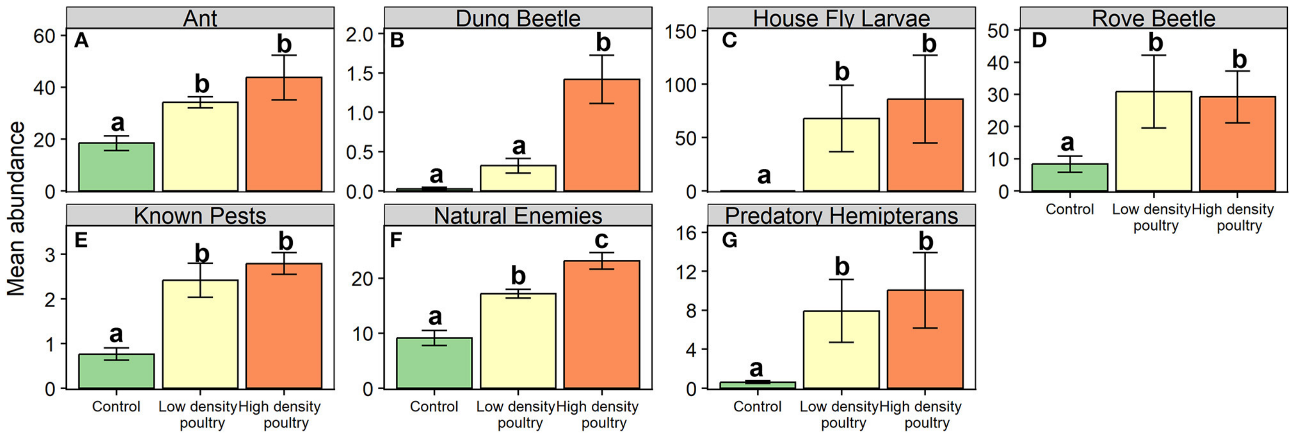

FIGURE 4
Ground-dwelling arthropod taxa of interest and functional groups. Mean abundances of ground-dwelling arthropod taxons **(A–D)** and functional groups **(E–G)**. Bars represent standard error bars while letters above bars indicate significant differences ($p < 0.05$) between treatments per Tukey HSD post hoc test (see Supplementary Table S5 for Tukey HSD post hoc results).

TABLE 2 LMM results for plant-dwelling arthropods.

	Response variable	*F*-value	*p*-value (adjusted)
(A) Orders	Coleoptera	15.91	**<0.001**
	Diptera	1.22	0.32
	Hemiptera	38.45	**<0.001**
	Hymenoptera	27.07	**<0.001**
(B) Functional Groups	Herbivorous Hemipterans	18.83	**<0.001**
	Known Pests	89.19	**<0.001**
	Natural Enemies	36.37	**<0.001**
	Predatory Hemipterans	16.76	**<0.001**

Linear mixed effects models for vegetation-dwelling arthropods. Each row represents a model for a different arthropod (A) order or (B) functional group as a response variable with treatment as a predictor. Each model included year and block as random effects.
P-values are adjusted with a Holm-Bonferroni correction.
Bold p-values indicate significant effect of treatment.

3.5. Community composition

The community composition of ground-dwelling arthropods, at the order level, varied with treatment (F-value $= 11.6$, $R^2 = 0.52$, $p = 0.001$; Figure 6A). A post hoc analysis showed significant pairwise differences between high-density poultry treatment and control treatment ($p = 0.003$) and significant pairwise differences between low-density poultry treatment and control treatment ($p = 0.003$) but not between low-density and high-density treatments ($p = 0.461$). For plant-dwelling arthropods, we found no differences in community composition, at the order level, across treatments (F-value $= 2.07$; $R^2 = 0.16$; $p = 0.091$; Figure 6B).

4. Discussion

This is one of the few studies that has investigated how the addition of poultry to an agroecosystem impacted the abundance and community composition of arthropods. We hypothesized that poultry would exhibit strong negative effects on arthropods given observations that poultry consume arthropods and trample and consume vegetation. Indeed, this study found strong negative impacts of integration on cover crop biomass and percent vegetative cover. However, integration of poultry to a mixed-cover crop system had a positive relationship on the abundance of several ground-dwelling arthropods, suggesting that poultry may promote ground-dwelling arthropod communities. Conversely, we observed a negative relationship between the abundance of several plant-dwelling arthropods and the addition of poultry to a cover-crop system, suggesting that poultry may reduce plant-dwelling arthropod communities. Potential mechanisms for these observed relationships can best be described as bottom-up and top-down effects for ground dwelling-arthropods and plant-dwelling arthropods, respectively (Hunter and Price, 1992).

4.1. Top-down effects

While the main drivers of these relationships remain unknown, it is likely that top-down effects by poultry on plant-dwelling arthropods are driven by chicken activity that results in the destruction of plant-dwelling arthropod habitat (i.e., plant stems and leaves) via trampling and consumption of plant material (Figures 7A–C). Poultry reduced cover crop biomass by 72 and 87% and in the low- and high-density poultry treatments relative to the control, respectively. This phenomenon is in contrast to observed relationships between wild birds and vegetation, as the presence of wild birds has been shown to increase plant biomass in addition to reducing leaf damage and plant morality in

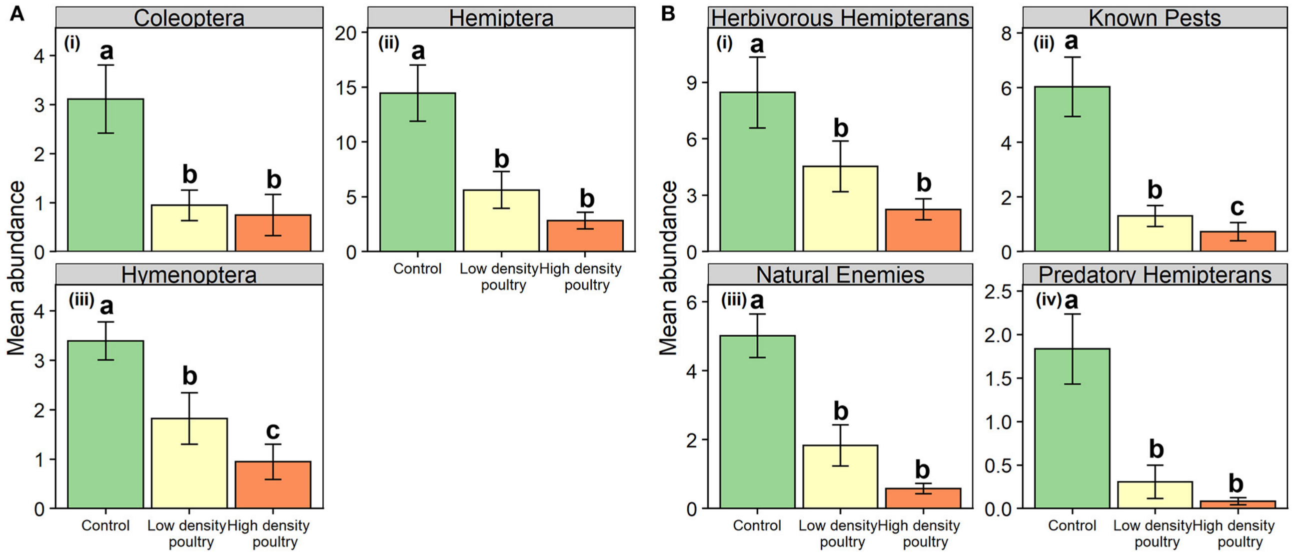

FIGURE 5
Plant dwelling-arthropod orders and functional groups. Mean abundances of plant-dwelling arthropod orders **(A)** and functional groups **(B)**. Bars represent standard error bars while letters above bars indicate significant differences ($p < 0.05$) between treatments per Tukey HSD post hoc (see Supplementary Table S6 for Tukey HSD post hoc results).

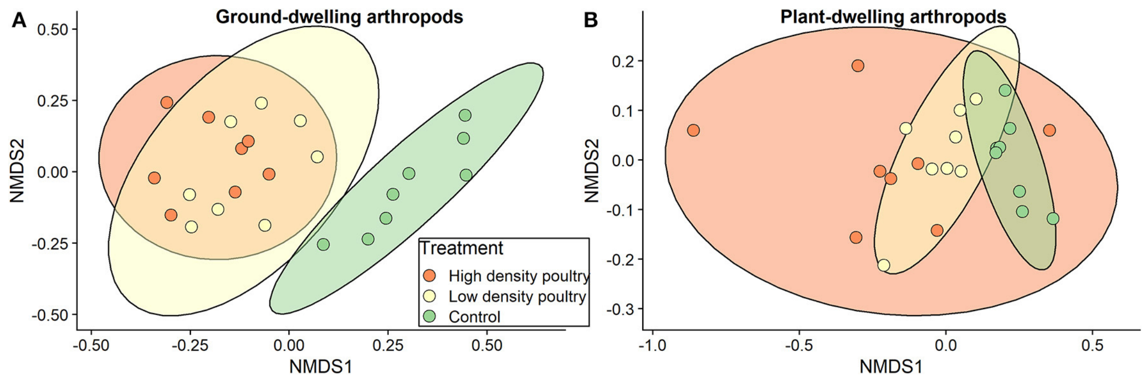

FIGURE 6
Composition between treatments. NMDS plots for **(A)** ground-dwelling and **(B)** plant-dwelling arthropod communities at the order level. Number of reduced dimensions for both was k = 2. Stress = 0.105 for **(A)** and stress=0.056 for **(B)**.

both agricultural and natural systems by consuming herbivorous arthropods (Mäntylä et al., 2011). There is also a possibility that poultry may be consuming plant-dwelling arthropods (Figures 7A, D, E). However, this likely only contributes a small fraction to the observed reduction in plant-dwelling arthropod abundance given the drastic reduction in plant cover that was observed across the study and the lack of insect foraging behavior displayed by the chickens. Likewise, consumption of ground-dwelling arthropods is likely also occurring (Figures 7A, D, F), although the effect of this consumption is greatly outweighed by the positive relationship between chicken fecal deposition and ground-dwelling arthropod abundance.

Future studies should compare how arthropod abundance changes in response to vegetation removal or destruction in the absence of poultry (e.g., by mowing, artificial trampling) to better estimate how much chicken insect foraging contributes to changes in arthropod communities. Additionally, it is also possible that the chicken tractors themselves contributed to decreases in plant-dwelling arthropods relative to control treatments (without tractors). The movement of these tractors, the short-term shading (1–3 days), and even the increased human activity (to care for poultry) near tractors may have impacted the arthropods and cover crops. Additionally, future studies should aim to establish perennial forages that are better suited for the destructive nature of pastured poultry and are more typical of pastured-poultry operations. Indeed, extension specialists suggest that forages for pastured poultry should consist of plant species that are tolerant to scratching and biting, have large leaf to stem ratios, and can recover from grazing and trampling (Jacob et al., 2017). This study utilized annual cover crops established after spring vegetable production to address over-arching questions related to the integration of poultry into vegetable rotations. However, a

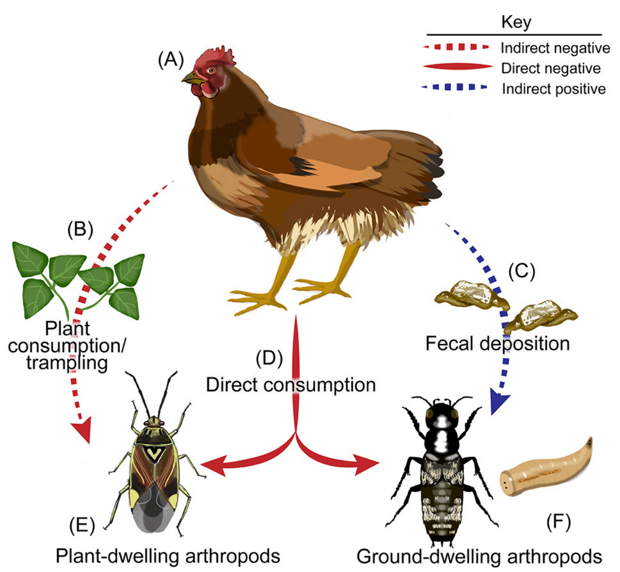

FIGURE 7
Potential pathways in which poultry may impact arthropod communities. A diagram showing the potential indirect and direct pathways in which pasture-raised poultry may impact arthropod communities. Poultry such as **(A)** chickens may **(B)** consume or trample plant habitat of **(E)** plant-dwelling arthropods, thereby reducing their local populations indirectly. **(A)** Chickens may also alter arthropod communities via their **(C)** fecal deposition which may increase the abundance of **(F)** ground-dwelling arthropods. Finally, **(A)** chickens may also **(D)** directly consume both plant-dwelling and ground-dwelling arthropods. Illustrations by K Garcia.

clear challenge to this short-term rotational integration was the establishment of an annual cover crop capable of withstanding poultry integration, evidenced by the drastic reductions in cover crop biomass. The rotational plan that was implemented across this project was designed to capture replication of experiment plots in order to replicate the impact of poultry integration on arthropods, cover crops, and crops as part of a larger project (Supplementary Figure S1). For this reason, we had rapid rotations of spring vegetables, summer cover crops, fall poultry, and winter cover crops. Many farmers that integrate poultry often utilize perennial pastures that are in place for a year or more before transition vegetable crops. While this research does not exactly represent these longer-term integration strategies, it is perhaps even more impressive that this short-term rotational integrated system found such strong changes in the arthropod communities, given the high-level of disturbance.

Whatever top-down effects poultry may be having on plant-dwelling arthropods, either directly or indirectly, appear to be impacting both beneficial (i.e., natural enemies) and crop pest insects; the relative abundances of both of these functional groups were greater in the control treatments relative to the poultry treatments (Figure 5B), suggesting that poultry activity comparably affects these functional groups in this cover-crop system. Similarly, plant-dwelling herbivorous hemipteran and predatory hemipteran abundances were also greater in the control treatment relative to high- and low-density poultry treatments. Thus, in this cover crop system, the addition of poultry non-discriminately negatively impacts the relative abundances of both beneficial and crop pest insects. Conversely, ground-dwelling natural enemies and known pests both increased with the addition of poultry (Figures 4E–G). Thus, it remains unclear whether natural enemies that are being promoted by poultry are providing sufficient, if any, pest suppression. Future studies should determine whether increased populations of ground-dwelling natural enemies (via addition of poultry) are providing pest suppression of insect pests through ecological approaches such as sentinel prey experiments which are often used to measure biological control activity by predators (Chisholm et al., 2014). Additionally, future research should quantitatively investigate whether poultry are consuming plant-dwelling arthropods and vegetation, such as with the use of DNA metabarcoding-based diet analysis (Crisol-Martinez et al., 2016; Mata et al., 2021). If pasture-raised poultry are indeed found to be consuming insects at significant rates, this might be beneficial to farmers as past research has shown that adding insect meal to poultry diet can improve growth performance (Benzertiha et al., 2020), nutrient digestibility and immune function (Elahi et al., 2022), and gut health (Biasato et al., 2018; Józefiak et al., 2020).

4.2. Bottom-up effects

Despite the evidence of top-down effects on plant-dwelling arthropod communities, the bottom-up effects on ground-dwelling arthropods were most staggering. Most impressive of all was the impact of poultry on house fly larvae (Figure 4C). Indeed, of the 11,069 house fly larvae that we collected in our pitfall traps across both years, only four were collected from control treatment plots. Despite these sharp differences in abundance between poultry density treatments, it should not come as a surprise since house flies are considered to be major pests of animal husbandry operations including poultry farms where they consume foodstuffs and wastes (Axtell, 1999; Malik et al., 2007). House fly larvae are known to be consumed by a variety of insects, including some hister beetles (Coleoptera: Histeridae) and the larvae of other flies (Malik et al., 2007). Indeed, the predatory functional groups: ants, rove beetles, spiders, predatory hemipterans, and natural enemies had greater relative abundances in poultry plots relative to control plots. It is plausible that these predators recruited to poultry plots to take advantage of dipteran prey. Additionally, the close proximity of research plots may have facilitated some mobile coprophagous insects being lured from control plots to experimental plots, thus inflating the observed differences. House flies may be so highly mobile that this would not play a role. Other less mobile groups may have moved from control plots to experimental plots. Ultimately, changes in relative abundance of arthropods reflects local population growth and colonization of plots to target resources, and it is likely that this phenomenon would be observed regardless of plot placement.

To our knowledge no other study has documented the bottom-up impacts of pasture-raised poultry manure deposition on arthropod communities. However, studies have investigated how applying poultry manure as fertilizer, *sans* poultry, impacts arthropods. Brown and Tworkoski (2004), for example, found that the application of composted chicken manure resulted in increased arthropod predators, less herbivores, and reduced abundances of key apple pests in apple orchards (Brown and Tworkoski, 2004). Additionally, a systematic review by Rowen et al. (2019) on fertility management for insect pest control found that manure fertilizer increased generalist predator activity in 6 of 13 studies. It is theorized that manure and other detritus increases populations of decomposer arthropods, whose populations can then assist in sustaining generalist predator populations (Halaj and Wise, 2002; Rowen et al., 2019). Our study appears to support this hypothesis, as we observed increased abundance of fly larvae and predatory groups (spiders, rove beetles, natural enemies, and predatory hemipterans).

In addition to potentially sustaining generalist predator populations, house fly larvae can aid in decomposition and nutrient cycling, and have in some instances been used to convert raw poultry manure to fertilizer (Calvert et al., 1970). However, given the documented role of house flies as vectors of food-borne pathogens to fresh produce including the transmission of *Escherichia coli* O157:H7 to spinach (Wasala et al., 2013) and the transmission of *E. coli* O157:H7 and *Salmonella enterica* to lettuce (Pace et al., 2017), special consideration should be given to the spatiotemporal separation of pastured poultry and fresh produce. Future studies should aim to investigate what spatial configurations between pastured poultry and fresh produce pose the least threats to food safety and what the role of house flies may be in heightening such risks. Further, when incorporating livestock and their raw manure into any crop rotational system, there are temporal aspects to consider. For example, USDA's National Organic Program standards call for a 120-day interval between the application of raw manure and harvest of crops whose edible portions come into contact with soil and a 90-day interval for crops whose edible portions do not come into contact with ground (USDA AMS NOP, 2023). Anecdotally, we observed large masses of maggots in residual poultry food that was exposed to rain after tractors had moved in the experimental rotation. Thus, it is possible that discarded poultry feed also impacted the abundance of house fly larvae.

Despite the large effect sizes observed between treatments, it remains unclear whether these changes in arthropod communities will persist over time. In this study, insect collection was done shortly after poultry had been occupying the collection areas in a 3-week time period. Thus, these results only highlight short-term effects on arthropod communities by poultry. It should be noted that within this study's rotational system fields were mowed and tilled immediately after broilers reached market weight and were processed which would act as a major disturbance to both the ground and vegetative arthropod community. Thus, it remains unclear what longer-term net effects of pastured-poultry activity could have on arthropod communities the following spring when vegetables are planted, and whether the effects of some interactions (Figure 7) would have longer-term effects than others (i.e., would the reduction of plant-dwelling arthropods persist longer than the increased abundance of ground-dwelling arthropods or vice versa).

Additionally, both the short-term nature of this study and our anticipation of strong top-down impacts on arthropod communities were similar to expectations of wild bird exclusion studies, although our results revealed additional bottom-up effects. Studies of the net effects of wild birds also show contrasting impacts via direct consumption of crops and indirect benefits to crops through trophic cascades (Pejchar et al., 2018). In some instances, birds can provide protection to crops by consuming insect pests. However, in some instances birds can constrain pest control services by consuming arthropod natural enemies (Martin et al., 2013). For the most part, studies that focus on the net effects of birds on agroecosystems focus on top-down effects such as predation of insects or crops. However, outside of agroecosystems, studies show that some gregarious bird species can have bottom-up effects as well. Seabirds, for example, typically forage across a vast marine area and then deposit marine nutrients (guano, carcasses, food scraps, reproduction byproducts) on their nesting islands (Kolb et al., 2012). The nesting activity of seabirds can also result in disturbances to the local environment (Kolb et al., 2012). For example, nesting great cormorants (*Phalacrocorax carbo* L.) in the Stockholm archipelago of Sweden were found to negatively impact plant species richness, percent vegetative cover, and plant species composition (Kolb et al., 2012). In regard to arthropod communities, Kolb et al. (2012) found varying responses for abundance of functional groups with herbivorous coleopteran abundance decreasing in islands with nesting cormorants but with fungivorous coleopteran and scavenging coleopteran abundances increasing (Kolb et al., 2012). These results from wild gregarious bird studies align with our findings, in which we found that there were not only top-down forces on plant vegetation and arthropods associated with vegetation, but also bottom-up forces on other arthropod groups.

5. Conclusion

This study revealed the drastic changes that can occur in arthropod community structure when prompted by the addition of pasture-raised poultry to a crop rotation. While it is thought that the addition of poultry to a crop rotation system may bolster yield through nutrients delivered from fecal deposits, this study shows that poultry activity may also stimulate ground-dwelling arthropods through bottom-up ecological mechanisms. However, this study also found that the addition of poultry decreased the abundance of plant-dwelling arthropods. The implications that these changes in arthropod abundance may mean for both short- and long-term biological control remain to be explored. Nonetheless, this study has contributed to our understanding of how poultry may impact arthropod communities.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at the University of Kentucky.

Author contributions

DG developed the methodology. KG, VH, KT, and DG collected the data. KG and DG analyzed the data. KG wrote the initial manuscript. DG and JD supervised the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Soil health and synergy of ecological determinants of green cocoa productivity in different soil ecotypes in Ghana

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Introduction: Soil health is critical for the efficient management of soil fertility and crop yield in "green" cocoa (GC) (*Theobroma cacao L.*) agroforestry systems. However, knowledge about agroecosystem factors that affect healthy soil productivity in "green" cocoa agroforestry systems is patchy in West Africa. Based on organic cocoa (OC) and conventional cocoa (CC) agroforestry systems in Ghana, this study examined the soil health and synergy of ecological factors that determine the yield of GC.

Methods: Using multi-stage random sampling, 11 CC and 11 OC farms were sampled from three soil types (ferralsols, lixisols, and leptosols) within selected agroecological zones. Socioeconomic and farm data, including bulked soil samples, were collected at 0–30 cm depth for analysis of soil chemical and physical properties.

Results: The results showed intricate relationships between the ecological factors and the yield of GC (1.07 t ha^{-1}), which comprised dry beans of OC (1.24 t ha^{-1}) and CC (0.89 t ha^{-1}). The green cocoa yield increased for fields owned by female farmers and for native farmers who inherited or outrightly owned farmlands. The cocoa yield was also positively related to physicochemical factors such as soil organic carbon (0.21%), pH (5.8), and carbon–nitrogen ratio (40.8%). The carbon–nitrogen ratio and pH together exerted the highest positive influence (0.62%) on the yield. Biological factors such as plant density (>7 cocoa trees per 23.4 m^2) and black pod rots reduced the cocoa yield.

Discussion: This study provides comprehensive empirical determinants of green cocoa productivity and offers a more reliable estimate of cocoa plant density. The findings suggest that Ghana's cocoa can be much greener if stakeholders promote healthy farm soil productivity and empower women who engage in soil organic carbon-conserving agroforestry.

KEYWORDS

cocoa agroforestry, crop yield, soil fertility, soil organic carbon, ferralsols, lixisols, leptosols, plant density

1. Introduction

Cocoa (*Theobroma cacao* L.) produced on healthy farm soil supported by an agroforestry system is considered a "green" product (Schroth et al., 2016; Sdrolia and Zarotiadis, 2019). This is because it is believed that the process of production and the product are ecologically friendly, carbon-neutral, or climate-smart. The green products of cocoa agroforestry systems (CAS), such as cocoa yield, are produced in a way to prevent, reduce, and correct harmful environmental impacts of production systems (Rajab et al., 2016; Schroth et al., 2016). This involves the cultivation of cocoa with non-cocoa tree species of different canopy levels (multi-strata woody vegetation), with or without livestock simultaneously on the same unit of land (Tscharntke et al., 2011; Blaser et al., 2018; Castle et al., 2021, 2022). Evidence suggests that farm soils in CAS are healthier than those in cocoa monocropping systems (Rajab et al., 2016; Asare et al., 2019). A healthy cocoa farm soil can "sustain productivity, diversity, and environmental services of terrestrial ecosystems" (FAO ITPS, 2020). Hence, cocoa stakeholders are increasingly sourcing beans from "green" cocoa agroforestry systems that have healthy farm soils (Tscharntke et al., 2011; Fountain and Huetz-Adams, 2020). Nonetheless, previous studies show a paucity of empirical information on "green" cocoa farm soil productivity enhancement strategies (Hartemink, 2005; Fountain and Huetz-Adams, 2020; Amponsah-Doku et al., 2021). Therefore, it is important to examine the soil health and synergy of ecological factors influencing "green" cocoa farm soil productivity in agroforestry systems.

Healthy farm soils and trees of cocoa agroforestry systems can sequester 7 and 50 metric tons (t) of carbon (C) per hectare (ha), respectively, which provide ecosystem "green" benefits (Ofori-Frimpong et al., 2010; Lal, 2016; Quarles, 2018). Through photosynthesis, the trees absorb atmospheric carbon and store half of the carbon as soil organic carbon (Ofori-Frimpong et al., 2010; Rajab et al., 2016; Ingham, 2019). The soil organic carbon (SOC) constitutes a major component of cocoa farm soil health indicators (Amponsah-Doku et al., 2021; Arthur et al., 2022; Doe et al., 2022). The SOC serves as food for microbial organisms such as fungi and bacteria that play a crucial role in the mineralization of soil nutrients such as nitrogen (N), phosphorous (P), and potassium (K) for plant uptake (Ingham, 2019; Esmaeilzadeh-Salestani et al., 2021). The SOC also facilitates the release of soil nutrients through microbial actions, which improve soil structure and water and nutrient retention capacities (Rousk et al., 2010; Asigbaase et al., 2020, 2021). Other "green" benefits of CAS are temperature regulation, biodiversity, water cycling, and soil nutrient cycling (Asase and Tetteh, 2016; Rajab et al., 2016; Asigbaase et al., 2021).

A cocoa cropping system that makes efficient use of resources for optimum yield while maintaining soil health is also ecologically sustainable and may be described as green (Ofori-Frimpong et al., 2010; Dobermann et al., 2013; Sumberg and Giller, 2022). The same applies to a sustainable conventional cocoa (CC) cropping system, which aims at optimizing "green" cocoa yield by minimizing environmental liabilities through the rational use of timber species, food intercrops, synthetic pesticides, and mineral fertilizers. Similarly, a sustainable organic cocoa (OC) system uses non-synthetic inputs such as bioinsecticides, manure, or organic fertilizers with timber species (shade trees) and food intercrops to achieve an optimum "green" yield and soil health. Essentially, both cropping systems aim at optimizing the "green" benefits of cocoa agroforestry ecosystems (Sumberg and Giller, 2022). Hence, the composite of sustainable CC and OC is termed green cocoa (GC) in this study. The GC is akin to "mass balance," where certified and non-certified sustainable cocoa beans are aggregated after harvest in Ghana and elsewhere (Mol and Oosterveer, 2015).

Ghana is the second-largest producer of the world's cocoa beans. In the year 2020, the country produced ∼870,000 t of cocoa beans (FAOSTAT, 2021). In the same year, the cocoa sector employed 80,000 smallholder farmers and accounted for 19% (US$ 2.3 billion) of the country's export earnings (Fountain and Huetz-Adams, 2020; Amponsah-Doku et al., 2021). However, the suitability of climate and soil conditions for growing the crop is declining in the cocoa agroecological zones of the country (Läderach et al., 2013; Dossa et al., 2018a,b; Doe et al., 2022). As a result, despite improved innovations, resource inputs and several interventions in the cocoa sector (Fountain and Huetz-Adams, 2020), cocoa yields are reported to be declining with each harvest in farmers' fields (Appiah et al., 1997; Hartemink, 2005). At the national level, the cocoa yield barely increased (0.03 t ha^{-1}) from 0.52 t ha^{-1} in 2017 to 0.55 t ha^{-1} in 2020, against a potential yield of 3.5 t ha^{-1} (FAOSTAT, 2021; Asante et al., 2022). The rigidity of national-level yield to rise and the declining yield at farmers' fields are inherently linked to ecological factors. Among the potential ecological factors are climate change and variability, soil type, plant age and density, pests and diseases, socioeconomic factors, and unsustainable management practices (Abdulai et al., 2020; Amponsah-Doku et al., 2021; Asitoakor et al., 2022).

However, studies on the determinants of cocoa yield have focused on single factors and soil fertility. For example, Aneani and Ofori-Frimpong (2013) adopted a socioeconomic approach to examine cocoa productivity and did not consider soil parameters. Those studies that examined soil fertility (Ofori-Frimpong et al., 2010; Ahenkorah, 2016; Asare et al., 2017; Kongor et al., 2019) did not consider soil health, social factors, and the magnitude of factor effects. The combined effects of the factors may be synergistic, which requires a more holistic study. In addition, the ecological concept of farm soil productivity suggests manipulations of social, biological, chemical, and physical ecological factors jointly impact crop yield and soil health. The complex interactions of these ecological factors generate stimuli at certain thresholds, below or beyond which desirable or undesirable feedback impacts the yield and soil health (van Ittersum and Rabbinge, 1997; Folke et al., 2010). For soil chemicals, the thresholds may be ideal or toxic to farm soil health (Tscharntke et al., 2012; Asare et al., 2017; Doe et al., 2022). Hence, limited predictive knowledge about the thresholds of these ecological factors hinders healthy cocoa farm soil productivity management.

The paucity of predictive knowledge of green cocoa farm soil productivity undermines local and international stakeholders in decision-making, aimed at enhancing the green benefits of cocoa agroforestry systems (van Vliet and Maja Slingerland, 2015; Dossa et al., 2018a). Particularly, it limits the work of smallholder cocoa farmers, buyers, extension officers, policymakers, and environmentalists, operating within different "soil ecotypes." In this study, "soil ecotype" refers to a landscape characterized by a specific soil type in a specific agroecological zone or ecosystem. The present study, therefore, examines the soil health status of CC, OC, and GC systems, and the synergies and magnitudes of the ecological factors determining (±) green cocoa yield in Ghana.

2. Materials and methods

2.1. Study area

The study was conducted on smallholder cocoa farms in four communities, spread across three soil ecotypes in Ghana (Figure 1). The soil ecotypes were (i) Ferralsols (FR or Oxisols) of Wet Evergreen (WE) soil ecotype (FR.WE) in Elubo and Boinso cocoa districts of Western Region, (ii) Lixisols (LX or Alfisols) of dry semi-deciduous inner zone (DSIZ) soil ecotype (LX.DSIZ) in Suhum cocoa district in Eastern Region, and (iii) Leptosols (LE or Entisols) of moist semi-deciduous south-east (MSSE) soil ecotype (LE.MSSE) in Papase cocoa district of Oti Region. Average annual rainfall and cocoa production vary in the sequence of FR.WE > LE.MSSE > LX.DSIZ. Mean daily temperature (25°C) and annual rainfall (1,270–1,651 mm) of the LX.DSIZ are lower than those of the FR.WE (26°C and 1,732 mm). The mean daily temperature and annual rainfall are approximately 25°C and 1,400–1,800 mm, respectively, in the LE.MSSE. Soil quality varies in the different soil ecotypes, generally declining in the order as follows: LX.DSIZ > FR.WE > LE.MSSE (FAO and ITPS, 2015).

2.2. Study design, small size, sampling, and management practices

The study was designed as a quantitative cross-sectional agroecosystem analysis (Conway, 1987), which combined smallholder cocoa farmer socioeconomic and farm habitat survey data. Cocoa farmers and their respective cocoa farms were selected using a multi-stage stratified random sampling. In the first stage, three soil ecotypes were purposively selected out of six cocoa agroecological zones and seven soil types because they had both organic and conventional CAS. For each soil ecotype, 11 OC and 11 CC agroforests were randomly selected, making 33 CC and 33 OC systems, when combined 66 = GC farms (Figure 2). The OC system was managed by certified organic smallholder farmers of Yayra Glover Limited (YGL), while the CC system was managed by non-certified smallholder farmers in the same community.

The treatments were actual farmer practices in organic (OC) and conventional (CC) cocoa cropping systems in Ghana. Many previous studies such as Arthur et al. (2017) and Asigbaase et al. (2020, 2021) have examined differences in the effects of agronomic practices by OC and CC farmers on their soil physiochemical quality and yield. Generally, the OC farmers use non-synthetic (organic) agrochemicals while the CC farmers use synthetic (inorganic) agrochemicals, which is the pivotal difference between organic and conventional agriculture. However, using copper-based fungicides (CBF) to control the fungal (*Phytophthora* spp.) disease of cocoa black pod rot is common in both OC and CC systems. The use of CBF in conventional and organic farming is common in many European countries (European Commission, 2021) and well accepted under the Japanese Agriculture Standard (JAS) for organic plants. The same applies to the United States Department of Agriculture (USDA) organic standard and the Rainforest Alliance Sustainable Agriculture standard.

In terms of actual management practices, the OC farmers used organic fertilizers such as Elite granular organic (NPK3:4:4 + 9Ca + 1 Mg + 0.04B + 0.08Zn + 11 Organic matter) and PhytoGreen liquid organic fertilizers. In addition, they use biosolids, organic manures, and compost. Copper-based fungicides such as Champion, Nordox, and Kocide were used to manage cocoa black pod diseases. Insect pests such as capsids (mirids) and aphids were managed using organic bioinsecticides such as pyrethrum, extracts from the seeds and leaves of a neem tree (*Azadirachta indica* A. Juss.), and AgroPy 5EW supplied by YGL through the Ghana COCOBOD. The CC farmers used mineral fertilizers such as Assase Wura (NKP0:22:18 + 9CaO + 7S + 6MgO), Sidalco (NPK10:10:10), and Cocofeed (NPK0:30:20). Copper-based fungicides such as Ridomil Gold Plus (metalaxyl cuprous oxide) and Fungikill as well as pesticides such as Confidor (imidacloprid) and Akatemaster (bifenthrin) supplied by the Ghana COCOBOD. The treatment given to the GC by combining the CC and OC yields is akin to the practice of mass balance in sustainable cocoa trading (Mol and Oosterveer, 2015), integrated soil fertility management (Quaye et al., 2021), and integrated organic farming (Layek et al., 2023). All the cropping systems were rainfed, zero-tillage with at least 18 timber species ha^{-1} (CHED/WCF, 2016).

2.3. Data collection, soil sampling, and measurement of variables

The socioeconomic data of the farmers were solicited using a semi-structured questionnaire, while the biological, chemical, and physical characteristics of their cocoa farms were collected using a checklist (see online Supplementary material, subsection 1).

2.4. Measurement of cocoa biological variables and yield

The farm biological data covered cocoa tree age (CAge), cocoa variety (cultivar), number of black pod rot diseases per tree (*Bpod*), planting density (Pltdn), and cocoa yield. The cocoa fields studied had irregular spacing of cocoa trees as pertains to most smallholder cocoa fields in Ghana. Therefore, to determine the cocoa plant density, which is representative of the entire farm, a 24 m transect line was laid within each farm, with three sets of regular hexagons (Figure 3), and the hexagons were 3 m apart along the transect line. Each hexagon was made up of three congruent rhombi (Figure 3A) or six equilateral triangles (Figure 3B) of 3 m sides (23.4 m^2). Based on the recommended 3 m apart planting distance (3 m×3 m) for cocoa (CHED/WCF, 2016), each hexagon, rhombus, and triangle is supposed to have seven, four, and three cocoa trees, respectively. The average cocoa plant density (Pltdn) per hexagon was computed by dividing the sum of cocoa trees within the delineated hexagons by the number of hexagons (Figure 3C).

The total count of black pod rots (*Bpod*) on the cocoa trees within the hexagons was divided by the number of cocoa trees to obtain the average number of black pod rots per tree. The age of the cocoa trees (CAge) and cocoa varieties (cultivars) were provided by the farmer and verified through observation by the researchers

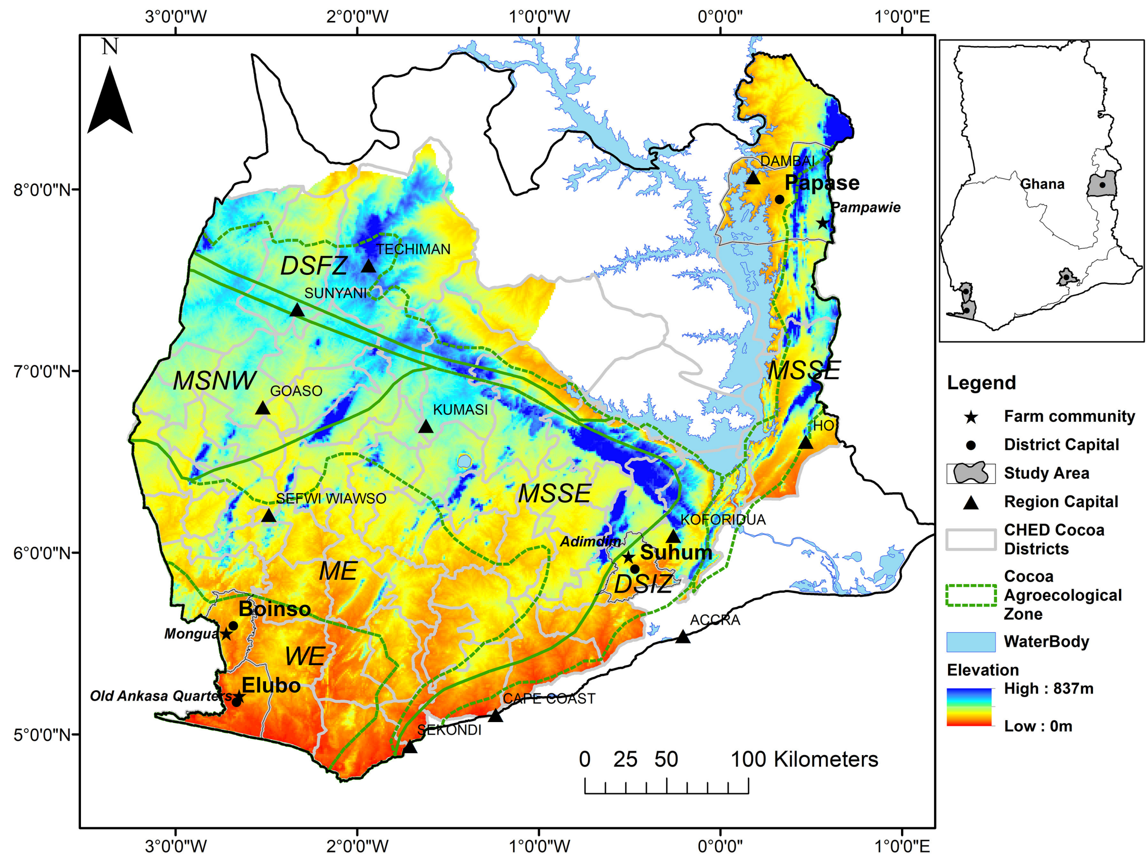

FIGURE 1
Map of the study area.

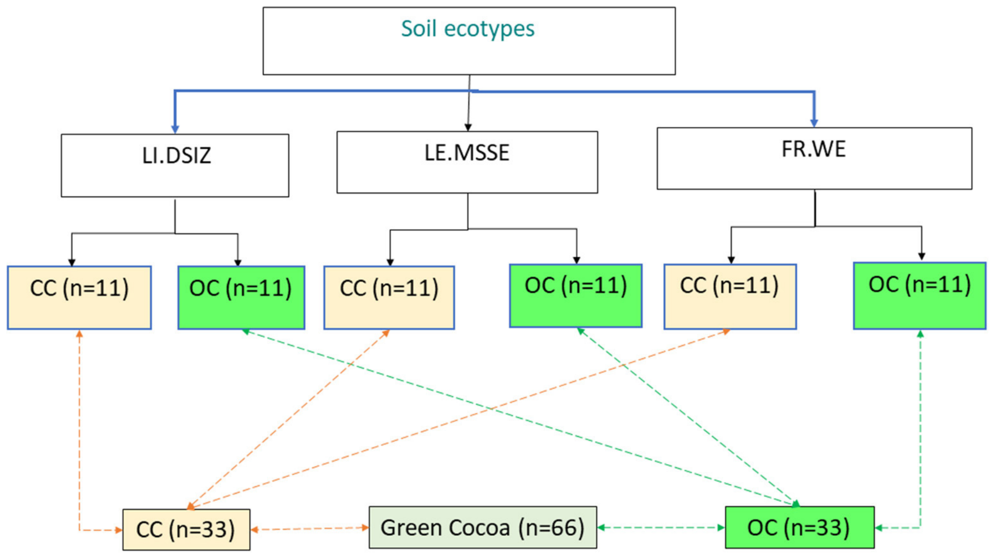

FIGURE 2
Distribution of the study samples.

and cocoa extension agents. Cocoa cultivar indicator variables such as Amazonia, Hybrid, and their mixtures were also measured as dummy variables (1 = *Yes* or 0 = No).

Information on the annual cocoa production output was quantified as the number of bags of dry cocoa beans (a bag is 64.5 kg or 0.0645 t) obtained from the farm by the farmer during the last

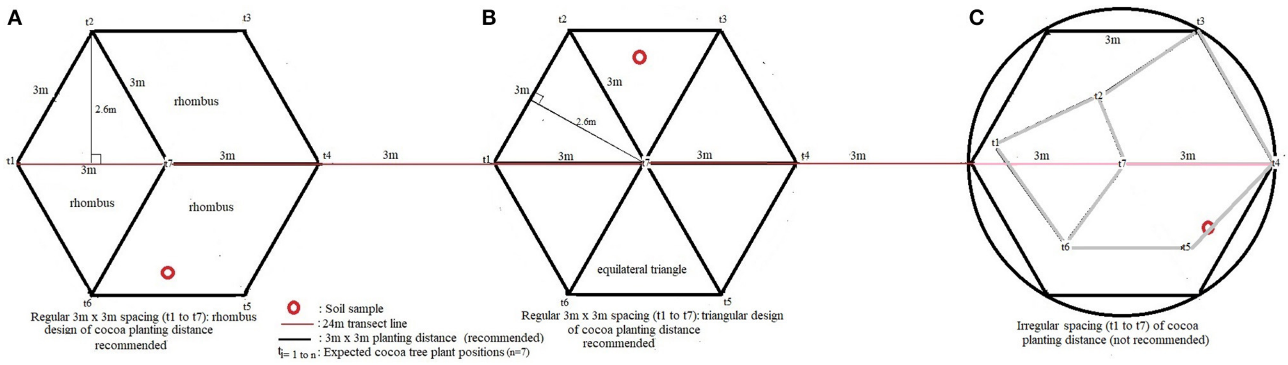

FIGURE 3
Schematic representation of expected cocoa plant position, plant density and soil sampling points for fields with **(A)** regular rhombus spacing, **(B)** regular triangle spacing, and **(C)** irregular spacing of cocoa planting distance.

cropping calendar and verified by the cocoa passbook(s) of the farmer. Williams et al. (1989) and Wang et al. (2022) define crop yield as the magnitude of dry weight (t) of cocoa beans harvested per ha of land cultivated using a given cropping system. Therefore, the total annual cocoa yield in t ha^{-1} was computed by dividing the product of the number of bags of dry cocoa beans and 0.0645 t by the given farm size (ha) in the present study. The farm size was measured using a handheld GPS device (GPSMap 64, Garmin Ltd., United States).

2.5. Measurement of soil chemical and physical properties

One core of soil sample was taken at 0–30 cm depth from each hexagon along the transect line, mixed thoroughly in a plastic bucket and bulked to make a composite sample. The composite soil samples were well labeled and transported to the Ecological Laboratory of the University of Ghana for processing and analysis. In the laboratory, the samples were air-dried, crushed, and sieved through a 2 mm mesh after removing all visible plant materials for the determination of the soil's chemical and physical properties.

The soil chemical properties measured were pH, SOC, N, P, Ca, Mg, K, Na, Al, and H, and the physical properties were texture and soil ecotype. For the measurement of exchangeable bases (Ca, Mg, K, and Na) in $cmolc\ kg^{-1}$, 10 g of the soil sample was extracted with 100 ml of 1 normal ammonium acetate (NH_4OAc), buffered at pH 7, and measured using an atomic absorption spectrometer (PINAAcle 900T, Perkin Elmer Inc., Waltham Massachusetts, United States). The sum of Ca^{++}, Mg^{++}, K^{+}, and Na^{+} yielded the total exchangeable bases (TEB). Effective cation exchange capacity (ECEC) was calculated by adding up TEB and acidity ($H^{+} + Al^{+++}$), all in $cmolc\ kg^{-1}$. The acidity of the soil was measured by the titration method (McLean, 1965). Base saturation (Bs) was expressed as a percentage of the TEB to the ECEC. Bray-1 method (0.03 M NH_4F + 0.025 M HCl) (Bray and Kurtz, 1945) was used to determine available soil P ($cmolc\ kg^{-1}$). Soil organic carbon (SOC) g/kg (%) was determined following Walkley and Black method (Walkley and Black, 1934). Soil CN was calculated based on the ratio of SOC to total N. The total N% was determined using a semi-micro Kjeldahl procedure (Bremner, 1996). The soil pH [water] was measured in 1:2 soil–water suspension using a well-maintained and calibrated electrode digital pH meter (EC PCS Testr35, Eutech Oakton Instruments Ltd, United States). The soil's texture (sand, silt, and clay) was determined using the pipette method (Robinson, 1922). All the properties are presented as continuous variables (X_i). The soil ecotypes were determined by superimposing shape files of the soil sampling points on the soil type (LX, FR, and LE) and agroecological zone (DSIZ, WE, and MSSE) maps using ArcGIS 10.4 and labeling the area of intersections (LX.DSIZ, FR.WE, and LE.MSSE) as a factor variable.

Soil biological properties were not measured. However, the soil pH, acidity (AI + H), and SOM/SOC are well known for influencing soil microbial activities and are often used as proxies for soil biological properties (Moebius-Clune, 2016). Thus, the soil parameters were chosen because they are well-known indices of soil health, soil fertility, and crop yield (Moebius-Clune, 2016). The soil health status was assessed based on the ideal range (Table 1) of the soil fertility indices or otherwise the extremes beyond which the indices become toxic (Gaspar and Labosk, 2016; Asare et al., 2017; Doe et al., 2022).

2.6. Measurement of socioeconomic variables

The socioeconomic data include the farmer's age (FAge) in years, number of cocoa training (Ext) attended by the farmer, gender (FSex), nativity (being an indigene or a migrant), and farmland tenancy (LT). Binary numbers 1 = *Yes* and 0 = No were used to represent social indicator variables such as gender (FSex) where female farmer = 1, male farmer = 0, and being a native farmer = 1 or otherwise = 0. The same applies to land tenancy (LT) arrangements such as outright and inherited farmland ownership. Sharecropping systems such as "abunu" where 2/3 of the cocoa yield goes to the farmer and "abusa" where 1/2 of the yield goes to the landlord were also treated as dummy variables.

TABLE 1 Soil health status and fertility indices of cocoa cropping systems for the soil ecotypes.

Ecological factors			Soil ecotypes						Combined
			LX.DSIZ		LE.MSSE		FR.WE		
(Ideal range)	Unit	Stat	CC ($n = 11$)	OC ($n = 11$)	CC ($n = 11$)	OC ($n = 11$)	CC ($n = 11$)	OC ($n = 11$)	GC ($n = 66$)
SOC	%	μ	1.5	2.0	2.3	2.5	1.7	2.5	2.1
(2.5–3.5%)		SD	0.6	0.6	0.7	0.5	0.5	0.3	0.7
		CV	40.0	30.0	30.4	20.0	29.4	12.0	33.3
CN	%	μ	30.5	55.8	42.2	41.6	31.0	43.4	40.8
(20–40%)		SD	7.4	16.8	9.3	14.2	8.2	5.7	13.8
		CV	24.3	30.1	22.0	34.1	26.5	13.1	33.8
pH		μ	6.1	6.0	5.9	6.3	5.0	5.4	5.8
(5.6–7.5)		SD	0.4	0.5	0.6	0.5	0.5	0.5	0.7
		CV	6.6	8.3	10.2	7.9	10.0	9.3	12.1
P	cmolc kg^{-1}	μ	29.3	32.4	30.5	28.4	20.7	30.7	28.7
(20–50)		SD	9.4	14.1	17.8	13.2	6.0	9.6	12.3
		CV	32.1	43.5	58.4	46.5	29.0	31.3	42.9
K^+ (4–8% of Bs)	cmolc kg^{-1}	μ	0.8	0.9	1.0	1.1	1.5	1.6	1.2
		SD	0.3	0.5	0.3	0.4	0.8	0.7	0.6
		CV	37.5	55.6	30.0	36.4	53.3	43.8	50.0
Mg^{++} (12–25% of Bs)	cmolc kg^{-1}	μ	35.7	33.2	41.5	37.6	50.3	49.1	40.9
		SD	4.6	5.1	9.3	8.5	14.6	10.6	11.0
		CV	12.9	15.4	22.4	22.6	29.0	21.6	26.9
Ca^{++} (65–80 of Bs)	cmolc kg^{-1}	μ	60.6	62.9	54.6	58.8	41.3	42.8	53.9
		SD	4.7	5.0	10.5	9.0	18.2	13.1	13.5
		CV	7.8	7.9	19.2	15.3	44.1	30.6	25.0
Na^+ (0–1 of Bs)	cmolc kg^{-1}	μ	2.9	3.0	2.9	2.5	6.9	6.4	4.0
		SD	0.9	0.8	1.3	0.6	3.6	2.5	2.6
		CV	31.0	26.7	44.8	24.0	52.2	39.1	65.0
Acidity (0–10 of Bs)	cmolc kg^{-1}	μ	5.2	5.1	5.7	5.2	26.1	14.7	10.1
		SD	1.9	1.5	2.3	2.1	25.8	9.5	7.2
		CV	36.5	29.4	40.4	40.4	98.9	64.6	71.3
ECEC	cmolc kg^{-1}	μ	19.9	19.2	19.7	19.9	11.2	10.2	16.6
		SD	5.2	4.9	5.3	4.6	4.3	3.6	6.2
		CV	26.1	25.5	26.8	23.0	38.3	35.7	37.2
Bs	%	μ	95.1	95.2	94.7	95.1	81.8	87.7	91.7
		SD	1.7	1.4	2.0	1.8	13.8	7.0	7.9
		CV	1.8	1.5	2.1	1.9	16.9	8.0	8.6
Sand (35–65%)	%	μ	46.5	54.2	22.8	26.6	23.7	51	37.7
		SD	12.6	10.3	5.7	11.7	9.1	10.3	16.6
		CV	27.1	19.0	25.0	44.0	38.4	20.2	44.0
Silt (30%)	%	μ	35.5	29.2	60.1	54.8	54.8	27.8	43.5
		SD	7.3	6.1	9.1	11.6	8.9	8.8	15.7
		CV	20.6	20.9	15.1	21.2	16.2	31.7	36.1
Clay	%	μ	18.2	16.5	17	18.4	21.4	21.2	18.7
(25–40%)		SD	7.0	5.0	6.0	3.9	4.3	3.7	5.3
		CV	38.5	30.3	35.3	21.2	20.1	17.5	28.3

The ideal ranges are based on Ahenkorah (2016), Gaspar and Labosk (2016), Asare et al. (2017), and Doe et al. (2022). μ, mean; SD, standard deviation; CV, coefficient of variation ($SD/\mu*100$). Low CV ≤15% and High CV ≥36% (Wilding, 1985).

2.7. Specification of the green cocoa farm soil productivity function

Plant production ecology theory underpins predictive crop production function and farm soil productivity (Odum, 1968; Scow, 1997; van Ittersum and Rabbinge, 1997). The theory suggests the interaction of biotic and abiotic ecological factors mediates yields of soil productivity. A certain threshold of the interaction generates stimuli (β_i) that may trigger a positive (+) or negative (-) magnitude of crop yield and other ecosystem services (Folke et al., 2010; Lal et al., 2015; Lal, 2016). These interactions can be modeled using ecological functions (Odum, 1968; Zuur et al., 2009). In this study, the interaction of the agroecosystem (socioecological) factors (X_i) impacting the yield of green cocoa farm soil productivity (Y_i) was expressed according to Zuur et al. (2009) and Zhao et al. (2017) as follows:

$$Y_i = f(X_i), \qquad (1)$$

where Y_i is the response (dependent) variable and X_i is an explanatory (independent or predictor) variable. The functional form of Equation 1 (Figure 4, fully expressed in Supplementary material) depends on the type of relationship between Y_i and X_i and the distribution of Y_i and X_i (Cobb and Douglas, 1928; Lloyd, 2001). We applied both double-log ($A{X_i}^{\beta_i}$) and semi-log ($\alpha_i X_i$) functional forms as shown in Equation 2.

$$\log Y_i = logA + \ log{X_i}^{\beta_i} + \alpha_i X_i + \varepsilon_i \text{ where } \varepsilon_i \sim N(0, \sigma^2), \qquad (2)$$

where A denotes a positive constant (intercept). α_i represents a proportionate (%) change (±) in Y_i per unit change in a dummy (or factor) variable, $X_i = 1$ as opposed to $X_i = 0$, holding other factors constant. β_i is technically known as elasticity or responsiveness (%) of Y_i to changes in X_i. In other words, β_i measures the percentage change in the yield of green cocoa due to a 1% change in each factor, holding other factors constant. The ε_i denote random error term.

The double-log functional form (with the lowest Akaike information criteria) was chosen for this study because of its useful properties, which generate information for managing Y_i and X_i to the optimum levels. In a strict double-log functional form, the sum of β_i ($\sum \beta$) is 1, but when generalized, the sum may be $\lesseqgtr 1$. $\sum \beta$ also denotes a certain degree of homogeneity (λ^{β}) factor for scaling up or down the Y_i (Cobb and Douglas, 1928; Lloyd, 2001).

2.8. Statistical estimation and analysis of the data

The statistical analysis of the variables and estimation of the green cocoa productivity function were performed using R version 4.1.3 (R Core Team, 2019). Descriptive statistics such as the mean (μ), standard deviation (SD = σ) and variance (σ^2), percentages (%), and boxplots of the variables were computed to describe the attributes of the cropping systems. The coefficient of variability (CV) of the attributes was expressed as $\sigma / \mu \times 100$. A CV $\leq$ 15% is low (homogenous), while a CV $\geq$ 36% is high (heterogeneous) (Wilding, 1985).

Bivariate Pearson's correlation coefficients (r) of the relationship between any pair of factors, including the yield, soil ecotypes, and OC and CC systems, were used to explain the synergy. The significant predictors of green cocoa productivity and their magnitudes of effects were examined using multiple regression (t-statistics) estimates of Equation 2. The combined significance of the predictors was determined using F-statistic analysis of variance (ANOVA).

Equation 2 was estimated using ordinary least-square (OLS) regression. The semi-log estimate of Equation 2 is known for minimizing the non-normality of the data, while the double-log minimizes multi-collinearity as well as differences in units of measurement of the variables (O'Brien, 2007). We followed O'Brien (2007) caution to avoid multi-collinearity by dropping some variables. Dropping key variables limits the representativeness of the function to reality, and too many variables would create multi-collinearity. We cautiously dropped a few variables that experienced perfect collinearity, hypo-heterogeneity (too small σ^2), and hyper-scedasticity (too small R^2 and too large standard errors).

We perform post-estimate regression diagnostics to validate the assumptions $\varepsilon_i \sim N(0, \sigma^2)$, underlining the use of the OLS. We checked for residual (ε_i) normality (N), zero (0) mean, and constant variance (σ^2). We tested heteroscedasticity (multi-collinearity) using the generalized variance inflation factor (GVIF$^{(1/(2^*\mathrm{Df}))}$), equivalence of VIF to satisfy the VIF's "<10" rule of thumb (Fox and Monette, 1992). According to Fox and Monette (1992), VIF values <2, 5, or 10 have zero, minimum, and moderate (all acceptable) multi-collinearity, respectively. Obtaining these VIF values demonstrates that the estimated F-statistic (ANOVA) and T-statistic (OLS) are reliably valid for scientific interpretations and management decision-making.

3. Results

3.1. Soil health and fertility in soil ecotypes for conventional, organic, and green cocoa

Table 1 shows the soil fertility of the observed cocoa agroforestry systems within the soil ecotypes, with the ideal soil health indicators in parentheses. The SOC which largely defined the soil health of the systems was generally adequate (≥2.5%). It was, however, inadequate (<2.5%) for the conventional cocoa (CC) farms within the Lixisols of Dry Semi-deciduous Inner Zone (LX.DSIZ) and the Ferralsols of Wet Evergreen (FR.WE) soil ecotypes. The average CN ratio was mostly adequate (30.5–40.8%). The base saturation of all the systems and the soil ecotypes was within the range of 80–95% of the ECEC, indicating that all the systems had high soil fertility levels. The ECEC was lower (<16.6%) for the organic cocoa (OC) and CC systems in the FR.WE than in the other soil ecotypes. The FR.WE were more acidic (H^+ = 14.7–26.1 $cmolc\ kg^{-1}$ with pH = 5.0 to 5.4) than LX.DSIZ and the Leptosols of Moist-Semi-deciduous South-East (LE.MSSE) for both organic and conventional systems. The same applies to the Na. The texture of the investigated soils was generally loam based on United States Department of Agriculture (USDA) textural classes.

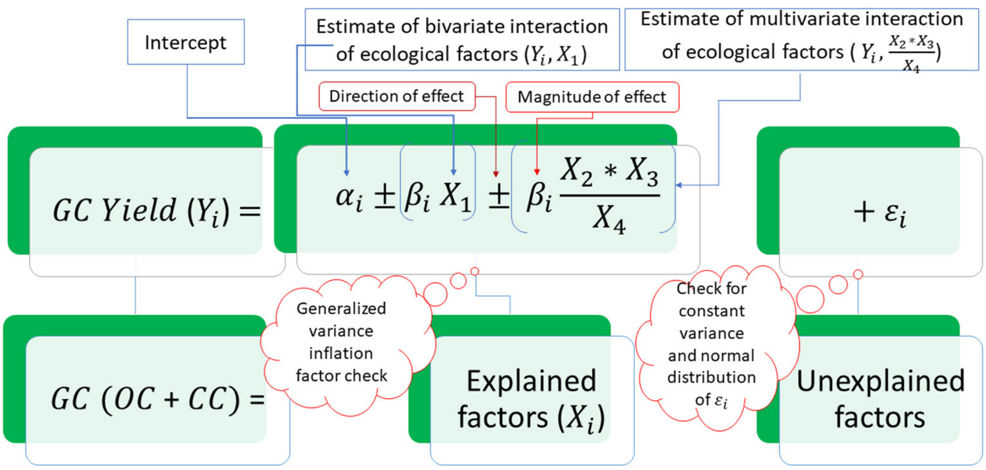

FIGURE 4
Schematic representation of multiple linear regression method for estimating factor effect on yield (Y_i) of green cocoa (*GC*) based on conventional cocoa (*CC*) and organic cocoa (*OC*) agroforestry systems.

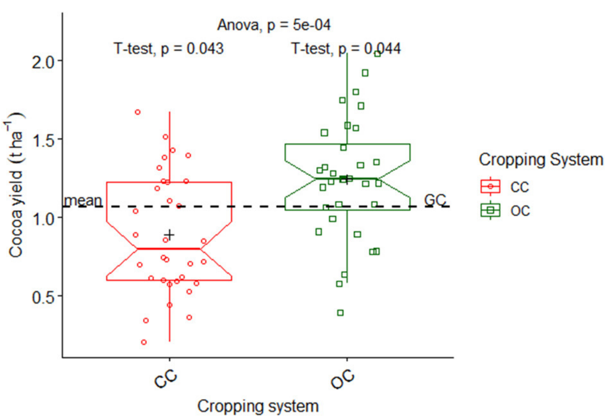

FIGURE 5
Yields of conventional cocoa (CC) and organic cocoa (OC); *t*-test compares the mean (+) yield of each cropping system to the overall mean (GC) represented by the dashed lines (—).

Silt (43.5%) predominated, followed by sand (37.7%) and clay (18.7%).

3.2. Estimates of conventional, organic, and green cocoa farm soil productivity levels

The total yield of the organic system (1.22 t ha^{-1}) was higher by 0.33 t ha^{-1} than the conventional system (0.89 t ha^{-1}). The average yield of OC and CC (dash line) which denotes the yield of green cocoa was 1.07 t ha^{-1} (Figure 5).

The cocoa varieties of the cropping systems were predominantly Amazonia (55.6%) and Hybrid (25.4%) cultivars. The mean cocoa tree plant density was 7.46 ± 2.58 per 23.4 m^2, which implies, for each hexagon (six equilateral triangles) or three rhombi, the planting density was exceeded by one plant. The organic system was lower in plant density per 23.4 m^2 (Pltdn = 6.43 ± 1.81) than the conventional system (Pltdn = 8.49 ± 2.85). The OC trees were older (17.40 ± 6.20 years) than the CC trees (13.70 ± 6.28 years). The mean number of black pods was 1 per tree (Bpod = 0.89).

The socioeconomic attributes of the farmers suggest that the cropping systems were predominantly managed by male cocoa farmers (79.9%) and a few females (20.1%). On average, both farmers were approximately 51 ± 15 years old. Approximately 57.1% of them had attended college (middle or junior high school). The farmers were natives (52.4%) and migrants (47.6%) who outrightly owned (25.4%) or inherited (44.4%) their cocoa farmlands. Each farmer had attended cocoa training (Ext) at least four times (Ext = 4.06 ± 2.24) during the last cropping calendar.

3.3. Synergy of bivariate correlation of the ecological factors

Figure 6 shows the bivariate (pairwise) correlation coefficients (r) explaining the relationships of the observed ecological factors. High blue color saturation signifies a high positive relationship. On the contrary, high red color saturation depicts a high negative relationship. The r illustrates the extent of desirability or undesirability of the relationships. There was a more desirable synergy of the factors, with the yield of OC and GC than the CC yield. For example, the cocoa yield had a significant positive correlation with female farmers (FSex) who also had a significant positive correlation with their farm soil organic CN ratio (Figure 6). In the three soil ecotypes, the cocoa yield increased significantly and insignificantly with aging of cocoa trees in the OC and CC systems, respectively (Figure 7). However, the correlation of the cocoa yield with soil available P was significantly positive for only the OC system in the FR.WE soil ecotype (Figure 8). The multiple regression analysis quantifies the effects of the factors.

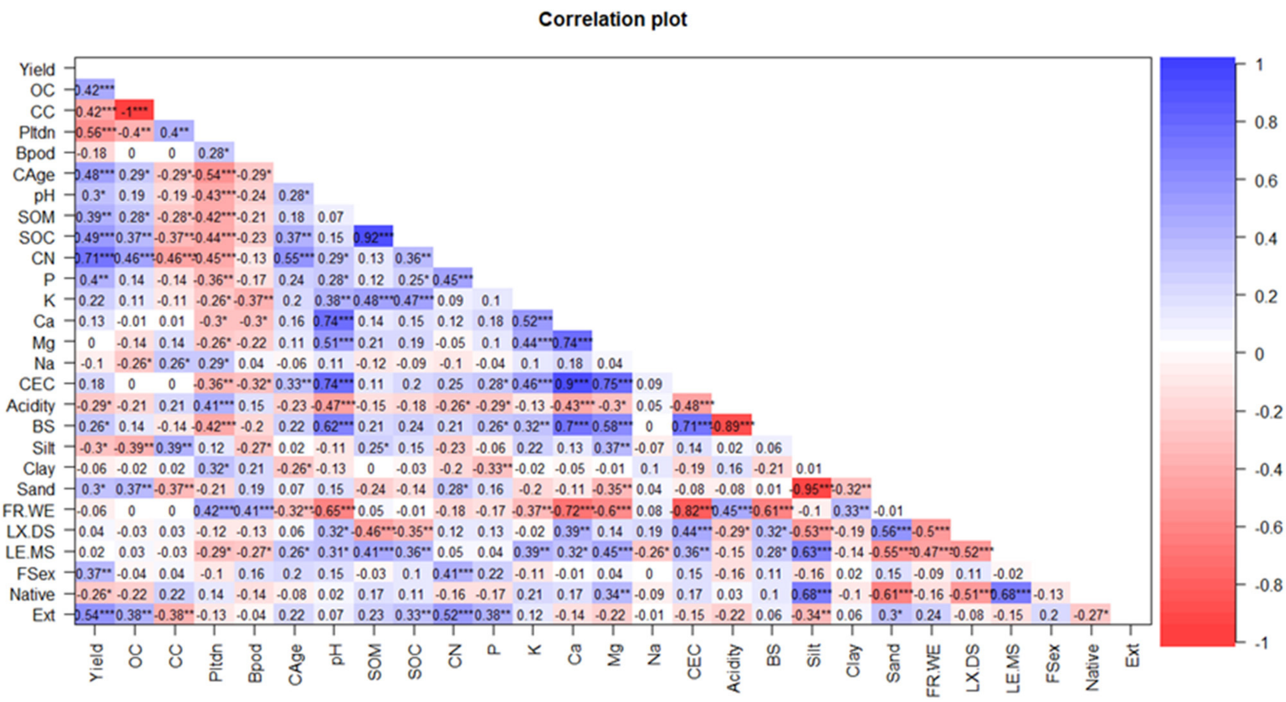

FIGURE 6
Pairwise correlation matrix showing the interrelationship of ecological factors within the observed cocoa agroforestry systems. High blue color saturation signifies a high positive relationship. On the contrary, high red color saturation depicts a high negative relationship. The values of the r show the extent of desirability or undesirability of the relationships, to illustrate the synergy of interactions of the ecological factors in the agroecosystems. Yield refers to green cocoa yield.

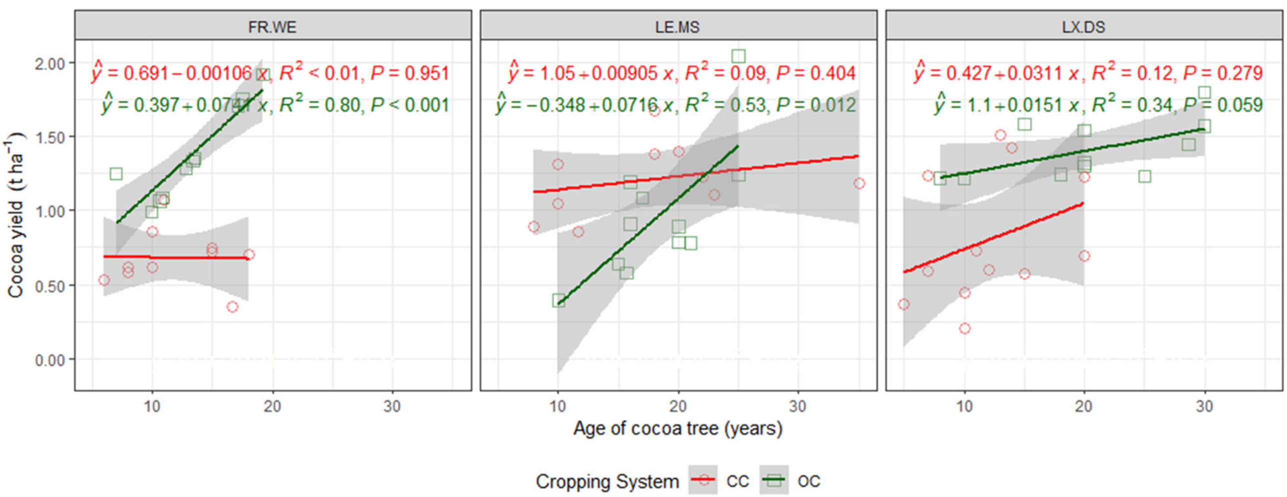

FIGURE 7
Trends of conventional (CC) and organic cocoa (OC) yields in relation to aging cocoa trees within the three soil ecotypes.

3.3.1. Estimate and diagnostic results of the green cocoa farm soil productivity function

Figure 9 and Supplementary Table 1 presents the OLS regression estimates of the green cocoa productivity function, and Table 2 shows the GVIF diagnostic results. As shown in Table 1, the mean GVIF$^{(1/(2^{*}\mathrm{Df}))}$ was close to 2 (2.034), which is equivalent to a VIF of 4.643, implying multi-collinearity was less than 5 (Fox and Monette, 1992; O'Brien, 2007). The diagnostics suggest that the $\hat{\alpha}_i$ and $\hat{\beta}_i$ are reliable for testing hypotheses and making scientific interpretations and decisions since all the assumptions of OLS (Zuur et al., 2009) have been met. For instance, the mean residual ($\hat{\varepsilon}_i = 0.005$) and standard error (0.2375) are close to zero (SW-test of residual = 0.991, $p = 0.931$). The heteroscedasticity (non-constant variance) was

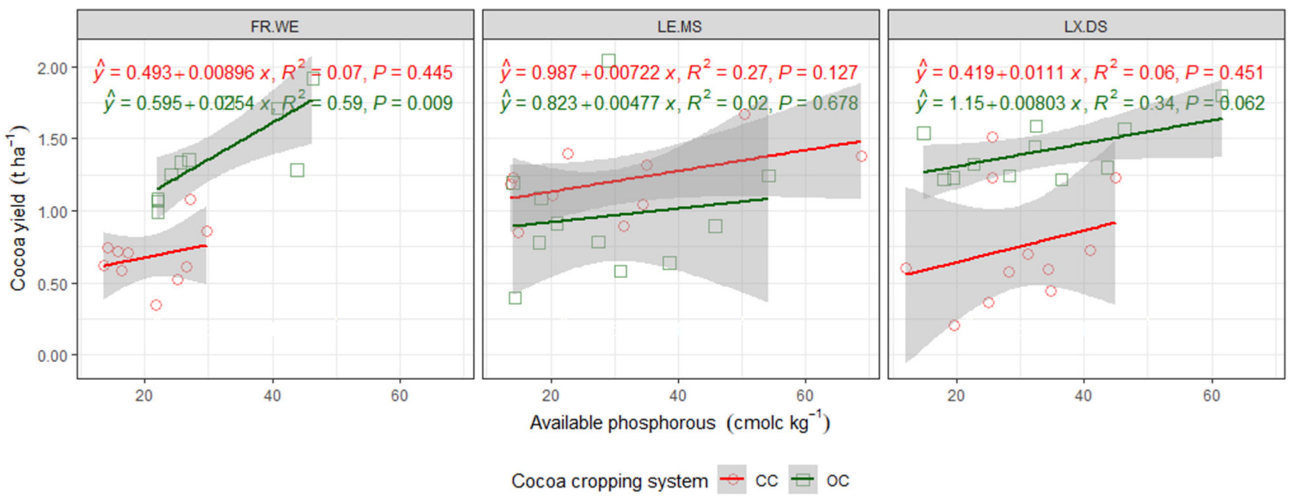

FIGURE 8
Trends of conventional (CC) and organic cocoa (OC) yields in relation to the concentration of available soil P (cmolc kg^{-1}) within the three soil ecotypes.

not statistically significant (chi-square = 3.155, Df = 1, p = 0.076). In addition, the ANOVA was significant (F-statistic = 10.98, $p < 0.0001$), indicating the function (regression) fits the data well. The adjusted R^2 (73.8%) suggests the explanatory variables jointly explained 73.8% of the variation in the observed cocoa yield.

3.3.2. Factors predicting the yield of green cocoa farm soil productivity and their magnitudes

There were 13 statistically significant ($p < 5\%$) predictors of green cocoa farm soil productivity and their magnitudes of determining the yield, as presented in Figure 9. Among the significant physical factors are CC ($\hat{\alpha} = -0.750\%$) and LE.MSSE ($\hat{\alpha} = -1.047\%$), which had negative effects on the yield, but their combined interactional (CC* LE.MSSE) effect was positive ($\hat{\alpha} = 0.732\%$). The interaction of CC and LE.MSSE had the largest positive effect (1.019%). The LE.MSSE (−1.047%) effect was negative. The positive significant socioeconomic factors were gender ($Fsex$: $\hat{\alpha} = 0.232\%$) and natives who had outright ($\hat{\alpha} = 0.745\%$) or inherited ($\hat{\alpha} = 1.009\%$) land ownership. The individual effect of being a native ($nativity$: $\hat{\alpha} = -0.589\%$) was negative. Of the crop biological factors, the cocoa black pod disease ($Bpod$: $\hat{\beta} = -0.154\%$) and the ratio of cocoa plant density to age ($\frac{Pltdn}{CAge}$: $\hat{\beta} = -0.164$) exerted adverse effects on the yield. In terms of the soil chemical factors, the responsiveness of the yield to $\frac{CN+pH}{10}$ ($\hat{\beta} = 0.616\%$), $\frac{K^*P}{BS}$ $\left(\hat{\beta} = 0.156\%\right)$, and $\frac{Ca+Mg}{K+P}$ ($\hat{\beta} = 0.191\%$) was positive. This implies, for instance, when the proportion of K and P in the Bs ($\frac{K^*P}{Bs}$) increases by 1%, the yield would increase by 0.156%, holding other factors constant. When $Bpod$ rises by 1%, the yield drops by 0.154%. The interaction of CN and pH exerted the largest impact of 0.616% among the soil chemical factors.

The double-log portion of the function suggests that the sum of $\hat{\beta}_i$ ($\sum \hat{\beta}_i = 0.8.71$) is <1, meaning there is a decreasing return to scale. In other words, when all the observed factors increase by $\lambda^{0.871}$, the GC yield increases by less than $\lambda^{0.871}$. Without the disease incidence ($Bpod$) and the interaction of cocoa plant density and age ($\frac{Pltn}{CAge}$), $\sum \hat{\beta}_i$ would be equal to 1.163. The 1.163 ($\sum \hat{\beta}_i$) denotes an increasing return to scale, suggesting that the yield would be more than double when all chemical inputs are doubled. Table 3 provides details on the marginal effects of each factor.

4. Discussion

4.1. Soil health status of the observed cocoa agroforestry systems

In general, the soil fertility indices (SOC, CN, pH, P, K, Na, H, Bs, and ECEC) measured in the three soil ecotypes were within the ideal soil health indicator ranges (Table 1). The findings are consistent with the reports of Ofori-Frimpong et al. (2010), Arthur et al. (2017), and Kongor et al. (2019) who also reported similar soil fertility indices. However, we found that the indices exhibited high variability, evident in the coefficient of variability (CV). The CV was either moderate (16–35%) or high (>35%) in SOC, CN, P, K, and ECEC. The moderate and high levels of variability raise soil health risks if the usual blanket approach to soil fertility management is to be applied (Dossa et al., 2018a,b; Doe et al., 2022). Hence, we recommend soil ecotype-specific soil fertility management or fertilizer regimes to minimize soil health risks and sustain healthy cocoa farm soil productivity in the study area.

4.2. The synergy of interrelationships of the factors affecting cocoa yield

The results showed significant ($p < 0.05$) correlations that demonstrate the synergy of interacting factors in the cocoa agroforestry systems, though most of the correlation coefficients

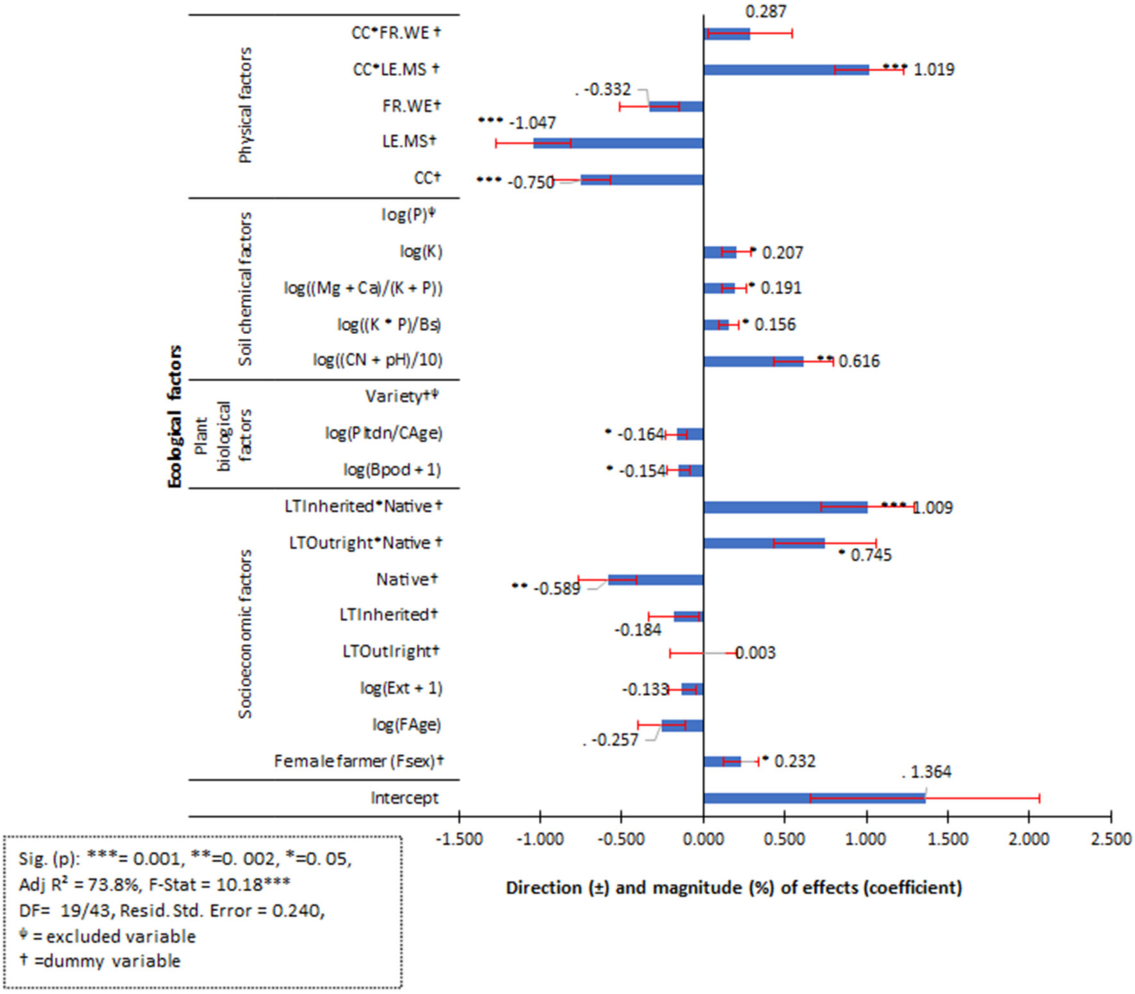

FIGURE 9
The ordinary least square regression estimates show factor influence on green cocoa farm soil productivity; † = dummy variable (Yes = 1, No = 0); φ = excluded variable.

($r\pm$) were weak (<0.5). The factors interacting in the green and organic cocoa systems were in more harmonic sync with cocoa farm soil productivity than the conventional system. For instance, the soil pH (r = 0.30), female cocoa farmers (r = 0.37), SOM (r = 0.39), P (r = 0.40), OC (r = 0.42), cocoa tree age (r = 0.48), SOC (r = 0.49), and CN (r = 0.71) correlated positively with the green cocoa yield. These factors were also positively correlated with the organic system but negatively correlated with the conventional system. These observations affirm Ofori-Frimpong et al. (2010) and Asigbaase et al. (2020, 2021) who recounted high SOC, SOM, pH, CN, P, and K in organic cocoa agroforestry systems.

In addition, the observed positive relationship (r = 0.41) between the green cocoa yield and CN/SOC in fields owned by female farmers suggests that CN/SOC accumulation was impacted by gender differences in cocoa management practices. This finding is congruent with the previous report by Mensah et al. (2021) that women were more environmentally conscious than men. The finding suggests that prioritizing gender support for women can improve soil health and productivity for green, conventional, and organic cocoa cropping systems. This could be achieved through intensifying agricultural extension services, financing, and mechanical or physical labor support for the women.

Corroborating Ofori-Frimpong et al. (2010), the SOC (r = −44) and CN (r = −45) were negatively related to overstocked cocoa tree density. Similarly, correlations of the green cocoa yield with black pod rot (r = −0.18), silt (r = −0.30), and planting density (r = −0.56) were negative. These findings confirm the report by Asante et al. (2021), who argued that poor cocoa planting distance and disease management are bottlenecks to closing the conventional cocoa yield gap in Ghana.

Consistent with Arthur et al. (2017) and Quaye et al. (2021), we observed that the green cocoa system had negative correlations with soil pH (r = −0.65), K (r = −0.37), and CEC (r = −0.82) in the Ferralsols of Wet Evergreen (FR.WE) soil ecotype. The FR.WE are usually acidic (<5.6) in nature due to high rainfall and leaching, and high acidity reduces their cation exchange capacity (CEC).

On the contrary, the Lixisols of the Dry Semi-deciduous Inner Zone (LX.DSIZ) (r = 0.44) and Leptosols of Moist-Semi-deciduous

TABLE 2 Estimates of generalized variance inflation factors for the green cocoa productivity function.

Ecological factors	GVIF	Df	GVIF^(1/(2∗Df))
FSex	2.148	1	1.466
log(FAge)	2.098	1	1.449
log(Ext + 1)	1.836	1	1.355
LT	33.754	2	2.410
Native	8.870	1	2.978
log(Bpod + 1)	1.618	1	1.272
log(Pltdn/CAge)	2.210	1	1.486
log((CN + pH)/10)	2.962	1	1.721
log((K ∗ P)/BS)	1.660	1	1.288
log((Mg + Ca)/(K + P))	2.355	1	1.535
log(K)	2.206	1	1.865
CC	9.350	1	3.058
SE	58.697	2	2.768
LT:Native	113.950	2	3.267
CC:SE	37.689	2	2.478
Overall mean GVIF	19.943		2.038

GVIF, generalized variance inflation factor; Df, Degree of freedom.

TABLE 3 Marginal effects of the determinants of green cocoa productivity.

Ecological factors		Marginal effect	Sig.
Socioeconomic factors	Female farmer (Fsex)†	1.181	*
	log(FAge)		
	log(Ext + 1)		
	LTOutlright†	1.455	
	LTInherited†	1.971	
	Native†	−1.203	**
	LTOutright*Native†	2.867	*
	LTInherited*Native†	2.867	***
Plant biological factors	log(Bpod + 1)	−0.076	*
	log(Pltdn/CAge)	−0.385	*
	CAge	−0.024	
	Pltdn	0.011	
Soil chemical factors	log((CN + pH)/10)	0.365	**
	CN	0.465	
	pH	0.465	
	log((K * P)/Bs)	0.388	*
	Bs	−0.002	
	log((Mg + Ca)/(K + P))	0.119	*
	K	1.106	*
	P	−0.001	
	Ca	0.014	
	Mg	3.036	
Physical factors	CC†	−1.604	***
	LE.MS†	−3.500	***
	FR.WE†		
	CC*LE.MS†	2.661	***
	CC*FR.WE†		

Sig. code (p): = 0 "***", 0.001 "**", 0.01 "*" 0.05, "." 0.1.
†A dummy variable (Yes = 1, No = 0), ψExcluded.

South-East (LE.MSSE) ($r = 0.36$) were positively correlated with CEC and soil pH. While the LE.MSSE had positive correlations with soil pH ($r = 0.31$), SOC ($r = 36$), K ($r = 0.39$), and SOM ($r = 0.41$), the LX.DSIZ had adverse correlations with soil pH ($r = -0.32$), SOC ($r = -35$), K ($r = -0.02$), and SOM ($r = -0.46$). These findings are consistent with FAO and ITPS (2015), who reported that the correlation between the observed soil ecotypes and their soil properties is due to parent materials and the climate of the soil ecotypes.

4.3. Yields of organic and conventional cocoa combined to form green cocoa yield

The observed average green cocoa yield (1.07 t ha^{-1}) exceeded the national mean of 0.50 t ha^{-1} for Ghana (FAOSTAT, 2021), and the farm level yield (0.21–0.65 t ha^{-1}) was reported by Abdulai et al. (2020). However, it is lower than the 23-year experimental cocoa yield (1.37 t ha^{-1}), reported by Ramírez-Argueta et al. (2022), and the potential/water-limited yields (2.5 to 3.5 t ha^{-1}) reported by van Vliet and Maja Slingerland (2015) and Asante et al. (2022). The average crop yield curve of farmers usually plateaus (flattens) around 75-80 % of the potential yield ceiling (Dobermann et al., 2013). The maximum water-limited/potential yields are usually high because they are obtained in a controlled environment (climate), with sufficient plant water, soil nutrients, and low pests and diseases Dobermann et al., 2013; Van Ittersum et al., 2013). Smallholder farmers generally operate under rainfed conditions, characterized by all the potential yield-limiting factors, including soil ecotype and socioeconomic constraints. To reduce the yield gap, high-yielding local cocoa varieties that are resistant to abiotic and biotic stresses and healthy soil fertility management practices are required. In addition, financial constraints, limited availability of organic and inorganic fertilizers, and agrochemicals to smallholders (Amponsah-Doku et al., 2021) need to be addressed per soil ecotype. Given the conditions described above, we recommend an integrated ecological cropping system such as the green cocoa cropping system to enhance the sustainability of agroecosystem services while closing yield gaps.

We also found a higher yield for OC (1.24 t ha^{-1}) compared with the CC (0.89 t ha^{-1}), which confirms the accounts of Badgley et al. (2007), Rajab et al. (2016), and Bandanaa et al. (2021) that OC systems and cocoa shade diversity are equally productive as the conventional monocrop cocoa systems. However, the results contradict the findings of Asigbaase et al. (2020) who found that the yield of OC was 30% lower than that of CC. Several ecological factors explain the aforementioned superiority of the GC and OC yields to the CC yield. Ofori-Frimpong et al. (2010), Tscharntke et al. (2012), and Somarriba et al. (2013) attributed high yield in OC systems to superior biodiversity and physicochemical soil quality build-up over time. On the contrary, Herzog et al. (2019) and Smith et al. (2019) found that the duration (age) of organic cocoa cropping systems had no relationship with the yield. The present study demonstrated that the cocoa yield of the organic cropping system increased with aging of the cocoa trees and their physicochemical properties compared with the CC system. This demonstration is consistent with the findings by Ofori-Frimpong et al. (2010), Tscharntke et al. (2012), Somarriba et al. (2013), and Asigbaase et al. (2020) who argued that the OC system builds up more soil physicochemical properties over time than the CC cropping system. Thus, improved soil physicochemical properties in the OC and GC systems simulate plant growth, prolong plant life, and increase yield. The build-up of physicochemical properties in the OC and GC systems emanates from integrated organic soil and crop husbandry practices. Integrated organic husbandry practices such as the use of bioinsecticides and biological pest control and application of organic manure and compost increase soil organic matter. The soil organic matter provides a binding agent that improves soil structure and stimulates soil microbial activities (Asigbaase et al., 2020, 2021). An improved soil structure retains soil moisture and nutrients, while soil microbial activities aid in the mineralization of other soil nutrients, such as plant available P and exchangeable K for uptake by cocoa plants. Furthermore, the use of bioinsecticides and biological (natural) pest controls in the OC and GC systems enhances the defense mechanisms of cocoa plants against insect pests that cause yield loss (Krey et al., 2020; Akesse-Ransford et al., 2021). On the contrary, the sole use of mineral fertilizers and pesticides in pure CC can kill or reduce the population of the natural enemies of the pests, leading to higher pest yield losses in pure CC relative to the OC and GG systems.

4.4. Ecological factors determining green cocoa yield and magnitude

For the ecological determinants (predictors) of green cocoa farm soil productivity, we found several significant factors based on the estimated cocoa productivity function. We found that the green cocoa yield responded positively to the LE.MSSE but negatively to the FR.WE. This is probably because of the higher concentrations of physicochemical properties in the LE.MSSE and LX.DSIZ than the FR.WE. The concentrations of P and K and percentages of CN, ECEC, and base saturation in the LE.MSSE and the LX.DSIZ were higher than those in FR.WE. These findings are not surprising because of the relatively higher rainfall regimes of the FR.WE compared with the LE.MSSE and the LX.DSIZ soil ecotypes. Doe et al. (2022) indicated that Leptosols in the Volta Region of Ghana have healthy soils due to their relatively larger clay loam content and deep SOM layer.

We also found that, while the age of the cocoa trees (CAge $= 16 \pm 7$ years) positively affected the yield, an overstocked cocoa plant density (per 23.4 m^2) and black pod rot disease occurrence inversely affected the yield, corroborating Ahenkorah (2016) and Kongor et al. (2018). The yield within the organic system appears more resilient to aging cocoa trees than the conventional system. This implies that older OC trees (17.40 ± 6.20 years old) were more productive than the younger CC trees (13.70 ± 6.28 years old). In addition, overstocking mature cocoa trees (>7 cocoa trees per 23.4 m^2) and having one black pod rot disease on each tree would reduce the cocoa yield. This observation underscores the undesirable humid condition associated with an overstocked cocoa plant population. The results reiterate the previous findings indicating that non-conformity to recommended regular plant distance (or plant density) reduces cocoa yield (Ofori-Frimpong et al., 2010; Asante et al., 2021). An overcrowded cocoa plant density redirects photosynthates and encourages competition (trunk and stem elongations) for sunlight at the expense of maturation, flowering, fruiting, and pod development. It retards ventilation and creates undesirable humid conditions that facilitate fungal diseases such as cocoa black pod rots (Ofori-Frimpong et al., 2010; Akrofi et al., 2015). Our findings suggest a need to intensify good agronomic practices such as regular pruning to improve plant spacing (Pltdn) and disease management using bioinsecticides.

The present study corroborates previous findings on planting distance (Ofori-Frimpong et al., 2010; Asante et al., 2021), and the novelty of our study lies in the method used to estimate the cocoa plant density on the field. Our method differs from the usual indicator variable approach (regular or irregular planting distance) reported in the study by Asante et al. (2021) and many other studies. Our method quantified the number of cocoa trees within a 23.4 m^2 hexagon, which is equivalent to three congruent rhombi of 3 m sides, which must ideally have seven cocoa trees, based on COCOBOD recommendation (CHED/WCF, 2016). This method unbiasedly permitted a more accurate measure of the cocoa planting density in often irregular stands of cocoa trees planted at stakes during the establishment by smallholder farmers. The method is, however, more laborious and time-consuming than the indicator variable approach, but it is certainly more accurate than the indicator variable approach.

Corroborating Asigbaase et al. (2021) and Li et al. (2021), we found that the combined effect of CN ($40.1 \pm 13.8\%$) and soil pH (5.8 ± 0.7) dominates the chemical determinants of green cocoa yield. The CN facilitates microbial activities for decomposing more SOM to enhance CEC and retention of cocoa plant nutrients (Somarriba et al., 2013; Rajab et al., 2016; Eddy and Yang, 2022). This explains why soil pH, acidity, SOM, and SOC management are widely accepted as a technique for restoring soil health and fertility for sustainable food crop production and mitigating climate change.

We also found that the overall base saturation (Bs $= 0.917$) was high (91.7%), but a majority of the individual elements (Ca, Mg, K, and Na) were insignificant. This implies that the decomposition of the individual effects of the elements is essential for farm-level soil fertility and productivity management decisions. However, the combined effect of the elements is even more essential. The positive effect of $\frac{K^*P}{Bs}$ is congruent with Ahenkorah (2016) and Dogbatse et al. (2021). This finding suggests that when the proportion of K^*P to Bs ($\frac{K^*P}{Bs}$) increases by 1%, the yield of GC would increase by 0.156%, holding all other factors constant. In addition, the significance of $\frac{Ca+Mg}{K+P}$ implies holding all other factors constant, this set ($\frac{Ca+Mg}{K+P}$) of determinants would generate a 0.191% extra green cocoa yield. Another implication of the interaction effects of both $\frac{K^*P}{Bs}$ and $\frac{Ca+Mg}{K+P}$ is that managing the proportion of $Ca + Mg$ to $K + P$ well is critical for green cocoa farm soil productivity. This is essential, particularly when the individual elements have insignificant effects on the yield.

We are not recommending a continuous application of P, Ca, K, and Mg beyond their maximum thresholds. As a rule of thumb, the theory of base cation saturation ratio (Gaspar and Labosk, 2016) limits the proportions of Na^+, H^+, K^+, Mg^{++}, and Ca^{++} to 0–1%, 0–10%, 4–8%, 12–25%, and 65–80% of the base saturation (Bs), respectively. The results showed that the proportion of K (1.2%) to the Bs of the observed soil was far lower than the ideal minimum of 4%. The same applies to the proportions of Mg (40.9%) and Ca (53.9%) to Bs. On the contrary, the Bs of Na and H exceeded their maximum proportions of 1% and 10%, respectively.

The observed soils appear fertile on the face value of Mg and Ca but limiting in K base saturations. Therefore, the positive influence of the combined effects of K, Ma, and Ca on green cocoa yield is only attainable through effective management of soil fertility and soil health. This can be carried out by enriching the SOC, Ca, Mg, and K contents while reducing the sodic (salty) and acidic effects of excessive Na and H, respectively. Common SOC sources in Ghana are organic manure, compost, rice husks, corncobs, biochar, cocoa pod husk potash, and empty oil palm bunches.

It was also noted that the marginal effect of exchangeable K on the green cocoa yield was greater than the marginal effect of available P, which implies P is the most limiting factor, as reported by Dossa et al. (2018a,b). According to Liebig's law of the minimum, soil productivity is determined by the most limiting soil nutrient or factor. The available soil P limitation can be addressed by tapping total P or developing a P-efficient cocoa variety to limit the amount of P lost to the environment.

Improving the efficiency of P-use can also be achieved using soil microorganisms in a root-foraging strategy, where plants uptake more P at a lower P critical level (Richardson et al., 2011). For instance, Richardson et al. (2011) employed P-mining strategies to enhance P-desorption, solubilization, and mineralization of soil nutrients when P was sparingly available in the soil.

Nevertheless, the dominant effect of the exchangeable K and the limiting effect of the available P have important implications for soil nutrient amendment and cocoa site-specific fertilizer formulation. The K effect suggests that the conventional farms in LX.DSIZ and FR.WE, require more P than K. The organic farms in the LE.MSSE require more of the same because the Ferralsols and Lixisols have a lower soil pH than the Leptosols. Liming materials (Calcipril, limestone, and dolomite) are effective in acidic soils and may help in P dissolution, unlocking available P, decreasing A^{+++}, and increasing exchangeable base cations (Ca^{++}, Mg^{++}, and K^+).

5. Conclusion

The study proved that the observed cocoa systems exhibit high soil health, fertility, and yield. The cocoa farm soil productivity was high in the order as follows: conventional cocoa (CC) < green cocoa (GC) < organic cocoa (OC), particularly in the landscapes of FR.WE, LX.DSIZ and LE.MSSE soil ecotypes of Ghana. For all three soil ecotypes, it was concluded that green cocoa yield responds positively to female cocoa farmers because female cocoa farmers correlate positively with the soil organic carbon and nitrogen ratio in their farms. The cocoa productivity within the organic system appears more resilient to aging cocoa trees than the conventional system. One black pod rot disease in an overstocked mature cocoa plant density per 23.4 m^2 can reduce cocoa yield. While soil ecotype predominates soil physical factor effects, the combined effect of soil chemical factors such as CN ratio and pH dominates the yield of green cocoa productivity. Doubling the sum ($\sum \hat{\beta}_i$) interaction effects of soil pH, CN, P, Ca, Mg, K ($\frac{CN+pH}{10}$, $\frac{K^*P}{Bs}$ and $\frac{Ca+Mg}{K+P}$) more than doubles the yield, holding limiting factors like diseased pods, aged and overcrowded cocoa tree density constant, among others. Thus, the critical determinants of green cocoa (integrated CC+OC) productivity are not limited to the individual effects of CN ratio, pH, P, Ca, Mg, and K but their combined synergy of effects including soil ecotype, cocoa plant density, black pod disease, and aging of the cocoa trees. In addition, gendered support for female cocoa farmers owning farmlands is likely to be a major determinant of green cocoa farm soil productivity. Hence, promoting female cocoa farmers with SOC, CN, and pH conservation is critical to soil health and green cocoa development in Ghana. These conclusions are pertinent to the LE.MSSE, LX.DSIZ, and FR.WE soil ecotypes.

The research has expanded the boundaries of ecological theory, specifically the concepts of cocoa green productivity, soil health, cocoa farm diagnostics, and yield improvement strategies in smallholder cocoa agroforestry systems. The study demonstrates a quantitative ecology methodological pathway for generating empirical evidence on the determinants of green cocoa productivity and cocoa agroecosystem assessment at the farmer/farm level. This includes methods for quantifying cocoa plant density in an irregularly spaced field of cocoa trees.

Limitations: we recognized that statistical regression models of crop production are a simplified version of the actuals. Topographic factors such as altitude and slope of farmland were held constant. Owing to the rainfed nature of cocoa farming in the study area, soil moisture, and climatic factors such as rainfall, relative humidity, sunshine, and evapotranspiration were assumed to be constants per soil ecotype.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee for Humanities, University of Ghana. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ED: research idea, concept and design, fieldwork, data analysis, and writing of the manuscript. EA, PO, and AQ: supervision, contributing, and commenting. BF-M: contributing, commenting, and editing. All authors reviewed and approved the manuscript submitted.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1169015/full#supplementary-material

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The evolution of organic food certification

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The surge in the development of the organic food movement is in response to mass industrial food production, prioritizing productivity and economic profit across the global food supply chain, the cost of individual human health, the nutritional value of products, environmental degradation, and climate change. In recent decades, bio-certified food has become especially important to farmers, consumers, and policymakers as a viable transition away from high-input, intensive farming methods to a more humane and sustainable food system. However, to create value and a point of distinction in the marketplace, a robust and valid operation system to verify organic standards throughout the supply chain is of utmost importance. In this study, we conducted two separate surveys. The first survey targeted active organic farmers from three countries. Based on the data obtained, we confirm, similar to other investigations, that the current system of bio-certification is not reliable with a certain degree of probability. The second survey consulted highly specialized experts in organic systems from around the world to identify how the bio certification system should be transformed. The results indicated that the average probability of unregistered violations can be 35.4% according to self-reporting by organic farmers. This together with results that found that 96.12% of experts believe that the organic certification procedure needs to change provides increasing evidence and justification for an overhaul of the certification system.

KEYWORDS

food certification, bio certification, organic food, organic certification, organic farming, organic agriculture, food supply chain, certification body

1. Introduction

1.1. Development of the organic certification

Organic farming was first mentioned in 1924. Rudolf Joseph Lorenz Steiner (Staudenmaier, 2008), an Austrian social reformer, held the first courses on organic farming among 111 attendees in the city of Koberwitz, Poland (Paull, 2011). His writing "Spiritual Foundations for the Renewal of Agriculture" on biodynamic agriculture was published in the same year in Germany, which was probably the first reporting of organic farming as a complex system (Vogt, 2007; Von Friedeburg, 2018).

Organic food and farming systems are now defined by not using chemicals, in particular, synthetic pesticides and fertilizers, toxic herbicides, chemical additives, hormones, solvents, and genetically engineered materials (Allen and Albala, 2007). Therefore, until relatively recently agriculture was in essence organic, but the modern-day term organic has emerged in response to the mass industrialization of the food system, as such a large supply of food products are now non-organic (Drinkwater, 2009). In fact, modern organic standards prescribe that "bio" products must be 100% organic and strictly only up to 5% inorganic inclusions are admissible for processed products (Council Regulation (EC) No 834/2007, 2007; Safe Food for Canadians Regulations, 2022).

The earliest documents regulating the production and labeling of organic products at the EU level were published in 1991. In 2023, revised regulations of the European Commission 834/2007 and 889/2008, and a new EU organic regulation 2018/848 of 30 May 2018 were implemented. These policies describe the basic requirements for healthy food production and particularly the labeling of organic products in Europe. In the context of the legislation, healthy food is referred to in many terms, such as organic food, ecological food, biological food, and their derivatives and diminutives, such as "bio" or "eco," alone or in combination (Regulation (EU) 2018/848, 2018). Such names are given to food and drinks which are produced in compliance with the standards of organic farming at all stages of production, preparation, and distribution. Organic products that are recognized by European standards are marked with an organic label which is often named "Euro-leaf" (Figure 1).

However, the rules of organic farming vary depending on the country of operation and the certification scheme itself. For example, in Germany, there were 17 bio-certification bodies in operation as of 2019 (Federal Ministry of Food Agriculture in Germany, 2020) and 46 organic entities in Spain as of 2022 (Organic Farming Information System Agricultural, 2022). However, all associated rules are united by common principles of ecological food production. These include the promotion of ecological balance, renewal and cycling of resources, conservation of biodiversity, restriction of chemical pesticides, toxic herbicides, synthetic fertilizers and additives, a ban on genetically modified organisms, ensuring crop rotation and companion planting, and enhancing soil fertility and water quality, among others (European Court of Auditors, 2018; Regulation (EU) 2018/848, 2018). Organic certification regulation in non-EU countries where a regulation has been developed does not fundamentally differ. For instance, in Canada, an organic product is an agricultural or aquacultural product that has been certified organic under Part 13 of the Safe Food for Canadians Regulations (SFCR; SOR/2018-108). To obtain the certification, operators must have their products certified by the Canadian Food Inspection Agency (CFIA), the accredited certification body, and develop an organic production system based on the Canadian Organic Standards CAN/CGSB 32.310 (311,312). The European Union (EU) and Canada recognize that their respective organic production rules and control systems are equal to each other (Canada.ca, 2016, 2023). This equivalency agreement means that certified organic products meeting Canadian or European organic standards may be sold and labeled as organic products in both the EU and Canada.

FIGURE 1
EU organic label.

The Directorate-General for Agriculture and Rural Development (DG AGRI) deals with managing and developing the EU organic platform of the Common Agricultural Policy. The Directorate-General for Health and Food Safety (DG SANTE) covers the enforcement of EU legislation on food safety, animal health, animal welfare, and plant health, providing validity to organic production by evaluating compliance with EU standards. In turn, Member States are responsible for monitoring and controlling compliance with EU standards and establishing the type of control system (private, public, or both) as well as the number of control entities (European Court of Auditors, 2018). The International Federation of Organic Agriculture Movements (IFOAM) is an umbrella organization that promotes the organic movement worldwide and offers organic accreditation to certification bodies (ifoam.bio). Finally, the European Organic Certifier Council (EOCC) is the head certification association that represents local organic control bodies within Europe, aiming "to increase the reliability of control and certification activities and decisions in relation to European organic agriculture" (eocc.nu).

The entire system of organic certification is based on the control of compliance with the criteria for organic production. This applies both to the initial receipt of an organic certificate and to the subsequent renewal of its validity every year. These tasks are performed by the certification bodies. A control or certification body (CB) is an independent third party or public administrative entity of a Member State that has been accredited for a sector and carries out local certification services. Within organic production, this includes making decisions for organic certification by satisfying at least a minimum set of formal requirements by conducting onsite inspections and sampling tests and establishing administrative methods of control. Where an operator is compliant, the CB issues a certificate that assures the adherence to the underlying organic standards and empowers the operator to put the Euro logo on their products (Gantz, 2014; Zezza et al., 2020).

1.2. The basic types of CB's control in the EU

The following types of control market operators are the main methods of certification bodies and are based on the key regulation documents: EC 2092/91; EC 178/2002; EC 2003/2003; EC 834/2007, EC 889/2008; 1235/2008; and EC 1107/2009. They are also summarized in the latest EU 2018/848.

1.2.1. Mandatory announcements from operators

This type of control occurs as part of the provision of mandatory required information. In particular, the bio-certified operator should immediately inform regulators about any irregularity or infringement concerning the organic status of its product/farm or organic products obtained from other economic operators or subcontractors. Moreover, bio-organic operators should record all data concerning production. Such records must provide at least the following information: (a) use of fertilizers: date of application, type and quantity of fertilizer, and land utilized; (b) use of plant protection products: reason and date of treatment, type of product, and method of treatment; (c) purchase of inputs to the farm: the date, type, and quantity of products purchased; and (d) harvest date and the type and quantity of organic or conversion crop.

1.2.2. Regular inspection

Physical inspection for each contracting operator is done at least once a year. In the case of agricultural producers who produce crops, it must be carried out during the growing season or until harvesting certified crops. A responsible representative of an operator must be present at the agreed date of the inspection, which will provide the inspector with binding information and allow access to the relevant documents. The inspection visit covers all processes that are associated with the production of organic products. In particular, (a) inspection of production facilities (storage facilities), inspection of premises and packaging facilities and control of stored products; (b) control of production processes including separate bio-production flows; (c) control of input and output documentations and products; (d) verification of supplier certificates; (e) control of the flow of goods; and (f) final interview.

1.2.3. Product taken for analysis

At a minimum, 5% of operators must have soil samples for analysis. The selection of operators to be sampled is based on a general assessment of the risk of non-compliance with organic production rules according to International Sustainability and Carbon Certification (ISCC) risk categories (ISCC, 2021). This overall assessment takes into account all stages of production, preparation, and distribution. The public or private inspection bodies take and analyze samples whenever there is a suspicion that products or processes that are not authorized for organic production are being used. This is conducted through a primarily unannounced inspector based on a general risk assessment of non-compliance to organic production rules.

1.2.4. Unannounced inspection

A minimum of 10% of contracting operators a year will undergo an unannounced inspection. CBs perform irregular and unannounced inspections, based on risk assessment, where operators with higher levels of risk should be included in the plan of unannounced inspection.

Table 1 lists the recognized types and subjects of control for a common European producer of organic grapes.

The other operation types, such as pruning (cut of shoots), general maintenance, wire adjustment, disbudding (removal of shoots), canopy management, shoot thinning, crop measurement, netting, and bunch counts, were not clearly identified in relation to any controlled criteria by relative baseline legislation and thus they are not covered under control measures.

1.3. Criticisms regarding bio-certification practices

There are a few studies in the literature that investigate the consistency of third-party certification. According to the investigation of Fouilleux and Loconto (2017), some variations in how CBs operate can lead to consumers' disappointment and even fraud. The reasons for the discrepancies in the evaluations of CBs may significantly depend on how CBs interpret the standards and non-quantifiable recommended practices (Fouilleux and Loconto, 2017). Bar and Zheng (2019) found that food operators are inclined to collaborate with those CBs who are the most loyal. The bio-certification model assumes that the financial success of CBs is also equally dependent on the loyalty of the operators themselves, as it directly depends on their fees and payments. Furthermore, Kononets and Treiblmaier (2020) found that in practical terms some large German retails do not trust most bio-certification schemes and, therefore, have instigated their own procedure for investigating the "purity" of producers and their products,

TABLE 1 Current control methods and basic criteria for activities on vineyards.

Operation type	Controlled relevant criterion	Relevant type of control
Herbicide Fungicide	Only the preparations listed in mentioned regulations may be used as basic substances (including lecithins, sucrose, fructose, vinegar, whey, chitosan hydrochloride, Equisetum arvense, etc.) Substances which should not be used as herbicides but only for protection against pests and diseases. Calcium hydroxide, when used as a fungicide, only on fruit trees.	Mandatory announcements; Regular inspections; Product analysis; Unannounced inspection
Fertilization (elemental *N*)	Mineral nitrogen fertilizers (N) should not be used	
Fertilization (elemental *P*, organic *P*)	Only phosphorus (P) fertilizer with a cadmium content not exceeding 90 mg/kg P205. Use is limited to alkaline soils (pH > 7.5)	
Fertilization (organic *N*, e.g., mulch)	170 kg of nitrogen (N) per 1 year/1 hectare of utilized agricultural area. This limit only applies to the use of manure from the holding.	
Pick (harvest fruit)	The inspection may take and analyze product samples based on the risk assessment to detect products which are not authorized for organic production, to verify production processes which do not comply with the rules of organic production, or to detect possible contamination by products not authorized for organic production.	Product analysis

Source: Council Regulation (EC) No 834/2007 (2007), Thompson et al. (2012), Glavan et al. (2020), and EC 889/2008, adjusted by the authors.

including analysis of production facilities and physicochemical analysis of products, before issuing a contract with producers.

Furthermore, recent investigations in Italy found that there is a direct dependency between the audit outcome and the CB performing the audit, and the probability to fall under sanctions of a CB directly depends on the share of unannounced spot inspections of a particular CB (Zezza et al., 2020). Earlier, Gambelli et al. (2014) studied a likelihood of detected violations in different adoption measures one of the local CB. In German food quality control industry were found the same regularities (Zorn et al., 2012; Bravo et al., 2013). However, based on the European Court of Auditors (2019), the control system for organic products has improved, but some challenges still remain. For example, in the Czech Republic, several cases have been found where the Certificate of Inspection stated results of laboratory analyses were not actually produced and vice versa. According to the European Court of Auditors (2019), there are two control bodies in Italy, which carry out most inspection visits each year; however, these inspections are uneven throughout the year and excessively inefficient in terms of plant production. Based on another report on the overall operation of official controls performed in Member States (2019–2020), it was found that EU programs were effective in meeting the targets on the prevalence of *Salmonella* bacteria. However, the rate of detection of Salmonella levels in samples taken by private third parties of control was on average essentially lower than that of the official tests by state authorities (EC Report, 2022). Therefore, considering that private third parties cover much more territories, this discredits the organic industry as a whole.

Based on key indicators globally and particularly in the EU zone during 2000–2020, organic agriculture has been growing rapidly for the last 20 years (Table 2). This presents increasing operational burden and bureaucracy on control entities, such as increased data volumes, which can exacerbate the current issues and criticisms of private CBs (Table 2).

1.4. Review of possible improvements of the organic certification procedure

To improve organic control mechanisms, a number of suggestions have emerged from the research literature. For example, a mechanism of enhanced supervision and prevention of both intentional and unintentional types of fraud (Padel et al., 2010). More recently, it has been proposed to reinforce the risk-based approach to controls and surveillance activity, particularly, to balance controls with vigilant risk analysis and standardization of procedures at both national and EU levels. The rotations of CBs and inspectors and further standardization of fees and procedures in combination with regular third-party audits, are some further focused measures recommended to improve the certification model for the organic market (Zezza et al., 2020).

The European Court of Auditors (2019) published recommendations for improving the control system for organic products in the EU, recommending to the EC to: (a) address remaining weaknesses in Member State control systems and reporting; (b) improve supervision over imports through better cooperation; and (c) carry out more complete traceability checks. In particular, the EC stated that too many products still cannot be adequately traced back to provenance. Furthermore, a recent EC report concluded that there was a need to improve the transparency and traceability of animal-origin proteins across the food supply chain (EC Report, 2022).

1.5. Research objectives

Previous research suggests the current system of bio certification is not 100% reliable, which makes it possible for violations and deviations from the principles of organic farming to occur. Therefore, collecting supporting evidence on the degree of such deviations in practice is the first objective of this investigation. The reliability of the bio-certification system cannot be, in some cases, supported by the faith and hope of final consumers that bioproducts are produced under conditions that fully meet the principles of bioproduction. Thus, the outcome of the first purpose of the study could be expressed by confirming or rejecting the following hypothesis.

Hypothesis: Accepted organic certification schemes do not guarantee 100% compliance with the principles and rules of organic production, regardless of where organic production is located.

The hypothesis can be considered confirmed only if 100% of randomly surveyed farmers in different countries with a valid bio certificate confirm a positive probability (>0%) of a violation of the principles of organic production without subsequent identification by the inspection body. Otherwise, the hypothesis is rejected.

TABLE 2 Key indicators on organic agriculture for the last 20 years in the EU zone.

Year	Organic area (farmland) [ha]	Organic area share of total farmland [%]	Organic producers	Organic retail sales [Million €]	Growth of the organic market for the period, %
2000	3’805’916.00	2.19	132’151.00	5’557.90	-
2005	5’860’227.04	3.57	159’818.00	8’848.10	+59.2
2010	8’374’614.45	5.10	215’472.00	16’069.98	+81.6
2015	10’639’203.07	6.54	265’677.00	24’896.44	+54.93
2020	14’868’779.52	9.16	349’499.00	44’829.75	+80.7

Source: Research Institute of Organic Agriculture (2021); the column “Growth” is developed by the authors.

The second objective of this study is to investigate the optimal structure for collecting, storing, and distributing data from organic farm sites and the level of decision making for organic certification. These two factors depend directly on the degree of transparency of the entire bio-certification system. Of particular value is the combination of these two aspects into a single relationship. Thus, the final outcome of the second objective of the study will be the discussion of a possible new model with promising bio-certification procedures based on the obtained data. Any alternative organic certification system will need to provide a 100% guarantee of the organic origin of products. The current study also intends to investigate the emerging approaches in the evolutionary development of the bio-certification model and, in particular, try to determine the possible extent of the use of digital technologies into decision making in bio certification, which could have a possible effect on the degree of current objectivity in the given process and avoid the issues associated with human error. For example, Kononets and Treiblmaier (2020) stated a very high likelihood of 55% that digital contracts in the food supply chain will eliminate the impacts of human mistakes and intentional unfair practices.

2. Materials and methods

2.1. Anonymous survey of bio farmers

The goal of an anonymous survey of bio farmers was to determine the approximate level of self-reported violations by the producer of food products that for some reasons were not identified by a certification body. Since this kind of information is not currently available, an anonymous survey of farmers working under any bio certification label was utilized. Conducting scientific observations or practical tests in the field to determine the required data was not possible. Anonymous surveys were conducted only in certain countries of the EU based on the historical, geographical, and statistical data. Germany has the most developed culture of bio production with the oldest history and experience and the largest number of public and private bio certification organizations that authorize and certify bio productions (Organic Farming Information System Agricultural, 2022). The Czech Republic was also chosen as one of the largest shares (11.24%) of farmers managing only organic or partially organic land, with an overall European average of 2.37% in 2016 (Eurostat, 2016). In addition, the Czech Republic ranks fifth in Europe in the percentage (15.33%) of total organic area (fully converted and under conversion to organic farming) while Germany (9.59%) is the closest to the European average of 9.08% among the EU-28 countries (Eurostat, 2020). Of additional scientific interest in these particular countries is the data that show that Germany has the highest percentage increase of 65.75% in the total organic area (fully convertible and under conversion) between 2012 and 2020. At the same time, the Czech Republic, one of the leaders in the percentage of organic farmers and organic land, showed the worst growth rate of equivalent area, only 15.3% over the same period (Eurostat, 2020). Although it is worth noting that the Czech Republic and Germany do not represent all the countries of the EU, however, they can be seen as the most representative not only in terms of statistical data and one of the most ancient traditions of eco-farming but also in terms of their location in the temperate transitional climate within Europe.

To test our hypothesis that violations of the rules and principles of organic certification are likely in any developed country, including a non-European country, we intended to find an agrarian country with different but also developed organic certification standards. Canada met this criterion well. Canada has one of the largest organic area share of total farmland among the countries of North and South Americas at 2.44% (Research Institute of Organic Agriculture, 2021).

Therefore, territories of these three countries (Germany, the Czech Republic, and Canada) were chosen for the survey where organic farmers operate. However, it is important to emphasize that this research does not aim to study the levels of possible violations with the highest degree of accuracy. This cannot be verified using anonymous questionnaires. The investigation, however, seeks confirmation in the form of personal testimonies of real bio farmers. This evidence aims to deny or reinforce existing assumptions about possible violations and adds to the body of evidence of older and more recent findings of other investigations: Zorn et al. (2013), Bar and Zheng (2019), European Court of Auditors (2019), Karalliyadda and Kazunari (2020), Zezza et al. (2020), Miśniakiewicz et al. (2021), and Nowicki et al. (2021). Finding effective ways to prevent and significantly reduce the likelihood of possible violations by some organic farmers is the motivation for the second objective of this study.

The questionnaire contained non-personalized but one key specific question: "*How likely (from 0 to 10) do you think it is that a farmer may violate any rules or principles of organic farming and bio-certification organizations will not detect it? * 0 is unlikely (0%), 10 is highly likely (100%).*" This question is not only aimed at measuring the reliability of the organic certification system but also at understanding the perception of organic farmers toward the reliability of CBs and the behavior of other organic farmers. Organic farmers are the key stakeholders and primary target audience and their perception of how likely it is that they themselves are in breach of the rules of organic farming is an important indicator of the rigor of the certification system. As the regulatory criteria between the EU countries and Canada are generally replaceable and mutually recognized in the legal field (Canada.ca, 2016, 2023), the results of the first survey among the given countries can also be considered valid for comparison.

Due to the high sensitivity of this question, providing an email address was not mandatory because respondents may not wish to disclose their identity. Email addresses and additional information were requested from the respondents as an additional measure of validation in case there were any concerns about the eligibility, authenticity, or competence of a farmer to complete the questionnaire. It is also possible that not all organic farmers use email or social media and respond to electronic requests; however, this factor should not and cannot affect the response itself. The question was deliberately depersonalized to enable farmers to answer truthfully. However, farmers' individual preferences regarding the disclosure of their opinion on this subject and their personal propensity to violate is expected to influence their answer.

Different tools were used for achieving the target audience. In particular, in the Czech Republic, bio certified farmers were contacted via email to a total of ∼1,300 bio food producers [available at the web portal of the Ministry of Agriculture of the Czech Republic (eAGRI)]. In Canada, a dual approach was used: (1) a direct email to around 160 bio farmers [contacts taken from the Organic Federation Canada (OFC) and the Organic Council of Ontario (OCO)] and (2) open public posts in Facebook groups that are dedicated to organic farming in Canada with an overall membership of 0.5 million. In Germany, around 200 bio farmers were contacted via email (from the Ecocert Group portal).

The questionnaire was delivered to participants in their local language (respectively for the Czech Republic in Czech, for Germany in German, and for Canada in English). Validation of farmers' responses was based on two criteria about the country of residence from the list of targeted countries and a positive answer to the question about the presence of any bio certificate relating to their farming activity. For each country, the average of the responses was calculated and the obtained result is presented as the estimated average for that country as:

$$L_{Av} = (\sum L_n) / Q$$

Where L_{Av} is the average likelihood of received responses for a country in %, L is the assessment of the likelihood of violating the organic principles by a farmer, and Q is the quantity of all the obtained and approved responses for a country.

2.2. The survey among international experts

The purpose of the second survey was to identify opportunities for further improvement of the model for bio-certification of food and agricultural production. Building on the results of the survey conducted with farmers, the authors identified two key questions for the analysis of the bio certification system:

- Where should the optimal body or decision point for bio certification of products be?
- Where should constant and variable data on food products be collected and processed?

This information was obtained by a survey with experts with the appropriate level of qualification and experience in the organic and certification sectors. The survey was conducted using an online questionnaire which was open between December 28, 2021 and June 7, 2022 (5 months and 11 days). Electronic requests were sent out to potential target experts, who were recognized researchers in the relevant field. The data to identify an electronic mailing list was taken from available information sources, mainly, scientific databases such as Scopus, Research Gate, and Web of Science. Most of the experts were identified by relevant scientific documents with key words such as "Certification of Food" and "Bio certification." In total, 5,848 emails were sent to invite research experts to fill in the questionnaire. Experts were identified and invited mainly from Europe, although there was no geographical limit. Moreover, the geographical affiliation of an expert was not considered in the analysis of the observed questions, but it was collected for general statistical information.

The involved experts were deemed to not have a conflict of interest. As far as the authors are aware, the experts are neither directly nor indirectly connected to the existing certification system. However, they have theoretical knowledge of the subject and may have relevant practical experience. Furthermore, they generally do not belong to any particular territory, which means that their answers are generally not influenced by any particular legislation, ethnic composition, religion, geographical location, etc. and can therefore theoretically be considered as objective as possible. Consideration of other international and local standards will not affect the results and conclusions of the study as it is considered as an ideal scheme in the view of admitted experts.

The following scoring system was created by the authors to validate each expert and the acceptance of their choice for further analysis. This was designed to enhance the overall quality of the responses and to maximize the validity of the data and the general credibility of the survey data. An expert was approved for the survey if the sum of their points for education and practical experience in the agricultural (food) industry equals at least 4 (total points $\geq$ 4). Thus, for the level of education in the relevant areas (Economics, Biology, IT, or Agriculture/Food) and practical experience in agriculture or food industries, the following points are assigned (Tables 3, 4).

The total points for education and experience cannot fall below the four-point threshold for an approved expert to consider their opinion. The validation system was designed to obtain as high competence as possible both in theory and practice (Table 4).

If a respondent scores 0 for one of the two criteria or their total score is $<$ 4, the expert was not validated for this survey. Their answer was recorded but was not included in the results of the study.

In total, the questionnaire (Table 5) for the survey of international experts had a total of seven questions, of which five are dedicated to validation and the remaining two are target questions.

Each choice option was assigned a specific text symbol to facilitate the explanation of the meaning of each choice. Therefore,

TABLE 3 Points for maximum education level in economics, biology, IT, or agricultural (food) background.

Not related to neither economics, IT nor agricultural (food) sciences	0
High school (I level, e.g., Bachelor)	1
Postgraduate (II level, e.g., Ing., Ms., Mg., etc.)	2
Postgraduate (III level, e.g., Ph.D., Dr., etc.)	3
Other (the scoring is evaluated individually)	0–3*

*The authors make equivalent for the relevant level of education listed above.

TABLE 4 Experience in the agricultural or food production sectors.

None	0
≤3 years	1
3–10 years	2
>10 years	3

TABLE 5 The final view of the questionnaire among international experts on the issue.

#	The questionnaire on bio certification of food	
A. Validation part Please fill the information to validate your expertise		
1	Name	*Short answer text*
2	e-mail*	*Short answer text*
3	Max education level in economics, IT or agricultural (food) background*	*Multiple choice:* • *Not related neither economics, IT nor agricultural (food) sciences* • *High school (I level, e.g., Bachelor)* • *Postgraduate (II level, e.g., Ing., Ms., Mg., etc.)* • *Postgraduate (III level, e.g., Ph.D., Dr., etc.)* • *Other*
4	Experience in the agricultural or food production sectors?*	*Multiple choice:* • None • ≤3 years • 3–10 years • >10 years
5	Country of residence*	*Short answer text*
B. Special questions Before answering, please take into consideration that "Decentralized" means that there is no single (centralized) point where the decision is made, but "Distributed" means that the data is shared and stored (duplicated) across multiple nodes (computers), but decisions may still be centralized (controlled by one party).		
6	Where do you think a decision on bio certification of food products should be made?	*Multiple choice:* • Licensed Bio certification bodies based on documentary assessment + onsite inspection (once a year) and additional controls based on likelihood of violations. (Code of choice: *A*) • Electronic automatic algorithm based on permanent data (e.g., soil, water, air, and crop analyses etc.) from spots of agricultural production. (Code of choice: *B*) • Both checks should be made, but priority decision should be made by licensed Bio certification body. Electronic system may take only assistant function. (Code of choice: *C*) • Both checks should be made, but priority decision should be made by permanent electronic algorithm. Bio certification body may take only additional check function (e.g., documentary assessment, onsite inspection, surveillance). (Code of choice: *D*) • Other (Code of choice: *A-D,* specific choice *E*)
7	In your opinion, where should all operation data from spots of agricultural and food productions be collected and processed?	*Multiple choice:* • In national bio certification organizations including centralized or centralized but distributed computer systems. (Code of choice: *W*) • In national government authorities including centralized or centralized but distributed computer systems. (Code of choice: *X*) • Central union data base (e.g., EU, OECD) including centralized or centralized but distributed computer systems. (Code of choice: *Y*) • In fully Decentralized electronic systems (e.g., based on blockchain technology) with no single control party. (Code of choice: *Z*) • Other. (Code of choice: *W-Z,* specific choice *V*)

a different "Code of choice" was assigned to each option. For the first question (Q1), there are codes with possible values from *A* to *E*, and for the second question (Q2), there are codes with values from *V* to *Z* relatively. The specific choice *E* is presented for question Q1, and *V* for question Q2, respectively. However, respondents can answer "Other," where a respondent indicates a conceptually different answer from all the listed answers.

2.3. Data analysis

2.3.1. The majority of choice by each question

The definition of an absolute majority on each of the key questions (Q_1 and Q_2) is the most predictable option among the involved experts in a given number. The share of votes for each issue is determined as follows:

$$\mathrm{V_{Cx}} = [(\mathrm{Q_{Cx}})/\mathrm{q}] \times 100$$

where V_{CX} is a share of experts' votes for a definite choice option in %, Q_{Cx} is the total quantity of responses on a definite choice, and q is the quantity of all validated responses.

2.3.2. The majority and the significance of the combination of choices for both special questions

This analysis implies ranking by the most frequent combination of answers to the main questions Q1 plus Q2. This means that not just the absolute largest number of possible choices per question was determined, but actually two absolute answers are taken into account within one questionnaire. It was worth considering that the absolute majority of answers to questions 1 and 2 together do not necessarily have to coincide with each of the absolute largest answer options for each of the main questions separately. Thus,

$$N_{1,2\ldots16} = (A, B, C, D)\ x\ (W, X, Y, Z)$$

where $N_{1,2...16}$ is the number of choices for each out of the 16 possible basic combinations of Q1 and Q2.

To consider the degree of competence of each expert and to derive the average competence of experts who chose a certain combination of answers, it was necessary to determine the average competency factor (*AvF*) of experts for profile experience and education for each possible choice of answers. Thus,

$$AvF = (\sum T_p)/\ Q$$

where Q is the number of respondents that chose definite combinations and T_p is the total points of each validated expert ($4 \leq T_p \leq 6$).

To determine the significance of a choice (Fs), taking into account the average competence of respondents in a particular choice and the share of such a choice among all respondents, we find:

$$Fs = AvF^{*}\ Vc_{12}$$

The current system of bio certification was represented by choice *A* (Licensed Bio certification bodies based on documentary assessment + onsite inspection once a year and additional controls based on the likelihood of violations) in question Q1 regarding the most preferred level for making decisions on bio certification, and choice *W* (in national bio certification organizations including centralized or centralized but distributed computer systems) in question Q2 dedicated to determining the most preferred level for managing data processing in the bio certification mechanism. Thus, it is possible to conduct a comparative analysis of the possible prospects of a widely functioning scheme with possible other options.

3. Results

3.1. Determining the likelihood of violating the rules and principles of organic farming without subsequent detection of the certification body

The survey of existing farmers working under any bio certification license was conducted between 9 December 2021 and 7 March 2022 and lasted for almost three calendar months. This survey aimed to understand the likelihood of violating the rules and principles of organic farming among bio farmers in the Czech Republic, Germany, and Canada. The survey was completed with 34 accepted and 30 approved responses, constituting 10 completed and approved questionnaires from each target country.

The coverage on social media outlets amounted to about 1 million users in the three countries in total; however, the actual success rate of completed forms via social media was 0.0015% or 15 questionnaires. This is likely due to the high sensitivity of the survey topic and the questions themselves, which can be seen to discredit farmers and the organic brand and certification process, which they work under. Sending direct targeted invitations to farmers' via email addresses proved to be more effective, where the "ignore" rate reduced from almost 99.99–98.7%.

Quantitative characteristics of answers can be the subject of reasonable criticism from experts, but there are some weighty reasons to consider them in our investigation. The authors had the task of identifying and directing inquiries to only organic farms. The percentage of organic farms in the total holdings in each country is much lower and is on average 2.37% with only organic or some organic areas across the EU-27 (Eurostat, 2016). According to recent research (Kononets and Treiblmaier, 2020; Kononets et al., 2022), the percentage of farmers in some European countries who use email on a daily basis is around 20%, which is quite low. Considering the nature and sensitivity of the first survey, it was quite valuable to obtain at least one testimony. A total of 10 such testimonies from each country form the basis for the scientific discussion. Finally, data from 30 responses were used to test our hypothesis and confirm the positive probability of violations.

In total, four completed questionnaires (11.76%) did not pass the verification process and, therefore, were not included in the results. Of them, three were not approved as they work without any bio-certificate and one respondent was from a non-target country, respectively.

Finally, Tables 6.1–6.3 show the responses of each approved organic farmer.

In addition, two bio-farmers from the Czech Republic left detailed comments (translated from Czech) on the issue:

> Extended comment 1: "*I can imagine that there may be 'organic farmers' who grow crops in the fields and fertilize with industrial fertilizers—I see large industrial farms as the most risky here, which will switch to organic for purely financial (greedy) reasons and are not really about this style of farming conviction*" (Respondent #29).
>
> Extended comment 2: "*However, it is clear to me that not everything organic is really 'organic'*" (Respondent #30).

Figure 2 shows the aggregated data from survey 1. As a result, German farmers believe that the probability of an unrecorded violation can be on average, 25.0%. In the Czech Republic, this figure is higher, at 33.0%. Canadian farmers think that this probability can be much higher, equating to 48.0%. Accordingly, the average across the three participating countries is 35.4%.

Hence, we obtained a complete confirmation of the hypothesis within the first objective of the study. This is because all 30 respondents (100% of farmers from three countries) positively estimated the probability (>0%) of a violation by a farmer without further consequences (overall, an average likelihood of 35.3% was obtained).

3.2. Identification of opportunities for the possible evolution of bio-certification procedures for food and agricultural products

3.2.1. Approved experts

The survey with international experts was conducted between 28 December 2021 and 26 June 2022. In total, 130 respondents

TABLE 6.1 Anonymous survey of farmers with a bio certificate in Canada.

#	No of acc.	Date	E-mail not mand.	Bio cert. label	Some information on bio certification scheme e.g., name of seal, product or farm, land square, specialization etc.?	How likely (from 0 to 10)* that a farmer may violate principles of organic farming and bio-certification organizations will not detect it?
1	1	09.12.21		Yes	OPAM, certified OG grains, 640 acres	**5**
2	2	11.12.21	Yes	Yes	Certified organic to COR standards for livestock and crops.	**4**
3	3	14.12.21		Yes	Dairy milk, oats, Peas, winter rye, pasture grasses, hay, and wet baleage	**7**
4	4	16.12.21	Yes	Yes	Eco Cert	**3**
5	5	16.12.21		Yes	Organic	**7**
6	6	16.12.21		Yes	Ecocert	**9**
7	7	16.12.21		Yes	TransCanada Organic Certification Services - for grain and livestock certification	**2**
8	N/v	18.12.21	Yes	No	Yes acres land size	**8**
9	8	27.12.21		Yes	Organic, certified by Pro-Cert	**4**
10	N/v	17.01.22		No	None, we grow naturally (what would be considered organic) but sell at farmers market and to restaurants so don't feel the need to certify	**2**
11	9	16.02.22		Yes	Provincial certification body, vegetables, seeds, and 17.5 acres.	**5**
12	N/v	16.02.22	Yes	No	Pro-Cert	**5**
13	10	16.02.22		Yes	Certified under the Canadian Organic Standard through Organic BC. We are a 10 acre mixed farm and a brewery, both certified.	**2**

*0 is unlikely (0%), 10 is highly likely (100%).
N/v, not valid response.

filled out the questionnaire, of whom 27 (20.76%) did not pass the validation process; therefore, their data were removed from the results. In particular, 17 experts were not approved due to absence of experience in the agricultural field, three respondents do not have appropriate education, six respondents have both insufficient experience and lack of education in relevant fields, and one expert did not identify himself neither by name nor email address and therefore his answers were deemed not reputable and reproducible. The respondents provided the required information and their email addresses for checking the results. In total, the results from 103 experts were accepted. Of them, 91 or 88.3% of respondents had the third level of postgraduate education (e.g., Ph.D. and Dr.) in a relevant area of science, while the remaining respondents (11.7%) had a second level of appropriate education in agricultural, biological, food, economics, or IT sciences (e.g., Ing., Ms., and Mg.). Concerning practical experience, 64 experts or 62.1% of respondents had more than 10 years of practical experience, 31 experts or 30.1% of respondents had between 3 and 10 years of work in the food and agricultural sector, and only eight respondents (7.8%) had <3 years of practical experience.

The geographical spread of experts was found to be quite large. Experts were engaged in research from the continents of Europe, America, Asia, and Africa. More details are provided in Table 7.

Table 7 shows that the participating experts were from 36 countries and four continents. For the purposes of this survey, this factor does not have a significant impact on the overall result of the survey. International experts were involved who are deeply focused both on organic certification and digitalization issues. In our case, there were as many as 130 such expert opinions. Of these, 103 approved responses were included in the final results of this investigation. From this point of view, the number of experts working in this field of science is quite sufficient for the purposes of this study.

3.2.2. Opinion of experts for improving the process of bio certification

The first research question was aimed at determining the degree of priority for electronic systems based on objective data in the process of making a decision on bio certification. The survey results showed (Table 8) that 59 of 103 (57.3%) agreed that a licensed body should play a key role in the decision to certify products, while electronic systems should play only a secondary or assisting function in the process. In turn, 26 experts (25.2 %) believed that the decision of bio certification should be made mainly by electronic algorithms, and bio certification organizations should only play a supporting role. At the same time, 14 respondents

TABLE 6.2 Anonymous survey of farmers with a bio certificate in Germany*.

#	No of acc.	Date	E-mail not mand.	Bio cert. label	Some information on bio certification scheme e.g., name of seal, product or farm, land square, specialization etc.?	How likely (from 0 to 10)** that a farmer may violate principles of organic farming and bio-certification organizations will not detect it?
14	1	11.12.21		Yes	Biokreis	**7**
15	2	12.12.21	Yes	Yes		**1**
16	3	12.12.21		Yes	Organic beekeeping with organic circle and EU organic certification. Our products; honeys, wax, and honey products	**2**
17	4	13.12.21		Yes	Bio-Kreis	**3**
18	5	13.12.21		Yes	EU-Eco, Biokreis, Bioland-Milch, and Bavarian eco-seal	**3**
19	6	13.12.21		Yes	Biokreis ev	**1**
20	7	13.12.21		Yes	Biokreis	**2**
21	8	14.12.21	Yes	Yes	Biokreis and Lacon as certification body	**1**
22	9	14.12.21	Yes	Yes	Bioland the entire operation is certified.	**3**
23	10	17.12.21	Yes	Yes	Lacon testing center	**2**

*The results of the fulfillment by the farmers are translated from the German language.
**0 is unlikely (0%), 10 is highly likely (100%).

(13.6%) believed that the decision should still be made only by the bio certification company, with only one expert indicating that the decision of certification would be better made by an electronic automatic algorithm based on entering data on soil, water, air, and product analyses. It is also worth noting that three experts marked the answer option "Other." The first of them (respondent #58) wrote:

> *"Data should be fused along the chain and shared among actors as well as with certification bodies. Only parties in the chain can ensure authenticity of the organic products eventually."*

Although this commentary is interesting, it reflects the property or technical characteristic of data management and not the preferred level of decision making. Therefore, this answer was not counted in the overall results. The second expert (respondent #67) noted that:

> *"Both checks, but the priority to either a certification body or an electronic algorithm depends a lot of the quality of both. I am not informed of the quality of electronic algorithms and in-field data collection for all the specific indicators and criteria in bio-cert-standards."*

This commentary indicates the desire of the expert to know more details regarding especially those options that require double-checking. Therefore, we assigned this answer to the E option, considering two preferred answers, C and D, making them the most preferable among the other answers A and B. The third expert (respondent #91) wrote:

> *"There should be differentiated requirements for audit/monitoring/inspection based on the scale of the operators and should be conducted by the public agencies."*

This is an interesting remark although it did not give a definite answer within predetermined ones and therefore it was assigned as "other" answer E.

The second specific question (Q2) of the survey was asked to understand experts' opinions on where food production data should be collected and processed, and who should be responsible for data management, storage, and transparency (Table 9). The results indicated the largest number of choices, namely 39 (37.9%), was made in favor of the answer *Y* that favors control under central united countries such as the European Union or other political entities, where centralized or distributed databases can be created within these databases. The second most favorable option with 23.3% of votes (answer *Z*) preferred data management in a completely decentralized electronic system, for example, under the control of the blockchain technology, where neither party of centralized control is in operation. The third most favorable option (answer *W)* was 21.4% of experts who preferred data to be processed solely in national Bio certification organizations. Finally, 14.5% of experts believed that data should be managed by local state control. A total of 2.9% of respondents chose the other option. For example, respondent #58 commented that:

> *"Central, secured cloud databases with decision support tools for use within supply chains by all actors + certification bodies."*

TABLE 6.3 Anonymous survey of farmers with a bio certificate in the Czech Republic*.

#	No of acc.	Date	E-mail not mand.	Bio cert. label	Some information on bio certification scheme e.g., name of seal, product or farm, land square, specialization etc.?	How likely (from 0 to 10)** that a farmer may violate principles of organic farming and bio-certification organizations will not detect it?
24	N/v	12.12.21		No		**10**
25	1	12.12.21		Yes	***Bio certification body "A"	**1**
26	2	29.12.21		Yes	Yes	**3**
27	3	30.12.21	Yes	Yes		**5**
28	4	18.02.21		Yes	They check everything in detail. They want to see everything.	**1**
29	5	21.02.22	Yes	Yes	*Extended comment 1 (below the Table)*	**5**
30	6	22.02.22	Yes	Yes	*Extended comment 2 (below the Table)*	**3**
31	7	22.12.22	Yes	Yes	Documentation check, sampling during harvest and determination of pesticide residues in harvested hops	**2**
32	8	22.12.21	Yes	Yes	Apples, pears, plums, and musts	**8**
33	9	28.02.22		Yes	Rendered butter certified under CZ-BIO-003	**3**
34	10	07.03.22	Yes	Yes	Control of organic farming Chrudim o.p.s.	**2**

*The results of the fulfillment by the farmers are translated from the Czech language.
**0 is unlikely (0%), 10 is highly likely (100%); N/v, not valid response.
***The name of CB is hidden due to sensitivity of data.

Although the comment describes the desired properties, it is generally clear that the expert prefers a central control entity with the active involvement of CBs, which in the predefined list of answers is closest to the choice *Y*. Respondent #67 stated that:

> "*Among the bio-cert organization, but this does not exclude the complementary or supportive use of decentralized electronic systems. I should add that I do not trust decentralized.*" Respondents #95 and #98 replied:

> "*Any of the options*" and "*I don't get the background of the question so I don't have any idea of my opinion,*" respectively. Expert #103 wrote:

> "*It should be a centralized but distributed (open access) computer system. The body is not so important as long as they are free of corruption (including grand-fathering).*" Therefore, these five answers were assigned as answer *V*.

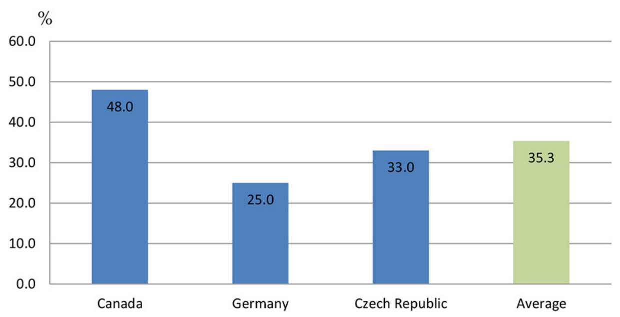

FIGURE 2
The average likelihood of bio certification reliability in countries. The average likelihood in (%), by opinion of bio farmers in given countries, that a farmer may violate any rules or principles of organic farming and bio-certification organizations will not detect it.

3.2.3. Analysis of the frequency of repetition of certain choice combinations when answering two special questions

Each expert chose the answer to questions 1 and 2 separately. However, the system functions in an interconnected manner and can be analyzed as a holistic relationship. Notably, there are combinations that are difficult to combine one with the other. For example, option *A* is for the first question and answer *Z* is for the second question. In particular, the preference for the granting of an organic certificate at the level of national bio certification organizations is difficult to combine with the storage and processing of data in a fully decentralized blockchain system, even though one expert answered this way in the survey. However, it is possible that both of these options can theoretically receive the largest number of answers separately. Therefore, Table 10 presents all possible basic options and their 16 possible combinations as well as the frequency of combination repetitions.

It follows from the presented data that the most frequently repeated combination of answers is *C* and *Y* out of 16 predefined possible combinations. The overall number of full combinations is

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97 as some answers were identified as "Others" in both questions and could not be assigned to the predefined list. CY received 19 repetitions, which is equivalent to 18.45% of the total number of respondents. This combination implies that a priority decision is taken by licensed bio certification bodies with electronic assistants as a supporting function, and at the same time, the operation data from the spot inspections of agricultural and food productions are managed by a central entity such as the EU or the OECD committee. The combination *CW* received 14 votes (13.59%) of the experts who prefer the same option (*C*) on decision making as the previous one but with the data being managed by local CBs. From five other possible combinations *DZ* and CX had 11 votes (by 10.68% of experts) and *CZ* and *DY* had 12 votes each (by 11.65% of experts). These were distributed fairly evenly with a difference in frequency within the statistical error and have minor differences in the significance factor *Fs*. They have quite close values between 0.54 and 0.61 among four combinations simultaneously. The other possible combinations were not statistically significant.

The average competency factor (*AvF*) for each combination option from 4 to 6 is also presented in Table 10, given that the value "6" is equivalent to an expert with more than 10 years of experience in the agricultural or food sector and having relevant education with a doctoral degree. In addition to the largest number of choices, the experts who voted for the *CY* option had one of the largest average competence factors of $AvF = 5.53$. The significance factor (Fs) was obtained by multiplying the *AvF* by the share of experts who chose definite combinations out of the 16 (Vc_{12}). In this aspect, the choice of the *CY* combination also strengthens its major combination among other combination variations with the highest Fs = 1.02. The second significant combination is CW among all possible combinations as it has a slightly lower level of

TABLE 7 Residence of experts by countries (A-Z).

Europe, 73 experts: Austria, Belgium, Czech Republic, Denmark, England, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Switzerland, and Ukraine
Africa, seven experts: Cameroon, Ghana, Kenya, South Africa, and Tunisia
S/N America, 15 experts: Brazil, Canada, Mexico, Peru, and USA
Asia, eight experts: Bangladesh, China, India, Taiwan, and Turkey

TABLE 8 Opinion of the experts among predetermined options A–D on where a decision on bio certification of food products should be made (Target question 1, Survey 2).

Choice Code Explanation (only one choice for each expert)	Code of choice	Number of votes for each option in the Q1 (overall share)
Licensed Bio certification bodies based on documentary assessment + onsite inspection (once a year) and additional controls based on likelihood of violations	A	14 (13.59%)
Electronic automatic algorithm based on permanent data (e.g., soil, water, air, and crop analyses etc.) from spots of agricultural production	B	1 (0.97%)
Both checks should be made, but priority decision should be made by licensed Bio certification body. Electronic system may take only assistant function.	C	59 (57.3%)
Both checks should be made, but priority decision should be made by permanent electronic algorithm. Bio certification body may take only additional check function (e.g., documentary assessment, onsite inspection, and surveillance).	D	26 (25.24%)
Other	E	3 (2.9%)
Totally:		103 (100%)

TABLE 9 Opinion of the experts among predetermined options W–V on where all operation data from spot inspections of food productions should be collected and processed (Target question 2, Survey 2).

Choice Code Explanation (only one choice for each expert)	Code of choice	Number of votes for each option in the Q2 (overall share)
In national bio certification organizations including centralized or centralized but distributed computer systems	W	21 (20.38%)
In national government authorities including centralized or centralized but distributed computer systems	X	15 (14.56%)
Central union data base (e.g., EU, OECD) including centralized or centralized but distributed computer systems	Y	38 (36.89%)
In fully decentralized electronic systems (e.g., based on blockchain technology) with no single control party	Z	24 (23.3%)
Other	V	5 (4.85%)
Totally:		103 (100%)

TABLE 10 The frequency of choice combinations to target questions 1 and 2.

No.	Possible combinations of choices (A-D:W-V)*		Frequency of choices, Q1+Q2	Share of experts' choices, $Vc_{1,2}$%	Average competen. factor of the experts, $4 \leq$ AvF ≤ 6	Factor of significance, Fs $= Vc^{*}_{12}$AvF
	Q1. Where a decision on bio certification of food products should be made?	Q2. Where operation data from spots of agricultural and food productions should be collected and processed?				
1	A	W	4	3.88	5.5	0.21
2	A	X	2	1.94	5.0	0.10
3	A	Y	7	6.8	5.86	0.4
4	A	Z	1	0.97	6	0.06
5	B	W	1	0.97	5	0.05
6	B	X	0	0	0	0
7	B	Y	0	0	0	0
8	B	Z	0	0	0	0
9	C	W	14	13.59	5.5	0.75
10	C	X	11	10.68	5.09	0.54
11	C	Y	19	18.45	5.53	1.02
12	C	Z	12	11.65	5.17	0.6
13	D	W	2	1.94	6.0	0.12
14	D	X	1	0.97	4.0	0.04
15	D	Y	12	11.65	5.25	0.61
16	D	Z	11	10.68	5.64	0.6
17	Other		6	5.83	5.67	0.33
Totally			103	100	5.42	n/a

*Choice Code Explanation (Tables 8, 9).

competence ($AvF = 5.50$) and a slightly smaller share in absolute choice (13.59%).

4. Discussion

4.1. Not everything "bio" is really bio

According to the obtained results, it is observed that even among relatively developed economies of Germany, the Czech Republic, and Canada, the percentage of possible unrecorded violations, estimated by the farmers themselves is 25, 33, and 48%, respectively, with an average of 35.3%. Canada has the highest self-reported average percentage (48%) of possible violations. This high self-reporting maybe due to a different agricultural mindset of farmers; the larger territory and geographical coverage of certification in Canada may complicate practical control and/or be reflective of the slightly different legislation regarding organic production compared to European countries. Regardless of the variation in responses within and between countries, these results confirm our hypothesis as 100% of farmers from these three countries believe that the likelihood of such violations is 35.3% on average and that their self-reporting of violation probability crucially does not depend on geography, economy, mentality, and current legislation.

In general, these results, although a very small sample of the farming population, indicate that bio seals maybe discredited in the eyes of European or North American organic consumers. It may also have implications for non-organic farmer motivation to convert to organic. Moreover, in countries with less-developed systems of quality control and accreditation around social and environmental responsibility, the figure maybe higher, potentially further undermining the credibility of "bio" products and causing significant harm to the established industry of organic certification.

Based on the extended comments of farmers #29 and #30, it is additionally evidenced that, in their opinion, not all bio-farmers comply with the rules and principles of "green" farming but maybe participating in organic certification for financial interest. This preliminary study demonstrates the need for more multi-actor research working with certification schemes and farmers to understand how the regulation and implementation of organic farming and certification can be better improved to optimize the robustness of the organic market and fairness for all organic farmers.

4.2. Evolution of bio certification model of food

A total of 59 experts (57.3%) are predominantly inclined toward double verification by both the bio certification company and electronic systems, with the bio certification companies prevailing. However, it is worth noting that the current long-established bio-certification scheme (represented by answer *A*) only received 14 votes out of 103 voters (13.59%). In fact, this means that 86.4% of respondents indirectly oppose the current procedure. A third party is overridingly the preferred option with 73 (70.87%) expert votes [options *A* (14) + *C* (59)]. However, it is 83.5% of experts (chosen options *B, C,* and *D* relatively) who choose the involvement of auxiliary sensors in assessing the ability to comply with ≪green≫ standards for food products. Hence, bio certification companies will inevitably debug electronic systems that capture objective data from soil, water, air, and other inputs and use them as an assisting tool in decision making on bio certification.

According to the 36.89% of votes in Q2 of survey 2 (Table 9), the role of managing and distributing data should be assigned to the central committee of countries or unions, demonstrating that experts tend to prefer this important function to be carried out by a unifying political body. At the same time, the existing actual model (represented by answer *W*) is a priority for only 21 experts (20.38%). Indirectly, the remaining 79.62% of respondents did not consider it appropriate to entrust the management of objective data and other information, such as the places of agricultural production to local bio-certification companies as is in operation currently (Figure 3).

Thus, additional consultations on a possible committee collecting and managing data from bio farmers are required.

Upon analyzing, the highest frequency of repeating combinations coincides with the maximum choice of answers to questions Q1 and Q2 separately, although this may not coincide. This coincidence of options *C* and *Y* significantly enhances the overall choice. Figure 4 depicts both the absolute selection of experts and the highest frequency combination of choices for the two questions together.

Therefore, there are two key structural differences proposed to the existing bio certification model. First, in the proposed experts' model, the data sources are expanded. For example, data metric indicators were added from points of real food production, which record data from the very 1st day of the production cycle. These data are as objective as possible and serve as a formal reason for a comprehensive analysis of the production point and consequently an objective decision on bio certification. Second, the data flow to the Central European Committee or other central body, where protection law, safety opportunities, and access rights are likely to be more effectively and transparently regulated according to the experts' vision. This will avoid or potentially eliminate a subjective impact from local bio-certification structures, unbalanced local regulations in favor of larger entities, or corruption in the food

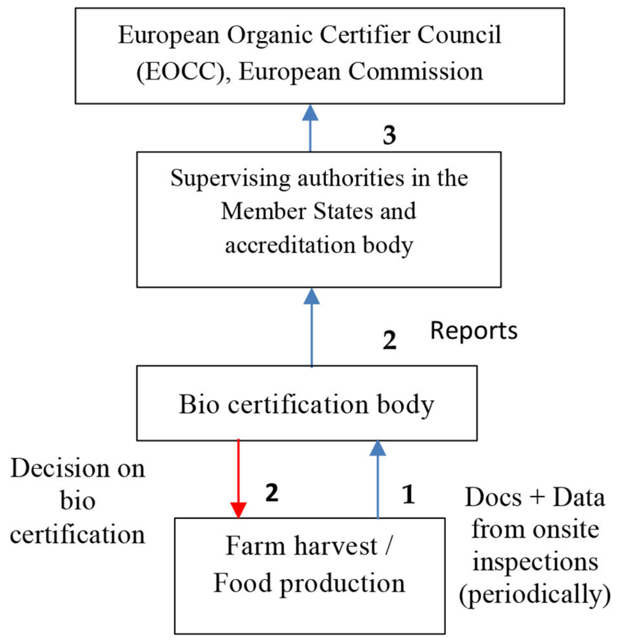

FIGURE 3
The typically accepted bio certification model in European area. Source: European Court of Auditors (2018) and European Court of Auditors (2019) modified by the authors.

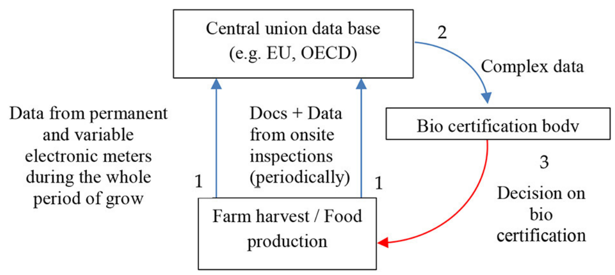

FIGURE 4
The certification procedure based on the absolute and combination choices of the experts (SQ1 and SQ2). Source: developed by the authors.

supply chain. It is also worth noting that almost a quarter, namely 23.3% of experts who chose the "*Z*" option (Survey 2, Q2), believe that such data should not be centralized at all or somehow controlled by someone. A control itself is always an opportunity to influence objective data, and the blockchain technology as a technology option is able to eliminate this type of risk.

Since the majority of experts have chosen the scheme *CY* as the better choice (*C* (Q1), both checks should be made but the priority decision should be made by a licensed bio certification body. An electronic system may take only an assistant function and *Y* (Q2) data should be collected in a central union database (e.g., EU and OECD) including centralized or centralized but distributed computer systems. Further research and evolution of the current bio certification system should consider exploring this operational strategy.

The characteristics of the newly presented bio certification scheme can manifest as follows:

- Electronic assistants and smart meters are becoming increasingly important and trusted in supporting objective processes, including compliance with organic standards. It is very likely that they will play a key role in evaluating how crops are grown and animals are raised. However, organic certification decisions are still for certifiers but not for digital algorithms.
- Storage and transparency of operational data from production sites will likely move toward central regulators to centralized or distributed computer networks rather than decentralized electronic systems (e.g., blockchain networks), as many experts advocate. This is fully supported by the conclusions of van Hilten et al. (2020) that state that transparency of the food supply system can be ensured by blockchain technology, but for many reasons, including economic feasibility, it does not have to be accompanied by the inclusion of the food supply chain in a fully decentralized system.
- This research supports the study by Havelka et al. (2022) that states that all microclimatic factors in agricultural production sites can now be coordinated electronically with certification bodies. This will lead to more precise regulation of microclimate in the areas of agricultural production.

We also emphasize that this study was conducted in relatively developed countries. Unfortunately, the results of a similar study in developing countries are not known, but obviously food security is at greatest risk in less-developed countries (Smith et al., 2019). Herein, we hypothesize that the likelihood of violations could be more dramatic, considering the results from the first survey on potential violations in organic production. Therefore, the positive effect of integrating the proposed new organic certification structure, formed after an international expert survey, would likely be even more significant. For example, the proposed bio certification model has the chance to partially or completely eliminate fraud in the data collection phase and the corruption phenomena in the organic certification decision phase. This is made possible by a collection, storage, and decision algorithm that has a relatively transparent synchronization between all stages, which is not the case with the classical accepted organic certification model.

5. Conclusion

In conclusion, the main objective of this article was to determine the most likely way for improving the accepted certification system, given that only 3.88% of expert respondents support the current system. It brings evidence from bio farmers themselves with regard to the possibility of violations within organic production under the current system. Further research should focus on understanding how farmers are able to violate the system, what these violations are, and how schemes can work with farmers and other actors to reduce or eliminate these risks. How the presented system is capable of improving requires additional research on the issue. For example, investigating the experimental cycle of the certification process of several agricultural producers to explore improvement options of the current model. These results indicate that the existing process satisfies neither research professionals in the field nor end-users which the certification process aims to serve, as 96.12% of respondents believe that the procedure should change, although we realize that this will remain the subject of possible criticism from some experts. However, essential rethinking is needed for improving the bio certification model if evidence continues to demonstrate issues with the current bio certification system. The European committees are not focused on changing the structural technology itself but concentrated on strengthening the control and quality of awareness and how operators work.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

YK: conceptualization, methodology, resources, and writing—original draft preparation. PK: validation, formal analysis, and data curation. PB: supervision, project administration, and funding acquisition. PS: review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Socio-technical transitions and sustainable agriculture in Latin America and the Caribbean: a systematic review of the literature 2010–2021

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The challenges and opportunities Latin American and Caribbean (LAC) countries face to meet sustainable development force nations to seek technological alternatives to ensure better policy design. It also includes technology transfer for the productive inclusion of the rural population in the region. This paper aims to characterize the conceptual frameworks applied to studying socio-technical transitions related to sustainable agriculture in the region. A systematic review literature (SRL) was conducted covering 2010–2021. The main findings suggest that the general ideas of socio-technical transition have been used to study sustainable agriculture in LAC. However, its use has been more implicit than explicit, with some predominance of the Strategic Niche Management (SNM) and the Transition Management Approach (TM) frameworks. In addition, the socio-technical transitions as a straightforward approach have started to be incorporated more clearly after 2020. Finally, the leading technologies to foster socio-technical transitions to sustainable agriculture in the region are related to pest control and soil conservation, so social practices such as certifications have had preponderance in this transition. This paper contributes to the existing literature, broadens the frontier of socio-technical analysis in the transition to sustainable agriculture, and expands our knowledge on applying socio-technical analysis in marginal contexts.

KEYWORDS

socio-technical transitions, sustainable agriculture, a systematic review of literature, Latin America and the Caribbean, agroecology

1. Introduction

This paper aims to characterize the conceptual frameworks applied to analyzing socio-technical transitions related to sustainable agriculture in LAC and identify the technologies supporting these processes. For this purpose, the paper conducts a systematic SRL based on a relevant post mapping approach. This SRL emulates the paper of El Bilali (2020), "Transition heuristic frameworks in research on agro-food sustainability transitions," which addressed the issue on a global scale. For this case it has been used four analytical frameworks most practiced in the analysis of socio-technical transitions (Markard et al., 2012): Multilevel Perspective (MLP), Technological Innovation Systems (TIS), SNM and Transition Management (TM) approach. The analysis in the LAC context has a special significance due to the challenges that the transition towards sustainability in food production must face considering the limited availability of economic and technological resources in most of their countries, in contrast to European or North American countries. In the Latin American region itself, differences can be observed in the availability of options for the approach: in Brazil, the process goes in hand with financing and technology provided by the governments and corporations from the food sector, while in Cuba the transition is driven by shortages in both aspects. The results of the analysis suggest that these circumstances influence the selection of the analytical frameworks of the sociotechnical transitions towards sustainability.

The characterization conducted in this paper provides a better conceptual understanding to promote a more comprehensive policy-oriented research agenda. At the same time, it sheds some light on the technological alternatives that would ensure better policy design regarding technology transfer for the productive inclusion of the rural population in the region. Also, it can help formulate public policies appropriate to each country's environmental, social, and economic environment, contributing to the formulation of their plans in terms of food security, poverty reduction and responsible production. This paper is intended to answer two main research questions:

1. What the literature in LAC countries indicates about the dominant approaches of socio-technical transitions on sustainable agriculture?
2. What does regional literature tell us about the leading technologies supporting a technological transition in sustainable agriculture in Latin America and the Caribbean?

According to the United Nations Development Program (UNDP), the LAC region comprises 42 countries and territories, currently home to 600 million people (Fifka et al., 2016). It has a rural population of 123 million (one in five workers are in the rural sector). The poverty rate is 45.7% of the population, with levels two to three times higher in rural areas (Cepal, 2019). Agriculture and practices related to the value chain of food production constitute a vital component of the regional economy linked to other sectors and services of the economy, such as trade, agribusiness, or tourism as the transition of Brazilian agriculture from low productivity and backwardness to its status as a significant player in international markets (Mueller and Mueller, 2016). In the same way, agriculture is relevant as part of the subsistence practices of rural communities and traditional societies that still exist in LAC, as the traditional maize agroecosystem in Mexico (Dominguez-Hernandez et al., 2018).

Moreover, to the systemic problem of rural poverty in LAC, new environmental challenges related to climate change, biodiversity conservation and resource depletion are significant challenges that should be considered and addressed. The response to these environmental challenges could be tackled from the production systems' "technological transitions" perspective (Geels, 2010). These changes in the production systems are labeled as "socio-technical" because they involve changes in technologies, markets, user practices, and political and cultural meanings (Geels and Schot, 2007).

Geels (2010) states that a transition occurs when the regime is destabilized through pressure. It also may be influenced by the interactions among three levels (niche, panorama, and regime), which occur until a new system state is reached. It is not attributable to a single interaction or driving pressure but to processes on multiple levels (Papachristos and Adamides, 2016). Within the studies of socio-technical transitions, two broad approaches can be distinguished: (1) Historical studies of completed socio-technical transitions (such as the replacement of horses by automobiles) and; (2) Studies on current social changes (energy consumption from fossil fuel to renewables sources; Sutherland et al., 2015).

After the introduction with the conceptual framework, definitions and concepts of socio-technical analysis and sustainable agriculture made in section 1, the section 2 presents the materials and methods used for the research design, as well as the inclusion and exclusion criteria of the SRL. Section 3 shows the analysis results using a descriptive approach to the publications, and some of the limitations found. The discussion of the results is presented in section 4, with citations of the most relevant findings of the analysis and the section 5 it is dedicated to the conclusions, with the answers to the research questions and suggestions of new perspectives for the analysis.

2. Materials and methods

This SRL started from a research problem translated into operational terms in research questions. Then, the scope of the systematic literature review was defined. Afterward, the reference databases' inclusion criteria for the search were defined. The primary searches were generated, and a characterization scheme was defined to support the analysis of the results.

For this SRL reviews papers that address the transition towards sustainable agriculture in LAC published between 2010 and 2021. Table 1 shows the search as it was conducted in the Scopus database based on the defined criteria [TITLE-ABS-KEY (transition) AND TITLE-ABS-KEY (sustainable*) AND TITLE-ABS-KEY (Agri*) OR TITLE-ABS-KEY (agro*)]. It was held on October 26, 2021. In this first search, 2,868 papers emerged. In a second step, the search was restricted to papers published after 2010, based on LAC, published in Open Access databases, either in English, Spanish or Portuguese, reducing the list to 187 papers. Finally, only 61 papers addressed the transition to sustainable agriculture in LAC. The inclusion and exclusion criteria used were the country subject of the research (LAC or one of the LAC countries as the subject), type of document (scientific/academic papers), year of publication (from 2010 onwards), and language (English, Spanish or Portuguese). The transition analytical frameworks were used as a reference for classifying the papers.

The search exercise used titles and abstracts of the papers as the first filter for selection. Then, the complete papers were read in a second filter. This exercise resulted in the final selection of 63 records. Table 1 describes the paper selection process, while Table 2 classifies the selected papers by year of publication.

Based on the framework of transition analysis and in the methodology, the topic addressed, and the region covered corresponds to step 6 (definition of analysis criteria), the papers' characterization to step 7 and based on such steps follows step 8 about the analysis of results (step 8).

TABLE 1 Systematic review literature (SRL) in socio-technical transitions and sustainable agriculture in Latin America and the Caribbean (2021).

Systematic literature review (SRL) step	Number of records selected	Process description
TITLE-ABS-KEY (transition) AND TITLE-ABS-KEY (sustainab*) AND TITLE-ABS-KEY (agri*) OR TITLE-ABS-KEY (agro*)	2,868	Records identified according to search criteria in Scopus
Record identification in Scopus	187	Refinement of the search limited to Latin America and the Caribbean, papers in English, Spanish and Portuguese
Removal of duplicates	186	One duplicate record removed
Selection of papers based on titles	133	Fifty-three records removed133 records focused on the transition to sustainability in sustainable agriculture.
Summary-based scrutiny and full-text records to determine eligibility	73	Sixty records were excluded, and 73 selected records focused on the transition to sustainable agriculture.
Inclusion of papers for systematic review	63	Nine records were removed, and the remaining 63 selected records focused on the transition to sustainability in sustainable agriculture in Latin America and the Caribbean.

Adapted from El Bilali (2019).

TABLE 2 Number of records included in a systematic review on the transition to the sustainability of agriculture in Latin America and the Caribbean (2021).

Year	Number of records	Reference
2021	6	Cunha et al. (2021), Monjardino et al. (2021), Palestina-González et al. (2021), Perillo et al. (2021), Pompeia and Schneider (2021), Rossing et al. (2021)
2020	17	Benítez et al. (2020), Boza and Kanter (2020), Chaibub et al. (2020), de Souza Amaral et al. (2020), Edivaldo and Rosell (2020), Gaitán-Cremaschi et al. (2020), Garrett et al. (2020), Gassner et al. (2020), Heredia-R et al. (2020), Lucantoni (2020), Mottet et al. (2020), Passos Medaets et al. (2020), Schiller et al. (2020a), Schiller et al. (2020b), Scotton et al. (2020), Tittonell et al. (2020), Van Loon et al. (2020)
2019	5	Coquil et al. (2019), Delgado Berrocal (2019), Paiva et al. (2019), Silva et al. (2019), Yagi et al. (2019)
2018	8	Casimiro Rodríguez and Casimiro González (2018), Coser et al. (2018), Dominguez-Hernandez et al. (2018), Fernandez et al. (2018b), Fernandez et al. (2018a), Ianovali et al. (2018), Teixeira et al. (2018), Withers et al. (2018)
2017	7	Da Silva et al. (2017), Garrett et al. (2017a), Garrett et al. (2017b), Gazzano and Gómez Perazzoli (2017), Latawiec et al. (2017), Reis et al. (2017), Santamaria-Guerra and González (2017)
2016	6	Hammond Wagner et al. (2016), Mueller and Mueller (2016), Pérez Sánchez et al. (2016), Salvini et al. (2016), Tejada et al. (2016), Hammond Wagner et al. (2016)
2015	1	Lima and Vargas (2015)
2014	5	Bonaudo et al. (2014), Jacobi et al. (2014), Leitgeb et al. (2014), Ramirez-Guerrero and Meza-Figueroa (2014), Sherwood and Paredes (2014)
2013	2	Rosas-Baños and Lara-Rodríguez (2013), Rondon et al. (2013)
2012	3	Das Chagas Oliveira et al. (2012), de Souza et al. (2012), Lovatto et al. (2012)
2011	3	Astier et al. (2011), da Silva et al. (2011), Rosset et al. (2011)
2010	0	There was no

3. Conceptual basis

3.1. Socio-technical analysis

Research in socio-technical transitions and innovation systems aims to understand technological changes by analyzing the causes that allow or inhibit a particular level of the system in long-term processes (Papachristos and Adamides, 2016). Below is a summary of the four chosen analysis frameworks for socio-technical analysis in this SRL.

The MLP understands transitions in terms of the interactions between niche, landscape, and regime (Rosenbloom and Meadowcroft, 2014). The distinction between the three levels is analytical and not ontological, as the levels are helpful for better categorizing and understanding socio-technical change (Raven et al., 2010). The MLP was created to understand technological transition but was later developed and refined to serve as a heuristic device to study sustainability transitions (Svensson and Nikoleris, 2018). It has developed mainly based on history rather than contemporary cases (Smith and Stirling, 2010), so it should be applied critically to modern transition cases from the social and technological context (Papachristos, 2014).

The TIS approach, is a widely applied framework for analyzing technology development in the context of sustainability transitions (Markard, 2020).The focus on TIS is defined as a network of agents interacting in the economic/industrial area under a particular institutional infrastructure involved in the generation, dissemination, and use of technology. The TIS framework is a practical tool for analyzing potential discontinuities and policy development possibilities regarding innovation systems across spatial scales (Lukkarinen et al., 2018).

The SNM perspective is designed to facilitate the introduction and dissemination of new sustainable technologies through protected social experiments. It is considered a research model and a political tool (Raven et al., 2010). It was developed by Kemp et al. (1998) to analyze how technological change and the acceptance of its social impact evolve together (Mirzania et al., 2020).

Transition Management was defined for the first time in 2000, based on the concept of transition, becoming later an operational model and political practice (Raven, 2005). It is a prescriptive framework that suggests policymakers can shape transitions through four sequential steps: (1) Strategic activities in a 'transition arena'; (2) Development of tactical activities for specific pathways while building agendas and coalitions to support such paths; (3) Operational activities on the ground such as innovation experiments and demonstration projects, aimed at learning by doing; (4) Reflective activities that lead to adjustments in visions and the articulation of best practices (Loorbach, 2010).

Loorbach et al. (2017) describe three different approaches in the science of transitions: Socio-technical, socio-institutional, and socio-ecological. The socio-technical approach emphasizes technological innovation, the socio-institutional approach emphasizes political and institutional change, and the socio-ecological approach the ecological thresholds between the extraction of fossil resources to renewable resources within closed cycles through adaptive management (Visser et al., 2019). Table 3 summarizes the socio-technical analysis's conceptual elements or dimensions that share or differ from the main transition analysis frameworks studied here.

In general, the different analytical frameworks presented in the literature can be ascribed to two major ontologies: (1) the Sociotechnical Transition (STT) and (2) the Socio-Ecological Systems (SES; Ollivier et al., 2018). Since socio-technical transitions are multidimensional phenomena and can be studied from various angles by different disciplines, each approach is supported by ontologies (Geels, 2010). Ontology is defined as "the assumptions about the nature of the (social) world and its causal relationships" (Geels, 2010, p. 2) that underpin and frame ways of looking at transitions (Ollivier et al., 2018). The MLP and the TIS approach correspond to STT ontology but in SNM and TM prevail a SES framework (Geels, 2010).

An important consideration is that not all emerging experiments are viable or have proved sustainable (Jurgilevich et al., 2016). There is no guarantee that proposals for implementing sustainable agriculture schemes will be accepted without a basis demonstrating their feasibility for critical actors. How sustainable agriculture practices will impact the relevant environmental indicators cannot be guaranteed. Here, the SES ontology makes a relevant conceptual and methodological contribution. One example is reducing the impact of climate change in a particular region. When agricultural practices in the traditional regime have a minimal impact on climate change, farmers are likely to show more resistance if they do not feel the guarantees that the transition would provide them with the tools to cope with risk.

Concerning SES, Biggs et al. (2012) identify three properties of the socio-ecological system to be managed: (1) biological and social diversity-redundancy; (2) connectivity between biophysical and social entities, and (3) the state of slow variables (organic matter, water, resources, management agencies, social values) that determines the dynamics of rapid variables (field management, water extractions, authorization to access resources) in complex systems.

Several authors have constructed their classifications on socio-technical systems. For example, starting from the approach, different systems are distinguished: socio-technical (energy, mobility, water, and waste), institutional or socio-economic (education, work, finance) and socio-ecological (forestry, fisheries, agriculture, culture). Røpke (2016) distinguishes resource and waste systems, supply or socio-technical systems, distribution and geography, governance, and economic and financial jurisdictions (cities, economies). Patterson et al. (2017) focused on change processes and distinguished four approaches: socio-technical transition, socio-ecological transitions, sustainability pathways, and transformative adaptation (Geels, 2019).

3.2. Understanding transitions

Regions are the source of niche innovations that will eventually transform regimes with actions that, while modest, are essential (Gibbs and O'Neill, 2014). Sustainability transitions are geographical processes: they are not ubiquitous, but rather, they occur in specific places, that is, in real geographical locations with materiality for them (Hansen and Coenen, 2015). The influence of the region can be seen in urban climate change experiments show that the actor constellations behind vary considerable between different parts of the world (Bulkeley and Castán Broto, 2013) or the important of geographical proximity between agents in the development of niches (Truffer and

TABLE 3 Summary of the conceptual elements or dimensions of socio-technical analysis that share or differ from the main frameworks of transition analysis.

Transition analysis framework	Type of cases	Applications	Approach
Multilevel Perspective (MLP)	Historical	Addressing the socio-technical change of large-scale infrastructures.	Socio-technical transitions/Technological transition/Sustainability transitions
Technological Innovation Systems TIS	Contemporary	Study of actors and institutions involved in the propagation of innovations.	Socio-technical transitions
Strategic Niche Management (SNM)	Contemporary	Introduction and dissemination of new technologies.	Socio-ecological system/Socio-technical transitions
Transition Management Approach (TM)	Contemporary	Modeling transitions through strategic, tactical, operational, and reflective activities.	Socio-ecological system/Social transitions

Author's elaboration (2021).

Coenen, 2012). In the case of transition to sustainability of agriculture in LAC, it is a force that comes from outside the niche (international organizations, NGOs, authorities, customers, and others), so the transition depends on the institution's strength that promotes it.

According to Geels (2010), in the regime, the elements can be tangible (laws, regulations, protocols, standards) or intangible (political paradigms, shared visions and beliefs, social norms, cognitive routines; El Bilali, 2019). Of the three types of rules in socio-technical regimes (regulatory, normative, and cognitive), academia focuses on regulatory ones because they are more tangible than the other two categories (El Bilali, 2019). The above characteristic may condition the success of the transition toward sustainability in agricultural practices. In LAC, except for the case of Brazil, is low the presence of a regime successful supported by the government, and the drivers are mostly export markets that condition niches to certain practices. One example is the regulation and standard for organic production.

3.3. Transition to sustainable agriculture

The analysis of socio-technical transitions from the perspective of the four selected frameworks applied in agriculture seeks to identify success stories that serve as models in the transition towards sustainability in agricultural production in LAC in their context, in accordance with the objectives of this SRL. The modernization of agriculture has resulted in a complete disregard for the negative externalities. The multiple ecological crises force us to ponder the transition toward sustainable agricultural systems by identifying alternative models that make them sustainable and exploring how to build them from the existing systems (Griffon et al., 2021). The triple threat of climate change, biodiversity loss, and food insecurity is a significant challenge to food system resilience (Hastings et al., 2021). Nonetheless, environmental issues became public problems, and stakeholders became aware of the connections between what they did and the ecological processes at various spatial and temporal scales (Steyaert et al., 2016). Within this framework, the idea of a 'green economy' emerges, which promises itself as a remedy to the ecological crisis, and is, simultaneously "in favor of growth, employment, and poverty reduction (Gibbs and O'Neill, 2014). According to Cooke, green economic development aims to mitigate the environmental damage caused by the overexploitation of waste and resources and moderate human contributions to climate change (Gibbs and O'Neill, 2014).

Achieving more sustainable food, feed, and bioenergy systems will require interventions such as increased recycling of nutrients and coordination of biomass flows among farms (Fernandez-Mena et al., 2020). Several technologies for sustainable agriculture have been proposed, including green fertilizers (GFT), biodiversity-based agriculture, and recycling. Legume production and consumption have been reinvented in many products and included in conservation agriculture, organic production, intercropping, and crop rotation (Ferreira et al., 2021). Two applications are crop waste as animal fertilizer or fertilizer released from control (Adnan et al., 2018) and biopesticides that act only against the target pathogen (Ram et al., 2018). However, the isolated application of these technologies cannot be seen as a panacea. For example, the adoption rate of GFT is unsatisfactory in most developing countries, given that the cost of production is considerably higher (Adnan et al., 2018). There is increasing interest in agroecology to move toward more sustainable agriculture and food systems, but its contribution to sustainability remains fragmented (Mottet et al., 2020).

4. Results

The selected papers highlighted the consistent increase in research on the transition to sustainable agriculture in LAC after 2016 when 49 of the 63 chosen works were published. Further, only 5 of these works used the framework of socio-technical transitions to analyze these processes. They were all be published in 2020 or afterward. Table 4 shows the distribution of the five papers using the approach to socio-technical transitions.

By examining the contents of the papers, using TM approach, Rossing et al. (2021) focus on co-innovation, governance, and management of ecological intensification in Uruguay and the European Union, showing more significant contributions to sustainability transitions were associated project preparation, a focus at the farm-level, connections with regional actors, and its interactions. Meanwhile, Scotton et al. (2020) investigated the influence of TM on the transition from conventional to organic agriculture in Mogi Guaçú, SP, Brazil, highlighted the influence of the management system employed, contrasting richness and diversity indices were higher under TM versus conventional

TABLE 4 Use of socio-technical transition analysis framework in papers on transitions to the sustainability of agriculture in LAC (2021).

Year	Transition framework	Document type	Reference	Case study	Country
2021	Transition Management (TM)	Paper	Rossing et al. (2021)	Use of co-innovation in eco-intensification projects	Uruguay/European Union
2020	Multilevel Perspective (MLP)	Paper	Schiller et al. (2020a)	Role of agroecology in agricultural transformation	Nicaragua
2020	Multilevel Perspective (MLP)	Paper	Passos Medaets et al. (2020)	Role of Good Agricultural Practices (GAP) and Organic Certification Programs	Brazil
2020	Transition Management (TM)	Paper	Scotton et al. (2020)	Influence of transitional management from conventional to organic agriculture	Brazil
2020	Technological Innovation Systems (TIS)	Paper	Schiller et al. (2020b)	Examining systemic barriers to the agroecological transition	Nicaragua

management. Passos Medaets et al. (2020) used the MLP to examine Brazil's Good Agricultural Practices (GAP) and organic certification programs, founding that GAP compliance programs represent an adjustment to refit modern agriculture to new expectations created at the level of the landscape and of the incumbent regime. Schiller et al. (2020a) used MLP to examine Nicaragua's barriers to agroecological transition, finding that although the term 'agroecology' is used widely by government, incentives for transitions to agroecology are weakly implemented. Also in Nicaragua, Schiller et al. (2020b) highlight the importance of using TIS approach to understand national agroecological transitions, where systemic barriers to the agroecological transition and cycles of blockages caused by barriers' interactions make change difficult. This sample of cases where the four analysis frameworks of sociotechnical transitions selected for the SRL were expressly applied show the potential of their use in the study of the transition towards sustainability of agriculture in LAC, presenting options to the researchers according to the context.

In addition to the four approaches for sociotechnical transitions analysis selected for this SRL, the other articles published between 2010 and 2021 can be associated with several frameworks. Table 5 lists the papers addressing the transition to sustainable agriculture in LAC with their respective associated approaches. For example, social practice approach groups the papers that deal with market concerns related to their own health, social commitment, food and nutrition security, adequate nutrition, alternative agri-food system, sustainable production models and food consumption. While agricultural techniques, soil technologies, software and simulations are referred to the use of different technologies to address transitions towards sustainability in agricultural production, the combined breeding and harvesting and agri-forestry deal with mixed production.

The most considerable number of papers on the transition to sustainability in agriculture, 23, were related to the approach of social practices, a study framework whose application lends itself to low-resource contexts like that of Cuba in recent years. The first of these in this country was by Rosset et al. (2011), which dealt with the impact of agroecology and the Campesino a Campesino movement, where peasants boost food production substituting more ecological inputs for the no longer available imports, making a transition to more agroecologically integrated and diverse farming systems, including additional benefits from resilience to climate change. Leitgeb et al. (2014) examined the themes, resources, sources, motives, methods, and results of farmers' experiments toward sustainable production, where results reveal those are an integral part of farming. Casimiro Rodríguez and Casimiro González (2018) share the experiences of a farm representative in Cuba's cooperative sector in a longitudinal study of the agroecological transition using the Socio-Ecological Resilience Assessment Methodology during three periods of transition between 1995 and 2015. These three papers are a sample of the benefits that the study of sociotechnical transitions to the consolidation of agroecology in each territory can bring.

Also in the approach of social practices group, the role of associativity in the transition to sustainability has some cases, like Mexico, where Rosas-Baños and Lara-Rodríguez (2013) analyze the creation of the Communal Forestry Company in San Pedro El Alto, which proposes a transition from subsistence agriculture to a type of production that would increase the quality of life and achieved a certain degree of development (Rosas-Baños and Lara-Rodríguez, 2013). In Brazil is addressed by Lima and Vargas (2015) a review of the case related to the Association for Sustainable Rural Development in Serra da Baixa Verde, it was observed, the critical importance of the role of the association to the farmers, without which, they could

TABLE 5 Research focuses on the transition to sustainable agriculture in Latin America and the Caribbean from 2010 to 2021.

Approaches	Number of records	Reference
Social practice approach	23	Pompeia and Schneider (2021), de Souza Amaral et al. (2020), Gassner et al. (2020), Gaitán-Cremaschi et al. (2020), Boza and Kanter (2020), Delgado Berrocal (2019), Paiva et al. (2019), Silva et al. (2019), Coquil et al. (2019), Ianovali et al. (2018), Teixeira et al. (2018), Casimiro Rodríguez and Casimiro González (2018), Santamaria-Guerra and González (2017), Reis et al. (2017), Da Silva et al. (2017), Hammond Wagner et al. (2016), Mueller and Mueller (2016), Salvini et al. (2016), Pérez Sánchez et al. (2016), Lima and Vargas (2015), Leitgeb et al. (2014), Rosas-Baños and Lara-Rodríguez (2013), Rosset et al. (2011)
Agricultural Techniques/ Technologies	10	Perillo et al. (2021), Chaibub et al. (2020), Van Loon et al. (2020), Edivaldo and Rosell (2020), Hammond Wagner et al. (2016), Sherwood and Paredes (2014), Ramirez-Guerrero and Meza-Figueroa (2014), Rondon et al. (2013), Lovatto et al. (2012), de Souza et al. (2012).
Transitional management Approach	5	Mottet et al. (2020), Heredia-R et al. (2020), Dominguez-Hernandez et al. (2018), Fernandez et al. (2018a), Fernandez et al. (2018b)
Soil Technologies	4	Cunha et al. (2021), Yagi et al. (2019), Withers et al. (2018), Garrett et al. (2017a)
Sustainability indicators	4	da Silva et al. (2011), Astier et al. (2011), Das Chagas Oliveira et al. (2012), Palestina-González et al. (2021)
Combined breeding and harvesting	3	Bonaudo et al. (2014), Latawiec et al. (2017), Garrett et al. (2017b)
Software and simulations/ Technological Innovation Systems	3	Tejada et al. (2016), Schiller et al. (2020b), Monjardino et al. (2021), Garrett et al. (2017b)
Agri-Forestry	2	Coser et al. (2018), Jacobi et al. (2014)
Strategic Niche Management (SNM)/Niche Studies	2	Benítez et al. (2020), Lucantoni (2020)

hardly make possible their production. Contrast the results of Da Silva et al. (2017) and Coquil et al. (2019) studies. The first reviewed the agroecological transition in France and Brazil where although PAIS can promote the adoption of more sustainable practices, is limited in promoting the agroecological transition, meanwhile two networks studied for the second contribute to the development of agroecological, more self-sufficient farming systems, which demonstrates that not all cases of transition towards sustainability will be successful.

Additionally, another group of papers incorporates the influence that the market can exert in the transition towards sustainability. de Souza Amaral et al. (2020) include an analysis of the impact of the short food supply chain created by the Center for the Marketing of Family Farming in Rio Grande do Norte, highlighting the role of farmers and their organizations in guaranteeing the volume and diversity of products and showing the impact of certification on organic production. Similarly, in Brazil, Salvini et al. (2016) evaluates the application of a role-playing game (RPG) to promote climate-smart agriculture in three groups of farmers in the southern Amazon, demonstrating this practice induced not only technical learning, but also socio-institutional learning and engagement for collective action. Mueller and Mueller (2016) analyze the transition of Brazilian agriculture from low productivity and backwardness to its status as a significant player in international markets, highlight the importance of the underlying institutional setting on the impact of agricultural policy and the need of inclusive and sustainable institutions created a fiscal, monetary, and political environment. Reis et al. (2017) explore the knowledge, attitudes, and practices of women farmers working in tobacco products on this activity's social, environmental, and health impacts, showing that an integrated approach is needed to deal with tobacco farmers' problems, considering a balance between their beliefs and government decisions.

A review of the social aspects of the transition to sustainable agriculture has focused on the issues of nutrition and food security. It includes an analysis of the food security and individual nutritional status based on the Body Mass Index in Antioquia, Colombia, by Pérez Sánchez et al. (2016), which showed agro-ecosystem features could threaten in the medium-term current food security conditions and the need of protection against this eventuality. Cepal (2019) analyze the establishment of the expression "adequate and healthy diet" in Brazil and the transition of the conception of healthy eating, incorporating the understandings and debates in the fields of food and nutrition security. Boza and Kanter (2020), discuss the key drivers of the transition to agroecological food systems through sustainable diets and provide viable solutions based on existing global experiences around the concepts of local diets, sustainable diets and agroecology practice, enhance the synergies between its. Gaitán-Cremaschi et al. (2020) empirically analyze plant food systems in Chile and assess their potential to support transition pathways to sustainability from ecological intensification, concluding that requires actions to remove barriers in the relations with the agri-food regime and among themselves.

Gassner et al. (2020) analyze how the United Nations Convention on Biological Diversity can influence international policy to favor local production and marketing capacity investments to replace imported food and beverages in the Southern Cone, inviting it to recognize the importance of mixed, diverse agricultural landscapes for their contribution to the conservation of wild biodiversity. Similarly, Pompeia and Schneider (2021) analyze how food and nutrition security narratives and adequate nutrition agendas have been mobilized and modified to respond to criticism and legitimize claims about Brazil's public policies and legislative proposals, concluding that commodity chains begin to privilege discourses that stress their contributions to the exports, while food to health gains momentum. Finally, Ianovali et al. (2018) evaluated the productivity and sustainability of different farming systems, including the migratory agriculture system and the economic impacts on Quilombola communities, recognizing that permanent agriculture was more efficient in terms of income and the use of labor than shifting cultivation system, but it is also part of a complex socio-environmental relations.

Also, in Brazil, Teixeira et al. (2018) developed a farm typology that combines participatory and quantitative methodologies to develop strategies to promote agroecological transitions, findings that farmers differ in their management strategies, had stronger engagements in a network composed of farmers' organizations, showed great potential to provide a wide range of ecosystem services and it is crucial to recognize peasant knowledge. Meanwhile, Silva et al. (2019) pointed out the microlearning process's importance in supporting agroecological transitions, showing that ecolearning processes foster robust ecologization processes and reinforce farmers' systemic visions of their activities.

In Panama, Santamaria-Guerra and González (2017) reconstructed the recent past and the current situation of agroecological initiatives, portraying the contribution of the incorporation of agroecological practices to small-scale family agriculture in this country. In Peru, Delgado Berrocal (2019) studied the landscapes created in the central Andes and the exemplary local conservation and territorial management practices that can serve as a model of socio-ecological transition to mitigate and adapt to the negative effects of anthropogenic climate change.

In the 12 years covered by this paper, several works related to agricultural technologies, fertilization and pest control were identified, including one by de Souza et al. (2012), which conducted experimentation in the coffee agroforestry system in Brazil using several technologies, finding agroforestry coffee (AF) was more profitable than sun coffee (SC). Another example can be found in Rondon et al. (2013), who studied the allocation of potato plantings to 1 of 4 transition systems and their impact on beetle control, providing information for growers making transition from conventional to organic potato production. Ramirez-Guerrero and Meza-Figueroa (2014) studied the effects of composting on soil and potato growth, development, and nutrition in Venezuela, finding that the values of phosphorus, calcium or magnesium content in the soil increased with the use of compost (chicken, bovine or and pigs). Sherwood and Paredes (2014) researched the impact of pesticide use on agriculture in Ecuador, showing the study how actors cooperate, collude, and collide in advancing certain technological agenda, even when against public interests. Hammond Wagner et al. (2016) presented a case study on pest management strategies in small-scale agriculture concluding that opportunities to transition to sustainable on this issue at the local level in Latin American through interventions countering the lock-in of synthetic pesticides. Edivaldo and Rosell (2020) studied the use of slash and burn in black bean production in Brazil, to which it corresponds to 30% of the total bean yield in Prudentópolis, playing a vital role for local food production and a sustainable eco-system. Van Loon et al. (2020) applied the Scaling

Scan tool to evaluate agricultural mechanization projects in Mexico, Zimbabwe, and Bangladesh, finding limitations for the development of suppliers of the value chain according to the market. Chaibub et al. (2020) investigated the application of biological pest control in rice production in Brazil, concluding that the treatments, microbiolized rice seeds or plant sprayed facilitate the agroecological transition. Finally, Perillo et al. (2021) focused their study on the GHG estimation of sugarcane cultivation in Brazil, formulated from the transitional management approach, finding that the gradual transition of pre-harvest burning contributes to the reduction of GHG emission.

The application of TM is recent in LAC and began with Fernandez et al. (2018a), who synthesized the successes and deficiencies of agroecology in Cuba, presenting specific information and experiences to discuss successes and challenges of transition to sustainability. Dominguez-Hernandez et al. (2018) evaluated the sustainability of the traditional maize agroecosystem in Ahuazotepec, Mexico, using the Framework for The Assessment of the Sustainability of Natural Resource Management Systems approach, showing that productivity was the most influential attribute. Heredia-R et al. (2020) evaluated the sustainability of smallholder farmers using a traditional agroforestry system (chakra) within the buffer and transition zones and core of the Yasuní Biosphere Reserve in the Amazon.

In the period covered, four papers were found addressing issues related to soil technologies. One corresponds to Garrett et al. (2017a), which addressed the integration of agricultural and livestock systems on the same ground, focusing on how federal policies in Brazil, New Zealand and the United States encourage or discourage this practice. Withers et al. (2018) analyzed Brazil's current and future phosphorus supply and explored the alternative use of livestock manure and residues from sugarcane processing as its substitute. Yagi et al. (2019) addressed soil fertilization using various proportions of bird manure in Brazil, identifying the benefits of the splitting of the poultry litter rate during the rainy season. In soil conservation practice in Piauí, Brazil, Cunha et al. (2021) evaluated the effects of monoculture on the soil organic carbon's microbiological characteristics, finding the transition to agricultural areas caused changes in the soil microbiological indicators.

Papers focusing on sustainability indicators include Astier et al. (2011), who applied a sustainability assessment framework for peasant systems in more than 40 case studies in Latin America, focusing on the choice of indicators, the effects of alternative strategies on agroecosystems' sustainability, and the trade-offs involved. Da Silva et al. (2011) diagnosed the Economic Sustainability of the properties in the Sanga Guabiroba micro-basin in Brazil using sustainability indicators (land and buildings, capital improvements, equity in machinery and equipment, property, and animals in permanent crops). Das Chagas Oliveira et al. (2012) used the MESMIS method to evaluate the degree of sustainability of peasant agroecosystems and their strategies to promote the emergence of innovations in Brazil locally, showing the relevance of local knowledge as a key factor in policies that promote the sustainability of family systems. Finally, in Mexico, Palestina-González et al. (2021) built a Sustainability Index for Traditional Agroecosystems composed of 16 indicators to analyze diversity-resilience, self-management-autonomy, integration, and self-sufficiency finding that these indicators increased the sustainability of home gardens.

Three papers were produced on the transition in activities that combine animal husbandry with agricultural production. Bonaudo et al. (2014) analyzed how agroecological principles can help farmers redesign and improve resilience, self-sufficiency, productivity, and efficiency within integrated crop and livestock systems (ICLC). Latawiec et al. (2017) used focus groups and semi-structured interviews with farmers in the state of Mato Grosso in the Amazon to identify the underlying factors that lead to or inhibit improvements in land management in pursuit of the transition that leads to the expansion of Brazilian agriculture with zero-deforestation. Garrett et al. (2017b) provided a comprehensive historical and international perspective on why integrated crop and livestock systems have declined in most regions and what conditions have fostered their persistence and resurgence.

Regarding using the TIS approach, Tejada et al. (2016) explore land-use modeling to simulate how the growing land demand could affect future deforestation trends in Bolivia. Schiller et al. (2020b) introduced the TIS approach to examine systemic barriers to agroecological transition in Nicaragua and cycles of blockades caused by the interactions of the barriers. Monjardino et al. (2021) applied an integrated framework that combines bioeconomic simulation, risk analysis, adoption theory, and impact assessment to investigate various combinations of conservation agriculture components in a case study from central Mexico.

Two of the papers published between 2010 and 2021 were on SNM. For instance, Location (2020) analyzed the agroecological conversion process implemented by a family farm in Cuba, and Benítez et al. (2020) conducted a case study of Cuba's Local Agricultural Innovation Project, focusing on gender-specific elements. Two papers addressing agroforestry used the SNM approach. The first paper by Jacobi et al. (2014) analyzes aerial and underground carbon stocks and tree diversity in different cocoa farming systems in Bolivia. The second paper refers to a comparison by Coser et al. (2018). They took the native vegetation of the Cerrado in Brazil to conduct a study to evaluate the transition from a low-productivity pasture to an agroforestry system.

The geographical distribution of these academic results can be seen in Table 6. It is noteworthy that 53 of the 63 papers had a single country as an object (84.1%), and nine involved two or more countries or regions (14.3%; see Tables 6, 7). Brazil hosted more than half of them (52.8%), leading the regional production of publications on the subject, followed by Cuba (13.2%) and Mexico (9.4%). The rest of the papers were distributed among Bolivia, Ecuador, Nicaragua, and Peru, with two papers per country and one for Chile, Colombia, Panama, Venezuela, and Uruguay.

Brazil participated in two multinational studies, one with France and another with the United States and New Zealand. Other studies were regional, one in South America and another in South America and Western Europe, including two global research studies. Another involved Uruguay with the European Union and Mexico/Zimbabwe/Bangladesh. Table 7 summarizes the findings of international research conducted in collaboration with LAC countries.

5. Discussion

Only five of the papers published on the transition to sustainable agriculture in LAC used one of the four frameworks for socio-technical transitions selected for this SRL to analyze it. The five papers were published after 2020 and represented 22% of the 23

TABLE 6 Distribution of country where research on the transition to sustainability in agriculture in LAC were developed.

Country	Number of papers	Percentage
Brazil	28	52.8
Cuba	7	13.2
Mexico	5	9.4
Bolivia	2	3.8
Ecuador	2	3.8
Nicaragua	2	3.8
Peru	2	3.8
Chile	1	1.9
Colombia	1	1.9
Panama	1	1.9
Uruguay	1	1.9
Venezuela	1	1.9
Total	53	100.0

TABLE 7 Multinational research on the transition to sustainability in agriculture.

Countries/regions involved	Number of papers
Brazil/France	2
United States/New Zealand/Brazil	1
Mexico/Zimbabwe/Bangladesh	1
South America	1
South America/Western Europe	1
Uruguay/European Union	1
Global	2
Total	9

analyzed papers. They were published in the last 2 years of the analyzed period, of which 17 were published in 2020 and 6 in 2021. It may presage a better future for applying these frameworks with greater rigor. Both the work by El Bilali (2019) and Giganti and Falcone (2022) shows the lag of LAC in using such analytical frameworks to study transition processes. Considering the purpose of facilitating the transition towards sustainability, the strict application of these methodological frameworks could increase their contribution to the transitions underway in contexts like those studied, whether it is addressing the socio-technical change, study of actors and institutions involved, the introduction and dissemination of new technologies or modeling transitions through strategic, tactical, operational, and reflective activities.

Looking at how transitions to sustainable agriculture have been framed within the approaches to studying socio-technical transitions, the 5 cases of stated application correspond to contemporary transitions. There is no historical analysis between them. However, this cannot be seen as a negative fact. According to Genus and Coles (2008), research on transitions has faced two challenges: (i) creating and improving the understanding of historical transitions and (Lukkarinen et al., 2018) (ii) advancing and refining the frameworks and tools used for the analysis of contemporary socio-technical transitions (Papachristos and Adamides, 2016). When the observation is expanded to establish trends, and all the selected papers published since 2016 are considered, some works explore historical-cultural aspects to respond to that first challenge. It includes Heredia-R et al. (2020) (traditional production systems of the Kichwa in Brazil); Garrett et al. (2020) (combined cultivation and livestock systems in Brazil), Delgado Berrocal (2019) (practices and techniques of the Waris and the Incas in Peru) and Ianovali et al. (2018) (Quilombola migratory cultivation system in Brazil). Transition research has a solid analytical core based on historical socio-technical data from the cases studies (Elzen et al., 2004; Geels, 2005). The trend that has followed the application of transitions towards sustainability in LAC is to take advantage of the study of historical cases to facilitate contemporary transitions.

Overall, identifying windows of opportunity and the first signs of an imminent transition is necessary to formulate policies to direct the system toward the desired trajectory, something that does not necessarily apply to historical studies (Papachristos, 2014). The 23 papers addressing social practices (Table 5), of which 19 were published after 2016 (83%), are hopeful signs because experimenting in niches is crucial to learning about social challenges and stimulating transitions (Raven et al., 2010). The SNM, the related approach to social practices, was used in 23 of 63 publications for 36.5% of the total sample.

The results suggest that socio-ecological and socio-technical systems similarly conceptualize their objects of study, showing complex, dynamic, multiscale, and adaptive properties (Smith and Stirling, 2010). Therefore, the leading technologies on which the experiences of technological transitions in favor of sustainable agriculture in LAC are based must include a look at social practices. In agricultural techniques/technologies, there is a wide range of alternatives. Some are relatively inexpensive, such as avoiding burning in cane cultivation to reduce greenhouse gas emissions (Perillo et al., 2021) to slashing and burning in the cultivation of black beans (Edivaldo and Rosell, 2020). Other low-cost technologies include chicken compost, bovine vermicompost, and pig vermicompost (Ramirez-Guerrero and Meza-Figueroa, 2014).

Table 5 shows that works related to agricultural techniques/technologies (10), soil technologies (4), combined breeding and harvesting (3), software and simulations/TIS (3) and agroforestry (2) represent 33% of the total selected cases. Techniques for pest control have high skill requirements, including the use of C24G agent as a biological control in rice plantations (Chaibub et al., 2020), the use of ground beetles in potato production (Rondon et al., 2013), and the use of sustainable pest management strategies (Lovatto et al., 2012; Hammond Wagner et al., 2016). There is also the call to pay more attention to the human face of socio-technical change against the actors cooperating, colluding, and colliding in favor of synthetic pesticides (Sherwood and Paredes, 2014).

As the only case in the SRL, the escalation in agricultural mechanization was seen in three different contexts. Van Loon et al. (2020) recognize that the availability of resources is a handicap in its implementation and propose using providers that offer the service to multiple users. In this way, the organizational point of view takes the technological aspect into the background.

Implementing integrated agricultural production systems is considered a promising strategy for sustainable agricultural intensification (Cunha et al., 2021). Several examples of integrated agriculture production systems can be found that fit agricultural techniques/technologies and soils. Bonaudo et al.'s (2014) were pioneers with their proposal to combine crops and livestock as an opportunity to improve the sustainability of agricultural systems. They were followed by Latawiec et al. (2017). The latter studied ways to improve land management from a producer perspective to understand better the importance of the underlying factors that lead to or inhibit improvements in land management. Garrett et al. (2020) concluded that combining crops and livestock is an activity that has come and gone in time. Hence, they analyzed the drivers of its decoupling and recoupling throughout history.

Coser et al. (2018) considered the contribution of agroforestry by combining agriculture with livestock. They evaluated the transition from a low-productivity pasture to an agroforestry system that combines two or more species with agricultural practices to potentially increase soil organic matter quality. In Bolivia, Jacobi et al. (2014) conducted a study that compared surface and underground carbon stocks and tree diversity in different cocoa farming systems and their links to tree diversity. They also highlighted the role of organic certification in transitioning from monoculture to agroforestry.

In the SRL, three papers were identified in which simulations were applied to analyze a transition instrument toward sustainable agriculture. Monjardino et al. (2021) lamented that despite the many benefits of conservation agriculture (CA), including land cover, crop diversification, and the cultivation of a new crop or variety, few farmers worldwide have simultaneously implemented all facets of the strategy. They applied an integrated framework to investigate how various combinations of CA components performed over a 10-year period found significant differences in profit, net value, downside risk, and risk-aversion cost between double-component scenarios and all other scenarios. Similarly, Schiller et al. (2020a) applied the MLP to the case of agroecology in Nicaragua, where although the government widely uses the term (agroecology), the transition incentives were weakly implemented. They summarize existing knowledge and gaps around service crops, arthropod-mediated functions, landscape and watershed regulation, graze-based livestock, nature-inclusive landscapes, and policy mechanisms to support transitions. Tejada et al. (2016) used the TM framework to create a land cover change model under different deforestation scenarios to simulate how growing land demand could affect future deforestation trends in Bolivia, beginning with Sustainability scenario, passing to the Middle and finishing at Fragmentation scenario of deforestation expands to almost all Bolivian lowlands. These simulation techniques are scarce in the literature, but they could facilitate and accelerate the transition toward sustainable agriculture by creating what-if scenarios that project the effects of decision making.

Regarding the research agenda, the findings of this SRL indicate that the knowledge gap in LAC is still huge, allowing advancement in regional and national agendas about sustainable agriculture transitions in the zone. At the regional level, although research has been progressing in technologies that improve sustainable soil management practices or pest control, LAC still presents opportunities in these fields, given its importance in terms of biological diversity and the challenges that deal with food safety and security as they are nutrition's, healthy diet and sustainable production and shown Pérez Sánchez et al. (2016), Paiva et al. (2019), and Boza and Kanter (2020).

At the level of national agendas, the challenge is just as complex since each country presents different starting situations and differentiated challenges regarding the sustainability of the rural sector and agriculture. To a considerable extent, the construction of national research agendas linked to public sustainability policy will depend on the type and level of linkage of the agricultural sector to the value chain of each country.

6. Conclusion

This paper has identified the technologies on which the experiences of the transition to sustainability in the region are based, showing a scarce use of the dominant approaches considered as reference (MLP, TIS, SNM, and TM). Results suggest a scenario of more outstanding production in the future where researchers addressing the socio-technical change, with better understanding of the context, introducing more newest and effective technologies and developing models to transitions using the those and other approach. Besides, there needs to be awareness in research on transitions about the diversity of food systems present in countries and how they interact.

The SRL shows a lag in work with an approach associated with TIS, limited by the region's conditions where the use of technologies in agriculture is scarce. The socio-technical transitions general ideas have been present in studies on sustainable agriculture in LAC but more implicit than explicit, with some preeminence of SNM and TM frameworks. Socio-technical transitions as a straightforward approach have been incorporated more clearly starting in 2020, waiting for its increase in the future considering state of the art on frameworks for approaching socio-technical transitions and the diversity of countries and authors who applied them. A stricter use of the analytical frameworks studied will improve the understanding of the analyzed contexts, the identification of more efficient technologies adapted to the specific needs and challenges of regional agriculture, as well as the comparison of research results carried out in this and other regions.

The literature about agricultural technologies in LAC countries dominates the biological control of pests and the fertilization of soils through composting. The evaluation studies in the region about the transition to sustainability are recent but diverse in methodologies. The technologies identified to make agriculture more sustainable have been focused more on reducing environmental impact than on increasing productivity, so social practices such as certifications have had preponderance in this transition.

About the region's countries, Brazil dominates the research on the transition to sustainability in agriculture and highlights the role that socio-technical analysis can play in developing agricultural plantations that harmonize environmental conservation with the satisfaction of developing countries' economic and social needs. Considering the vastness of the body of researchers in this country, the size of its economy, the weight of agriculture in it and the questioning of its impact on the conservation of its natural resources, are reflections of how the analytical frameworks of sociotechnical transitions considered in this SRL and others can facilitate the study of the transition towards sustainability in food production. These factors may influence the fact that Brazil's participation as an object of study for socio-technical transitions continues to predominate and the availability of resources and knowledge affect the technologies used for this transition.

One limitation of this SRL is the classification made of the selected papers, for which the authors did not refer their ascription to a specific framework of socio-technical transitions.

Author contributions

VG-V contributed to the conception and design of the SRL. VG-V and KR manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Circular economy in agriculture: unleashing the potential of integrated organic farming for food security and sustainable development

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Food is a basic human requirement which sustains the dynamics of the Earth's inhabitants by satisfying hunger, providing nutrition and health, and catering to culture, tradition, and lifestyle. However, the rising global population coupled with climate change including calamities, diseases, conflicts, as well as poor agricultural practices put a huge constraint on the quantity and quality of food. Modern agriculture propelled by the green revolution has somehow been able to meet the food requirements of the ever-increasing population and is heavily dependent on chemical fertilizers, pesticides, and machinery, reducing the quality of food, and simultaneously posing a great risk of environmental quality degradation and genetic diversity reduction. The Integrated Organic Farming System (IOFS) is a novel approach that holds the potential in addressing the challenge of reconciling food production with environmental preservation. As this approach embraces zero or minimal chemical use, adopting the reprocessing and reuse of agricultural residues has led to a sustainable system that can be viewed as the closest approach to nature and a circular economy. However, certain constraints need to be addressed, such as ascertaining the effectiveness of organic fertilizers, the complexities associated with weed management, and the inadequacy of proficiency, financial resources, and technical expertise required to implement the IOFS. Therefore, this study emphasizes the comprehensive benefits that could be derived from IOFS, particularly agroforestry, including efficient food production, improved food quality, biodiversification of crops by the adoption of lesser-known crops to cater to cultural requirements and minimal capital input to achieve environmental sustainability and a carbon neutral economy.

KEYWORDS

crop biodiversification, lesser-known crops, sustainability, 5R-concept, Circular-Organo-Agroforestry, organo-agroecosystem, agroforestry

Introduction

Agricultural systems are the foundation of human civilization, providing food, fiber, and fuel (Sharma U. et al., 2022). However, traditional farming practices have come under scrutiny due to their negative environmental impacts, involving soil degeneration in soil, water, and air with emissions of GHGs. This steered a proliferation of sustainable farming practices that balance food production with environmental conservation. Integrated Organic Farming Systems (IOFS) is an innovative approach that offers a promising solution to this challenge. Agricultural systems must have multi-functionality to achieve food security, economic gains, social functions, and environmental sustainability (Groenfeldt, 2005). In addition, an increase in the number of components and functions tends to enhance the stability of the food production and land use system (Price, 2000).

In the Indian context, IOFS is a novel concept of on-farm resource management strategy developed under the scheme (AL-NPOF)[1] All India Network Program on Organic Farming (IIFSR-ICAR, 2014), designed to realize remunerative and sustained agricultural out-turn to satisfy the heterogeneous needs of the farmers as well as the consumers. IIFSR-ICAR (2016) developed the IOFS model for Meghalaya and Tamil Nadu. The model for Meghalaya is [(Cereals + legumes + vegetable crops +fruits + fodder) + Dairy (1 cow + 1 calf) + fishery]. While the IOFS model for Tamil Nadu is [(Green manure-cotton-sorghum; okra + cilantro-corn + cowpea (fodder), *Desmanthus*, 1 dairy cow with heifer and young bull + biofence of *Gliricidia sepium* and coconut)].[2]

IOFS is a holistic approach that integrates various agricultural practices, such as crop cultivation, livestock rearing, and tree farming, in a synergistic manner. An integral feature of an IOFS involves two fundamental attributes: residue recycling, whereby the waste or byproducts produced by one constituent are utilized as inputs for the other constituent, and improved land-use efficiency, i.e., two subsystems occupying a portion, or the entirety of the space required for each subsystem (Paramesh et al., 2022). The goal is to maximize resource utilization and reduce waste, leading to improved soil health, biodiversity, and enhanced ecosystem services. IOFS also emphasizes the application of organic resources and minimizes the application of chemical pesticides and fertilizers, promoting soil fertility with the reduction of environmental pollution. Several authors outline the benefits of IOFS for food production and environmental sustainability (Garima et al., 2021; Sharma U. et al., 2022; Kumar et al., 2023; Verma et al., 2023a,b).

For instance, a study conducted in India found that IOFS increased crop yields, reduced input costs, and improved soil quality compared to conventional farming practices (Das et al., 2017). Another study in China showed that IOFS improved soil organic matter content, reduced nitrogen losses, and increased biodiversity (Chen et al., 2019). Thus, the adoption of IOFS has the capacity to propel toward food and nutritional soundness through the systematic employment of available assets and the incorporation of essential components such as agricultural crops (Altieri et al., 2012; Wezel et al., 2014). However, understanding the distinct functions performed by different elements of IFOS is crucial in catering to the unique requirements of smallholder farmers, as this knowledge is pivotal in fulfilling the dietary and sustenance needs of agricultural households. Simultaneously, due to the intricate nature pertaining to the projected growth of food and nutritional demand, it is imperative to recognize the paramount significance of region-specific IFOS systems throughout the globe. These IFS initiatives play a pivotal role in effectively catering to and satiating the demand, thereby assuming a critical position in the overall food security framework (Paramesh et al., 2022).

Specialized agroecosystems tailored to cope with climatic stress are vital to expedite food security and sustenance. IOFS also has the prospect to attenuate climate change by impounding carbon into soil and limiting GHG emissions. The carbon captured by primary producers during photosynthesis practically nurtures each ecological consumer-resource system by serving as the channel for the transmission of solar energy in the biosphere (Pelletier et al., 2011). A study in Italy showed that IOFS had a lower carbon footprint than conventional farming practices due to reduced use of synthetic fertilizers and lower energy consumption (Chiriacò et al., 2017). Similarly, an investigation in China reported that IOFS minimized greenhouse gas emissions by 13.4% compared to conventional farming practices (Zhou et al., 2019). If all agricultural systems adopt IOFS, the oversight in manufacturing synthetic fertilizer and application can curtail agricultural emissions by about 20%, of which 10% will be due to reduced NO_2 release while about 10% to low energy requirement (Niggli et al., 2009). Furthermore, these emissions reduction is again complemented by emission reparation by carbon sequestration of around 40–72% of the annual greenhouse gas emitted from food production systems. Overall, Barbosa et al. (2015) expressed that the adoption of IFOS presents a viable and encouraging approach for reducing greenhouse gas (GHG) emissions and curtailing nutrient loss. The optimization of available resources is achieved by implementing improved nutrient recycling techniques and utilizing crop residues as animal feed.

Despite the potential benefits of IOFS, its adoption has been limited due to several barriers, including a lack of knowledge and technical expertise, inadequate policy and institutional support, financial, sociocultural, and biophysical constraints, and market demand. Specifying the finitude of energy capital supporting food logistics combined with a restricted range of ecosystems to appropriate residues from energy production along with conversions, the ramifications for food systems deserve critical deliberation (Pelletier et al., 2008; Paramesh et al., 2022). Therefore, there is a need to undertake research and development in IOFS to universalize adoption. This review article intends to provide a conspectus of IOFS and its potential benefits for food production and environmental sustainability. The article will discuss the key principles of IOFS, its potential benefits and limitations, and the current state of research and development in this field. The article will also highlight the guide to the conversion of agrisystems to

1 https://iifsr.icar.gov.in/icar-iifsr/npof/

2 https://iifsr.icar.gov.in/icar-iifsr/npof/data/uploads/files/Tecnologies%20developed.pdf

IOFS that can promote the adoption of IOFS and the challenges that need to be addressed.

Approached methodology

Data collection

For identifying relevant studies for this review, we carried out a systematic search of electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar, using the following keywords: "Integrated organic farming," "Organic farming systems," "Sustainable agriculture," "Food production," and "Environmental sustainability." We also hand-searched the reference lists of selected articles for additional relevant studies. The search encompasses appropriate literary materials from as early as 1713 to February 2023.

Data analysis

The authors TS and LP individualistically partitioned the titles and abstracts of the articles identified to assess their relevance to the review topic. The full texts of potential articles were obtained and evaluated for eligibility. Eligible studies were those that reported on the implementation and outcomes of integrated organic farming systems in different regions of the world. Overall, this review article provides a meticulous audit of integrated organic farming systems with their potential to provide a sustainable and efficient approach to food production and mitigate environmental impact.

The crisis and constraints in food production and food security

Before the beginning of the 19th century, the human population's growth was relatively slow. It took over 2 million years to reach the 1 billion milestone (Population Division, UN); now, the worldwide total population is about 8 billion (countrymeters.info). UNDESA (2021) forecasted that there will be 9.6 billion people by 2050, and providing food for humans sustainably will become a goliath task as total food production volumes are expected to increase appreciably through 2050 to nourish the population (FAO, 2006; Godfray et al., 2010). Taking 2000 as the base year, the FAO (2011) projected that it is mandatory to surge food production by 70% globally and 100% in cash-strapped countries. Sustainable nourishment of the human population is an impending obstacle as resources are finite.

The rapid growth of the human population in such a short period, combined with natural calamities, poor agricultural practices, and World Wars I and II, has put a mammoth impediment on the food supply, causing widespread famine and malnutrition. The end of the Second World War marked the emergence of several agricultural improvements termed the Green Revolution (Gaud, 1968), which was hastened by the efforts of Dr. Norman Borlaug through the creation of HYVs along with disease-resistant varieties of cereals especially wheat and rice which have been genetically modified from the purebred (Borlaug, 1953). The introduction of the multiline and the intensification of agriculture practices have produced huge agricultural output and saved a billion people from starvation (Easterbrook, 1997). Specialization of agriculture with a focus on incremental production was undertaken with reparations of agroecological principles including crop diversity, especially pulses that could reduce synthetic inputs (Watson et al., 2017).

Reliance on a few crops has resulted in a narrow genetic base, and diseases combined with hanging environmental scenarios such as land degradation and climate change have made such crops susceptible to devastation. Mention may be made of the Irish potato famine of the 1840s, where one in eight Irish people died of starvation as the potato was their main staple, and potato blight disease caused major crop failure. Moreover, the Southern Corn Leaf Blight in 1970 due to an attack of race T of fungus *Helminthosporium maydis* on the Southern and Mid-West US Corn Belt brought frantic turmoil in the economy of America as 15% of the crop was wiped out. The farmers and lenders who suffered the ripple effect of the blight had to endure the consequences of trying to recover from their losses (Doyle, 1985). During the 1980s in California, USA, 2 million acres of grape vines were replanted in Napa Valley as the same high-yielding clonal variety was unable to withstand attacks by aphid (*Phylloxera vastatrix*), which had devastated England and Europe from the grafts brought from California in the late 19th Century (New York Times, 1985). These have led to an economic breakdown, as depicted in Figure 1.

To discourse the issue of food insecurity in several regions by famine, the first-ever World Food Summit of 1974 convened by FAO promulgated that every human has the absolute right to be free from hunger and malnutrition to develop their physical and mental faculties (UN, 1975). In subsequent years, concerted efforts from multiple agencies and governments have discussed the issue of food insufficiency to some extent. However, most of these movements have been incremental that have failed to bring about a transformative mode of agroecological approaches considering the dynamics and interactions with the cropping systems, producer, and consumer (Rosati et al., 2021).

Conventional modern agricultural practices have also been criticized for being intensive and having high dependence on non-renewable resources, chemicals, and fuels (Carson, 1962; Pimentel, 1996; Geiger et al., 2010; Winqvist et al., 2012), degradation of land and water resources (Carson, 1962; Pimentel, 1996; Winqvist et al., 2012; Petrosillo et al., 2023), negative impacts on the native bio-resources through reliance on a few staples (Jennings, 1988; Fischer et al., 2014; Petrosillo et al., 2023) along with the higher output of greenhouse gases especially methane (UNDESA, 2021), and compromised nutritional quality (Sands et al., 2009). The input on agriculture to bring about enough food for all is so high that 70% of global freshwater withdrawals from both groundwater and freshwater are attributed to the irrigation of farmlands or processing of food produces (UNESCO, 2020). And an input

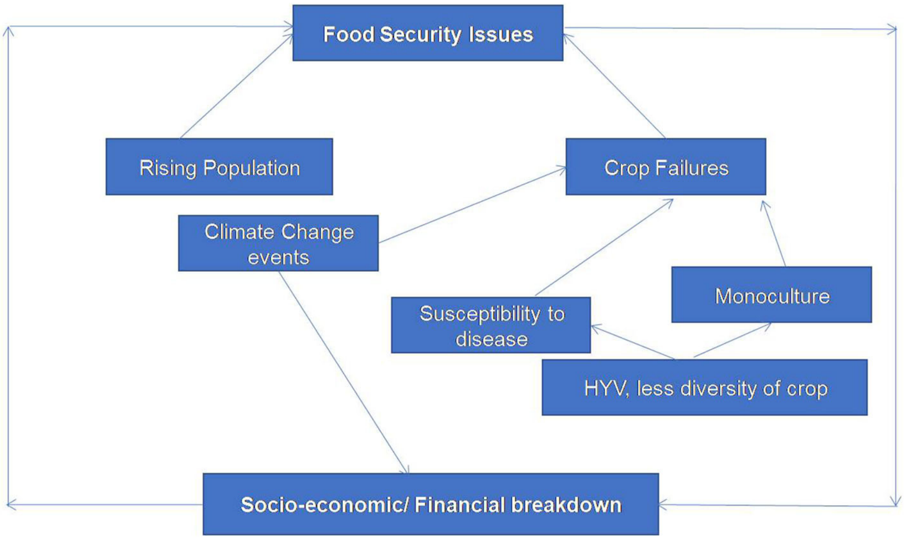

FIGURE 1
Collapse in socioeconomy as linked to food production.

of over 110 million tons of chemical fertilizers was made in the year 2018 (UNDESA, 2021). Kissinger et al. (2012) have attributed 80% of deforestation to agricultural land use. This projection was estimated using FAOSTAT (2009) deforestation values of 2005–2009 as a base, corresponding to the change in cropland areas in each country. The resulting ratio was furthermore employed to estimate deforestation values from area changes in the outlay. Meanwhile, half of the world's terrestrial habitable lands have been utilized for agricultural purposes, involving the conversion of huge areas (about one-third) of global forest lands (FAO, 2020a). The conversion of forests and thereby change in LULC to agroecosystems additionally poses a huge risk of loss of local biodiversity through habitat fragmentation and alteration (Gibbs et al., 2010).

AFOLU accounts for 25% of global human-induced GHG emissions (Ripple et al., 2014; Smith et al., 2014) of which 14.5% is contributed from livestock production. As specified by the IPCC, the agricultural industry was responsible for emitting an estimated annual quantity of greenhouse gases ranging from 5.1 to 6.1 gigatonnes of CO_2 (Barker et al., 2007). The estimate which does not include emissions coming from post-harvest processing and distribution as the greatest consumption of energy and emission of GHGs in the food system is regional and global food trade upshot "food miles" (Pretty et al., 2005).

Moreover, conventional modern farming practices exacerbate soil quality degradation through topsoil runoff and loss of SOM, forbidding the future sustainability of crop production already facing peril under extreme climatic events (Pimentel et al., 1995; Ashby, 2001; Gomiero et al., 2011; Porter et al., 2014; Petrosillo et al., 2023). There is a strong correlation between agriculture and climate change as agriculture is very much responsible for producing GHGs, and prevailing climatic conditions highly influence the success of an agricultural food production system. Climate change-associated events such as rising temperatures, erroneous precipitation patterns along with the increased frequency of extreme weather occasions imperil agricultural fecundity, the feasibility of farming spiraling vulnerability, and ultimately leading to food insecurity (FAO, 2007).

Food security is indispensable to achieving sustainable development (Popkova and Shi, 2022; Sharma U. et al., 2022) especially MDG1-Hunger, i.e., Eradicate extreme poverty and hunger. But if people engaged in the agricultural sector do not strive to "ensure environmental sustainability", i.e., MDG7- Environment, the efforts to increase food production are unsustainable (Rosati et al., 2021). Regrettably, the higher frequency of occurrence of climate change-associated hydro-meteorological uncertainties combined with rising global temperatures is exacerbating the existing food crisis (FAO, 2017). The predominant subsistence of over 60% of the population in Africa's Sub-Sahara region including 40–50% in Asia-Pacific regions is projected to be from the agriculture sector till 2030 (ILO, 2007). IPCC (2007) has also stated that by 2050 all global agroecosystems, inclusive of croplands, pastures, and meadows in temperate areas, are contemplated to be strained due to climate change. These can be supported by several reports of predictions on climate impacts and risks on agroecosystems by climate change through Table 1.

Additionally, The State of Food Security and Nutrition in the World 2020 and 2021 (FAO, 2020a; FAO et al., 2020) has pointed out that the world is "*not on track*" to realize Zero Hunger by 2030. As per this report, nearly 690 million people are hungry, ~8.9% of the total human population. It has also been highlighted that a global pandemic like COVID-19 can put pressure on health and nutritional security as the pandemic has disrupted production, supply chain, and adequate and appropriate food consumption (WHO, 2020).

TABLE 1 Regional impacts and risks of climate change to agriculture.

Region	Impacts	Risks to Agriculture and Food Security	Source
Africa	Temperature increase (x1.5) Precipitation is erroneous and scanty Increased intensity and incidence of droughts and floods	Agricultural productivity relinquished as a consequence of loss in cropland, shorter growing season, vacillation in crop choice Worsening food insecurity due to low crop yield, more people hungry Net returns from crops are projected to decrease by 90% by 2100	Boko et al., 2007; Christensen et al., 2007
Asia	Warming above the global mean in major portions of the continent, rapid glacier melts Longer Heat waves/hot spells in summer Increased precipitation—landslides and severe floods Increase in extreme events combined with El Nino events causing droughts during the summer month	Decreased crop productivity in Asia, risk of food insecurity Reduced soil moisture, land degradation, and desertification	Christensen et al., 2007; Cruz et al., 2007
Latin America	Warming above the global mean in Latin America, heat waves dry spells and droughts in Brazil Uncertainty in rainfall frequency, intensity, and distribution, tropical cyclones, glacier melts in the Andes due to El Niño Southern Oscillation (ENSO), landslides, and severe floods due to intense rainfall in some regions	Reduction in crop yields in some areas, although other areas may see increased yields. By 2050s, 50% of arable lands are likely to be subjected to desertification, erosion, and salinization leading to food security problems	Christensen et al., 2007; Magrini et al., 2018
Small Island Developing States	SIDs in the Caribbean, Indian Ocean, and North and South Pacific have warming lower than global. 10% reduction in average precipitation in and around the Pacific Increasing intensity of tropical cyclones, storm surge, and land inundation	Agricultural land loss by sea-level rise, seawater incursion causing inundation, salinization Risking farming viability-leading to food insecurity Fisheries affected by extreme events especially cyclones and rise in sea surface temperature	Christensen et al., 2007; Mimura et al., 2007

Adapted from UNFCCC (2007).

Integrated organic farming systems and circular -organo-agroforestry practices

Existing sustainable land use/farming practices

Organic farming system

"Organic agriculture promotes and enhances agro-ecosystem health, through a holistic production management system by emphasize the use of management practices in preference to using off-farm inputs, considering the use of locally adapted systems at regional level. This is carried out by means of, possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfill any specific function within the system." (FAO, 1999; IFOAM, 2002). Organic farming strives to eliminate the dependence on chemical inputs and, since being labor intensive, provides rural employment and development opportunities. IFOAM (2010, 2020b) recognizes, "Organic Agriculture as the producing system which sustains the health of soils, ecosystems, and people". These farming practices rely on the interactions between the components, promoting fairness of all life forms involved with the environment. It is distinguished by the prohibition of the input of chemical fertilizers and pesticides, particularly emphasizing soil health and cropping patterns, nutrient transfers, and oscillations within the agroecosystem (IFOAM, 2012). According to Pretty and Ball (2001), sustainable and organic systems in the United States demonstrated comparable yields to high-input industrialized systems while consuming 22–120% less energy. Simultaneously, according to several studies (Muller et al., 2012; Pimentel and Burgess, 2014), organic farming systems have been found to consume 20–50% less energy as compared to conventional farming systems (Zikeli et al., 2014). Moreover, the worldwide implementation of organic farming practices has the capacity to sequester an amount of greenhouse gas emissions equivalent to 32% of the total current human-induced emissions (Jordan et al., 2009; Zikeli et al., 2014).

Conservation agriculture (no-till agriculture)

At present, the cultivators-led transformation of agricultural land-use systems from tillage-based to conservation agriculture has garnered much thrust as a novel paragon for sustainable intensification in food production (Kassam et al., 2015). FAO (2014b) defined Conservation Agriculture as the approach of managing agroecosystems for greater and continuous productivity, improved profits, and food security while preserving and enhancing the resource base and the environment. This food production system is characterized by zero or minimal tool of soil to avoid disturbance of soil from cultivation, upkeep of permanent organic mulch over the soil with residues, crops, and cover crops, and enhanced crop diversity through mixing and sequencing of rotations especially of legume along non-legume. Kassam et al. (2015) have further added that CA can be complemented by the use of quality seeds, integrating management of water, pests, and soil nutrient. This particular land-use system can be executed on all land dimensions from an acre to some hectares (FAO, 2014c) and allows for maximum utilization of soil's ecosystem services with the use of locally adapted species. Simultaneously, conservative agricultural practices improve food accessibility and enhance the plasticity of the cropping systems against the perils of climate systems (Kassam et al., 2009; Friedrich et al., 2012; Farooq and Siddique, 2014; Jat et al., 2014; Sharma et al., 2020; Joshi et al., 2021; Sharma P. et al., 2022).

Natural farming system

In the Indian context, natural farming, an emerging practice, is providing new opportunities for efficient food production and environmental sustainability. The natural farming approach is a diversified farming system that incorporates crops, trees, and livestock, with a focus on functional biodiversity (Rosset and Martínez-Torres, 2012). According to Palekar (2005), the implementation of intercropping and mulching techniques, along with the substitution of chemical fertilizers and pesticides with locally produced alternatives such as Jeevamritham, Beejamritham, and Neemastra, results in a noteworthy reduction in production costs. Natural farming, which can also be referred to either as Eco-Agriculture or Eco-friendly Agriculture, is a modern methodology that seeks to improve both conventional and contemporary agricultural techniques while prioritizing the conservation of the environment, public health, and local communities (Mishra, 2013). Natural farming is a concept that is rooted in the principles of Masanobu Fukuoka, a Japanese farmer. It is based on the belief that it is essential to align oneself with the natural cycles and processes that exist in the environment. The potential effects of the extensive adoption of natural farming on greenhouse gas emissions are currently unclear. The anticipated outcome is a reduction in agricultural emissions per unit of land through the mitigation of fuel consumption, emissions from manufactured inputs, and N2O emissions resulting from a decrease in fertilizer use. According to CSTEP (2020), the implementation of natural farming systems has resulted in notable reductions in water consumption (50–60%), input energy (45–70%), and greenhouse gas emissions (55–85%). Similarly, Rosenstock et al. (2020) and Lohith et al. (2021) as well relayed a significant reduction in the GHG emission ranging from 23 to 60%.

Integrated farming system

Efforts for improvization of agricultural methods to increase food production and enhanced income from the farm has led many smallholder farmers to explore the integration of animal husbandry into traditional crop-based agricultural practices. Such efforts for efficient land use, to achieve sustenance and sustainability where the byproduct of one component became the input for another component and vice versa came to be known as Integrated Farming Systems. Several authors have defined IFS (Jayanthi et al., 2000; Singh and Ratan, 2009; Panke et al., 2010) but the core rationale of the definitions provided are maintenance of cropping patterns and combination along with optimal utilization of resources that accords in food security with income generation (FAO, 2001; Tipraqsa et al., 2007; Sasikala et al., 2015; Kumar et al., 2023). However, it ought to be reminded that the contradistinction between the integrated farming system and the conventional intensive farming system is not outright but is rather a matter of the degree of integration of resources in the farming practices (Tipraqsa, 2006) and hence environmental sustainability of IFS is not well-established.

Agroforestry

Agroforestry can be defined as the system of land usage or acreage where arboraceous perennials, such as trees, are raised along with herbaceous food crops in the same plot or piece of land that result in significant ecological interactions that further aid in maximizing financial returns to the practitioners (Young, 2002). In Lundgren's "Agroforestry Systems' (Lundgren, 1982), Agroforestry was defined as "a collective name for various land-use systems in which woody perennials (trees, shrubs, etc.) are grown in combination with herbaceous plants (crops, pasture) or livestock, in a spatial arrangement, a rotation, or both." Agroforestry systems provide the optimum output of food crops and animal husbandry, even in the face of shortages in wood produce (Mantel, 1990). FAO (2004) states that, "organic agriculture and sustainable forest management produce commodities and build self-generating food systems and connectedness between protected areas." The ubiquitous augmentation of these perspectives and their incorporation in landscape design is supposedly a cost-effective policy alternative for biodiversity conservation. A report of trial investigations by Ramesh et al. (2022) revealed that the inclusion of fodder in conjunction with the fodder trees is highly productive and could generate more effective farm income along with feed for livestock components.

The true essence of agroforestry lies in the anticipated position of on-farm and off-farm production from perennial woody components for facilitating sustainable land use and primal resources and maximizing economic returns for the farmers, which may be summarized as enhancing connectivity and stability among biodiversity, forests, landscape, and watershed (FAO, 2010; Nair, 2011; Murthy et al., 2016). An appropriate agroforestry system that usually follows organic norms can check against soil erosion with the help of the tree component and crop cover, maintain soil organic matter and augment nitrogen build-up through soil microbes and nitrogen-fixing species, and efficiently cycling of nutrients and energy within the system (Patiram and Bhaudauria, 2003).

Conforming and transforming agriculture systems to organic

Several organizations have given recommendations and suggestions to conform traditional and other sustainable agriculture systems to purely organic food production systems through conversion practices. PGS (Participatory Guarantee

System) of the IFOAM provides an alternative to third-party certification systems (characterized by complicated regulatory procedures) for better on-farm practice and marketing strategies for smallholder agriculture practitioners such that food produce by such practices is guaranteed giving consumer satisfaction (IFOAM, 2020a). PGS allows for purchasers and growers assent on the circumstances in existing local food systems and the scheme; these are substantiated through the direct participation of all stakeholders especially in the options and descriptions of the norms along with the review and decision process to recognize farmers as organic. This system ensures transparency and integrity (IFOAM, 2009), that is, particularly *adapted to local contexts and short supply chains,* and which is sometimes referred to as "participatory certification" (IFOAM, 2020a). Detailed step-by-step conversion from existing food production land use to 100% organic production system especially applicable to larger conventional agricultural practices can be achieved by following FAO/TECA ID 8364 Step-by-step conversion to organic agriculture (FAO, 2020b). In the simplest words, it has three steps, viz., (1) Collecting information, (2) Trying out identified promising practices on a small scale, and (3) Implementing the organic practices to the entire farm. Initially intending farmers must collect reliable information from successful farmers, agriculture departments, and through websites such as those listed in Table 2. This should be followed by choosing practices that are simple and within the financial, intellectual, and technical grasp, which would also show results in short rotation/duration. These chosen practices are to be tested in designated plots within the farm and during such phase, the operation taken should be purely organic. When success is achieved, the farmer may proceed to convert the entire farm to the chosen organic practice. This allows for a certain period of observation and choice of combination of crop/livestock/woody components/horticultural crops/aquaculture/fodder grass/cover crops, etc., to be adopted in the IOFS to enhance maximum output with minimal external input. This would make preparations for sustainable food production systems that could mitigate food insecurity and are environmentally friendly.

Gliessman's (2015) description of the transition of agricultural systems toward sustainable agrifood systems has described five phases during the transition from conventional to sustainable agriculture systems. The first three phases operate at the agroecosystem level, while the last two phases involve more interaction between the producer and consumers, as well as involving the active participation of locals during food species selection and diversification of the agricultural systems' components. Rosati et al. (2021) have argued that despite the efforts to enhance sustainability, even organic farming systems could be just considered incremental in nature as they focus more on refusing chemical inputs and replacements by agroecological alternatives and fail to be transformative. They added that integrating agroforestry practices into organic farming systems can bring about a wholesome, sustained agroecological system inclined toward diversification of crop components with amplified interrelationships between the farm system and nature. Their argument stands true when we look at the various workers' descriptions and definitions of agroforestry, as discussed in the previous paragraph. Thus, we can safely declare that including agroforestry as part and parcel of organic farming systems has a duality in being transformative as well as incremental in nature and can address improved food security that gives a minimum cynical impact on the environmental health. The positive impact of organic practice in agriculture practice diversification, including poly-cropping, agroforestry, and integrated farming systems of crop and livestock with local varieties and breeds, produces good quality organic food. Moreover, certified organic products provide for increment in revenue for growers thereby forming a market-based incentive for ecological stewardship (Scialabba and Muller-Lindenlauf, 2010).

UNFCCC (2007) has compiled reactive and anticipatory adaptation means for Agriculture to ensure food security, which is coherent with the IOFS approach. The adaptation measures for agricultural and food security are laid out in Table 3. It is evident that adaptation for sustainability and resilience of agricultural systems may be enhanced through organic conversion, promotion of agroforestry, and water management.

Concept of circular economy and sustainability in integrated organic farming systems

Geissdoerfer et al. (2017) defined Circular Economy as "a recovering system in which resource input and waste, emission, and energy leaks are curtailed by slowing, closing and reducing material and energy loops". The study adds that "a circular economy can be achieved through durable design, maintenance, repair, reuse, remanufacturing, refurbishing, and recycling." An integrated organic farming system follows the 5R-concept: Reduction of inputs, Reuse agricultural residues, Recycle water, Refuse inorganic fertilizers and pesticides, and Replace conventional practices with closer to nature practices. For an efficient circular economy, agriculture should be taken as a primary sector with sustainability as its prerequisite, forming the fundamental of the economy and the living system involved (Jurgilevich et al., 2016). Agricultural systems adopting a circular approach such as IOFS circulate waste from one process to create resources for other means. The feedback-rich systems inspire the circular economy in the biosphere, that there are no wastes that would remain as wastes as decomposers process them, and the nutrient made available for use by producers is thus the system providing for itself perpetually (Pearce and Turner, 1989; Ellen MacArthur Foundation, 2013; Nattassha et al., 2020).

Circular agricultural practices as multifaceted sustainability efforts (Govindan and Hasanagic, 2018) are designed in such a way that all key elements involved in food production, from cultivation, harvesting, post-harvest activities, transportation, marketing, consumption, and disposal (Irani and Sharif, 2018) promote sustainable development through food security and sustainability across all the elements (Fassio and Minotti, 2019; Nattassha et al., 2020; UNDESA, 2021). This process can be considered to follow a rigid Cradle-to-Grave or Life Cycle-Assessment (LCA) approach as agricultural systems follow organic farming protocols with agroforestry and residue recycling that can reduce carbon emissions and efficiently deploy natural resources significantly by curtailing material and capital inputs. Integrated farming systems,

TABLE 2 List of prominent NGOs, other official government organizations, and academic institutions promoting OA.

Name	Region/scale of operation	Function/role in OA	Website
BioTrade Initiative, United Nations Conference on Trade and Development (UNCTAD)	Switzerland, Global	Production, collection, and commercialization of goods and services from local and endemic biodiversity Trade- genetic resources, species, and ecosystems—coherent to the sustainability of the environment, society, and economy	http://www.biotrade.org/
CGIAR System-wide Program on Integrated Pest Management (SP-IPM)	Nigeria, Global	Within CGIAR, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Institute of Tropical Agriculture (IITA)—research on organic pest and disease management.	http://www.spipm.cgiar.org/Web/CGIAR%20IPM%20project.htm
Garden Organic	UK, Global	Organic gardening, farming, and food Promotion and facilitation of OA in Africa, Asia, and Latin America.	http://www.gardenorganic.org.uk/index.php
Global Horticultural Initiative (GHI), World Vegetable Center	Tanzania, Global	Remunerative options for growers and food security Focus area—underutilized crops.	http://www.globalhort.org/
Institute of Organic Agriculture, University of Bonn	Germany	Energy flows—Fruit & Vegetables—Livestock—Pest/Disease Management Nutrition, Quality, Health—Temperate and Irrigated Agroecosystem	http://www.iol.uni-bonn.de/
International Federation of Organic Agriculture Movements (IFOAM)	Germany, Global	Umbrella organization works with UN and multilateral institutions-organic agriculture movements globally. Provides common market guarantee and authenticity for integrity in organic claims—Organic Guarantee System (OGS) and PGS	http://www.ifoam.org/
(NCOF), Department of Agriculture & Cooperation, Ministry of Agriculture	India	Integrated Nutrient Management (INM) with bio-fertilizer Implementing IOFS in India	http://www.dacnet.nic.in/ncof/

Source: FAO-ORCA database in https://www.fao.org/organicag/oa-portal/orca-database/searchnew/en/.

when adopting the organic agricultural approaches, make the components of the farming systems synergistic (Csavas, 1992) and curtail risks, ensuring higher food production and economic profits in combination with proper utilization of organic crop residues (Radhamani et al., 2003; EC, 2012, 2014; McCarthy et al., 2019).

An ideal organic farming system assimilates the outputs or residue of one enterprise as feed for another enterprise in the confines of the same tillage (McDonough and Brungart, 2002; Argade and Wadkar, 2013). The long-term application of organic manure (Gomiero et al., 2011) in farms practicing livestock-based integrated organic farming was more stable and sustainable than conventional monoculture farming practices (Fließbach et al., 2007). The Brundtland Commission (Brundtland et al., 1987) defined sustainability as the "development that meets the wants of the current without compromising the capability of upcoming generations to meet their own needs," and Geissdoerfer et al. (2017) have stated that the notion of sustainability has its origins in forestry. To support their statement, they have used the conceptualization of silvicultural principles of "*Sylvicultura oeconomica*" (Von Carlowitz, 1713), in which the amount of wood being reaped in a cycle should not transcend the regenerative capacity of the woodland. The sustainability of any system may be expressed in terms of the equation "I = P x A x T", where I is the Environmental Impact of the system, P is Population (demographic factor), A is Affluence representing consumption, and T is Technologies involved (Holdren and Ehrlich, 1974). According to this equation, we can rate any agricultural land use system for its sustainability. Considering the parameters involved, we can conclude that Integrated Organic Farming Systems,

TABLE 3 Reactive and anticipatory adaptation measures for agricultural systems.

Reactive adaptation	Anticipatory adaptation
Erosion control, dam construction for irrigation, and Educational and outreach programs on conservation and management of soil and water Changes in utilization and application of synthetic fertilizers and maintenance of soil fertility and prolificacy Introduction of new crops, switching to different cultivars, and alteration in patterns of planting and harvesting period to suit the prevailing climate condition. Promoting agroforestry to improve ecosystem service	Progressive utilization of recycled water, upgraded system of water regulation, soil–water regulation. Diversification and intensification of food and plantation crops Identification of species having high resistance to diseases and climate stress Policy measures, tax incentives/subsidies, free market

Adapted from: UNFCCC (2007).

including Agroforestry land use systems (McAdam et al., 2009), can be declared highly sustainable and ideal for substantial food production with the least environmental impact.

Thus, the integration of circular economy principles to organic agriculture system with agroforestry can be expressed with an amalgamated term "Circular-Organo-Agroforestry" as depicted with components and a model in Figures 2, 3 respectively and may be defined as *"a system of agricultural farming practice which is organic and has rich species diversity which includes tree components, herb crop components and may include animal husbandry and fishery in which there are synergistic interactions* *between all components within the system following the principles of a circular economy that results in sufficient food production and helps in achievement of sustainable development."*

Concept of food biodiversity and nutrition enrichment from organo-agroecosystem

Guidelines on Assessing Biodiverse Foods in Dietary Intake Surveys (FAO Biodiversity International, 2017) defined "*food biodiversity"* as "the diversity of plants, animals and other organisms used for food, covering the genetic resources within species, between species and provided by ecosystems." A diverse diet augments the prospective of consuming sufficient nutrients essential to human health (WHO/FAO, 2003). Long-standing efforts to provide basic food for the human population have resulted in a biased focus toward more high-yielding starchy staples (Zhu et al., 2000). This further leads to dramatically simplifying landscape composition and a sharp decline in local biodiversity (Gomiero et al., 2011). This has put massive pressure on the diversity of food species from 5,000 to 70,000 plant species that have so far been documented as human food (Kunkel, 1984; Wilson, 1992; Schippmann et al., 2002, 2006), out of which almost exclusively three cereals, viz. rice, wheat, and maize, furnish 50% of the calories derived or having originated from the plant (FAO, 2015; Bailey, 2017; Futurism, 2017; FAO et al., 2021). Increased focus on the production of staples (Magrini et al., 2018) has generated reverberations in the ecological system and human wellbeing (IPES-Food, 2017). These reverberations can be attributed to the limitations of crop diversity combined with the intensification of livestock production methods (Tilman and Clark, 2014, 2015). Food security is not restricted solely to making food available and nevertheless must address the alarming sum of more than 800 million people in low-income countries suffering from chronic undernourishment and micro-nutrients deficiency (FAO, 2017), while overconsumption in high-income countries has propelled lifestyle diseases such as obesity, type II diabetes, and coronary heart disease (WHO, 2009; FAO, 2017). New solutions must develop solutions to overcome disparities in distribution and availability to food and nutrient requirements (Tilman and Clark, 2014). Healthy diets are unavailable to many people, especially the poor (FAO, 2020a), as over 3 billion people globally still get just adequate to fill their stomachs and do not have enough to purchase nutrient-rich food (FAO et al., 2020). This is very alarming as dietary guidelines that have been put forward in different regions of the world recommend a wholesome diet endowed with a variety of nutrients, which includes green leafy vegetables rich in vitamins, and micronutrients, whole grains and nuts, seeds, and pulses for proteins aside from starchy staples (Fischer and Garnett, 2016).

The Convention on Biological Diversity advocates the combination of the conservation and sustainable use of biodiversity in cross-sectoral plans to achieve sustainable development goals that include poverty eradication, adaptation/mitigation to impacts of climate change through sector-specific initiatives such as agriculture and forestry (CBD, 2011).[3] The Pacific Organic Standard emphasizes biodiversity enrichment through land use by requesting properties larger than five hectares to set aside a base land ratio of 5% of the certified area for wildlife excepting that the estates practice a traditional agroforestry or poly-culture system (Secretariat of the Pacific Community, 2008; Scialabba and Muller-Lindenlauf, 2010).

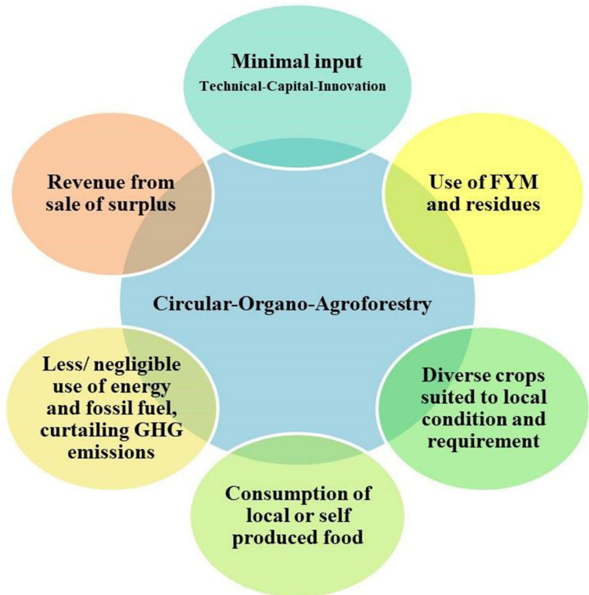

FIGURE 2
Components of Circular-Organo-Agroforestry to suit regional/local scenario for achieving MDGs.

FAO (2014a) defined a sustainable food system as "a food system that brings food security and nutrition for all such that the economic, social, and environmental bases to create food security and nutrition for future generations are not compromised". The FAO's Genetic Resources for Food and Agriculture Commission provided and adopted the Voluntary Guidelines for Mainstreaming Biodiversity into Policies, Programs, and National and Regional Plans of Action on Nutrition (FAO, 2016) supporting countries for incorporation of food biodiversity to encourage knowledge, conservation, promotion, and utilization of varieties and breeds used as food, as well as wild, neglected, and underutilized, underexplored species that could result in a better state of health and nutrition in respective countries. Species such as hanza (*Boscia senegalensis*) are a significant source of carbohydrates, Jessenia and patawa (*Oenocarpus bataua*) produce high-quality edible oil, Locust bean (*Parkia bicolor*) is rich in protein sources, Mongogo tree (*Ricinodendro rautenenii*) is a source of protein and fats, Bamboo shoots (Selvan and Tripathi, 2017), edible fungus, yams, etc. are significant lesser-known wild foods in which rural/forest dwellers extensively exploit in different areas around the world (Scoones et al., 1992; Selvan, 2022). Their utilization may be seen as signs of local populations' strategy to cope with food stress, and the incorporation of wild foods into local organic agricultural practices not only fulfills increased food production but enhances diverse nutrient availability (Nurhasan et al., 2022).

3 https://www.cbd.int

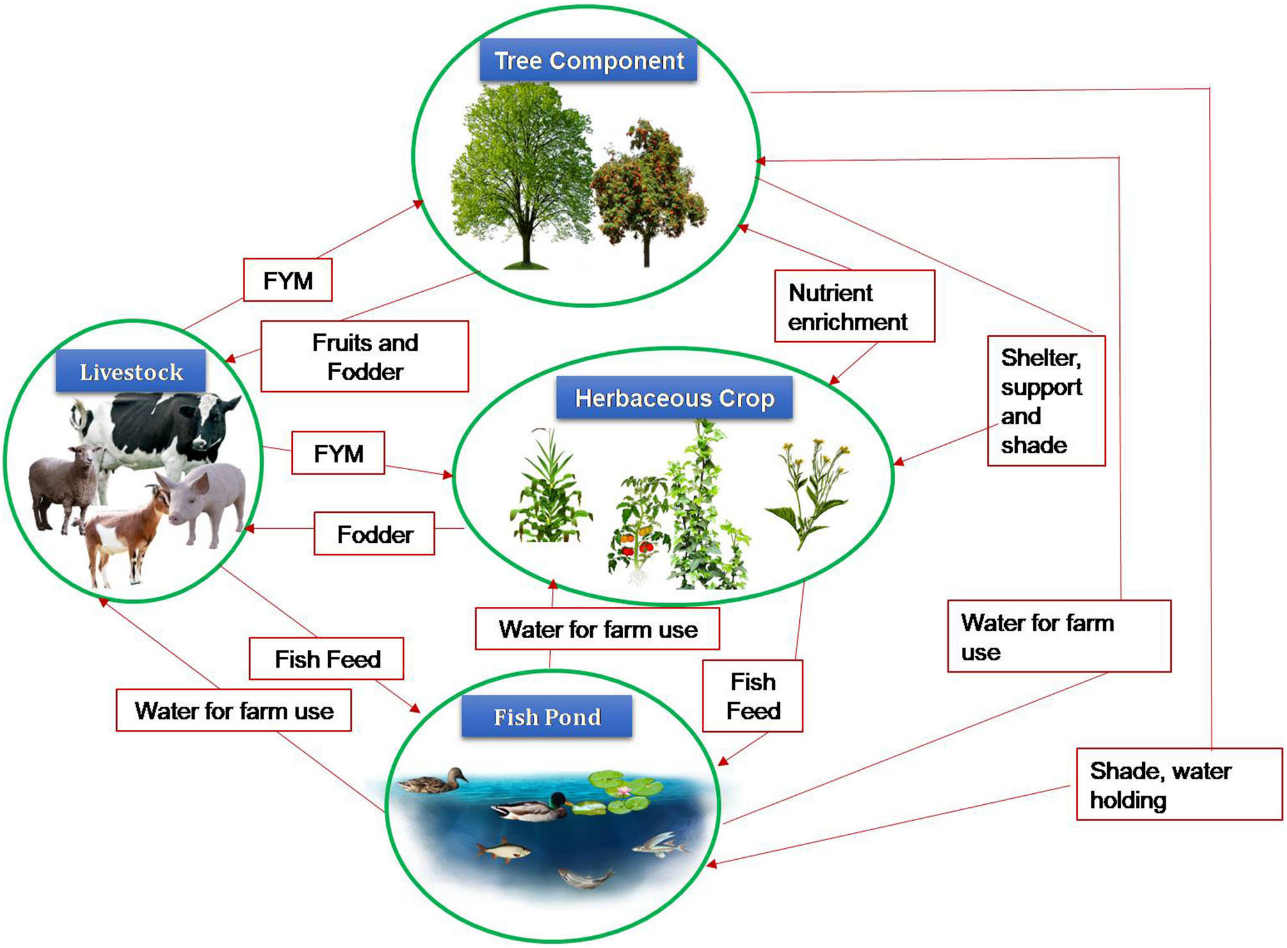

FIGURE 3
A model of Circular-Organo-Agroforestry.

According to IFOAM (2002), farmers are to ensure maintenance and improvement of agrilandscape and enhance biodiversity. Organic farming systems, including agroforestry worldwide, use species that caters to the requirements of the local populace that includes but not limited to food staple, medicinal plants, ornamentals, culturally and traditionally important species, and fuelwood species, the inclusion of species that could provide fuel and fodder which may be considered as a step forward (Rosati et al., 2021) and to be aligned with principles of certified organic farming (Maeder et al., 2002). The species and varieties incorporated into these systems have better adaptability and enhanced agroecosystem resilience to environmental factors as well as market uncertainties (Zhang and Li, 2003; Smith et al., 2007, 2014).

IOFS that include animal husbandry considers ethical concerns in the context of the wellbeing of the livestock, and hence, amidst varied animal husbandry systems, encouraging natural grazing in open pastures and meadows, inhibiting synthetic feeds fortified with vitamins, and promoting the use of organically sourced feed (Lund, 2006), which leads to improved consumer health, such as consuming meat from livestock that may contain traces of growth promoters and antibiotics from the artificial feed and injections. Greater species diversity in these systems is conceived to improve food security (Parrot et al., 2006) and enhance the systems' ability to resist shocks, thereby reducing vulnerability and increasing resilience while maintaining the stability of the natural ecosystem (Fraser et al., 2005; Whitfield et al., 2018; Sherpa, 2023).

The species selected for such land use are often tailored to the local climatic and environmental conditions and involve less technical or zero chemical input, partly due to a lack of finance, knowledge, and resources. For instance, in the Northeastern Region of India, almost all traditional farming systems are usually of agroforestry type. They are organic by default as the farmers lack technical resources and prefer to rely on their traditional methods of agriculture using farmyard manures, green manures, indigenous ways of weed and pest control, and selection of crop species or animal husbandry to cater to their local preferences (Das et al., 2018). Indeed, they practice a different type of organic agroforestry land use systems (Selvan and Kumar, 2017), one of the reasons why the food security problems in the region are not as serious. The careful selection of species by the farmers in the region has also made their land use systems advantageous and sustainable (Pretty and Bharucha, 2014).

TABLE 4 Synthetic inputs of fertilizers and pesticides in the food production systems.

Region	Fertilizer use Kg/Ha		Pesticide use Kg/Ha		Total increment percentage	
	2000	2020	2000	2020	Kg/Ha fertilizer use	Kg/Ha pesticide use
World	90.3	129	1.45	1.81	22.24	19.88
Africa	17.2	26.4	0.36	0.49	34.84	26.53
Americas	91.6	150.6	2.44	3.74	39.17	34.75
Asia	128.6	187.4	1.11	1.15	31.37	3.47
Europe	71.3	83	1.48	1.64	14.09	9.75
Oceania	92.9	82.1	1.42	2.13	−13.15	33.33

Adapted from FAO (2022).

Integrated organic farming uses agrobiodiversity to bring about variety and variability to increase yields, have higher disease resistance, requires less water input, have symbiotic relationship among the components of the system, produces food that has higher nutrient value, and produces more food groups to improve health of consumers (Bailey, 2017). Thus, it results in higher species richness and a greater abundance of taxa (Hole et al., 2005).

Status of organic food production systems

An area of 72.3 m ha of agriculture is under organic operations of which Australia alone has 35.69 million Ha which is ∼50% of the total; followed by Argentina at 3.69 m ha and Spain at 2.35 m ha. The fourth and fifth largest area under organic agriculture is observed in the USA (2.33 m ha) and India (2.3 m Ha). Meanwhile, Europe showed steady growth in organic agriculture possessing 23% of the total. The increase in land under organic agriculture increased 555%-fold in the last two decades, i.e., 1999–2019. The report further showed that Australia showed an increase of 200% in the last decade itself. Despite the increase in organic agriculture areas in all regions of the world, the intensification of conventional agriculture is also growing simultaneously. Entry of synthetic fertilizers and pesticides into the food production systems is showing an increasing trend globally and in all regions of the world. This trend of input Kg/Ha is shown in Table 4. An exception is seen in Oceania for Kg/Ha fertilizer use from 2000 to 2020 which is actually −13.15%. This may be attributed to the proliferation of organic cropland areas.

However, it should be noted that only 1.5% of total global agricultural land are following organic practices despite the massive increase. Liechtenstein has more than 41% of agriculture land in organic agricultural practices (Schlatter et al., 2021; Willer et al., 2021).

Furthermore, it is reported that there are above 3.1 million organic producers worldwide of which 91% are concentrated in Asia, Africa, and Europe of which major import and export share is from tropical fruits, organic vegetables, fruit drinks, etc. (Sahota, 2021). An encouraging fact is that India itself has about 1.37 million organic producers (Sahota, 2021). The total organic food retail sale exceeds 106 billion Euros in 2019. The US is the major market for organic food at 44.7 billion Euros, which is even higher than the combined market share in the European Union, i.e., 41.4 billion Euros. The 3rd biggest market in the world and Asia's largest organic food market is China at 8.5 billion Euros (Sahota, 2021).

Limitations and constraints in the adoption of IOFS

Application of organic farmyard manures and vegetative residues are often-times proven to enhance sustainable and circular utilization of resources in integrated organic farming practices. Challenges exists in determining the fertilizing efficiency of organic materials as it depends on the mineralization processes, absorption along with losses such as gaseous emissions, and leaching or runoff (Burton and Turner, 2003; Sørensen et al., 2013; Bernal, 2017). A drawback of the organic land use systems is the difficulty in controlling of weeds as weeding is done manually which is labor-intensive increasing operation costs. Meanwhile, the use of vegetative mulches and FYMs with bio/green fertilizers must be taken with proper care as some ingredients may even exhibit allelopathic effects on the crops (Bond and Grundy, 2001). Muller et al. (2017) made a conservative estimate of organic yields exhibiting a median yield gap of 25% from the data in Seufert et al. (2012)'s "Comparing the yields of organic and conventional agriculture". Clark et al. (1998) stated that the increase of soil organic matter after the transition from conventional to organic systems occurs rather too slowly and takes too long to manifest significant positive outcomes. Adoption of novel practices of transformative agricultural practices requires interactive discourse between local farmers and researchers/advisors for better acquisition of knowledge (on the complexity of the species compositions, functions, and services) which is itself a time-consuming and critical challenge (Geels and Schot, 2007; Hauggaard-Nielsen et al., 2021). Bernal (2017) further emphasizes that the contemporary lack of data and limited research on consequences to the agroecosystems due to increased microbial activities associated with organic fertilizers may cast doubts on the sustainability and put constraints on the adoption of IOFS. Moreover, locality factors play a crucial part in ascertaining the accomplishment of any particular farming systems as what is deemed sustainable could unfortunately yield desirable outcome in a different region or even within the same

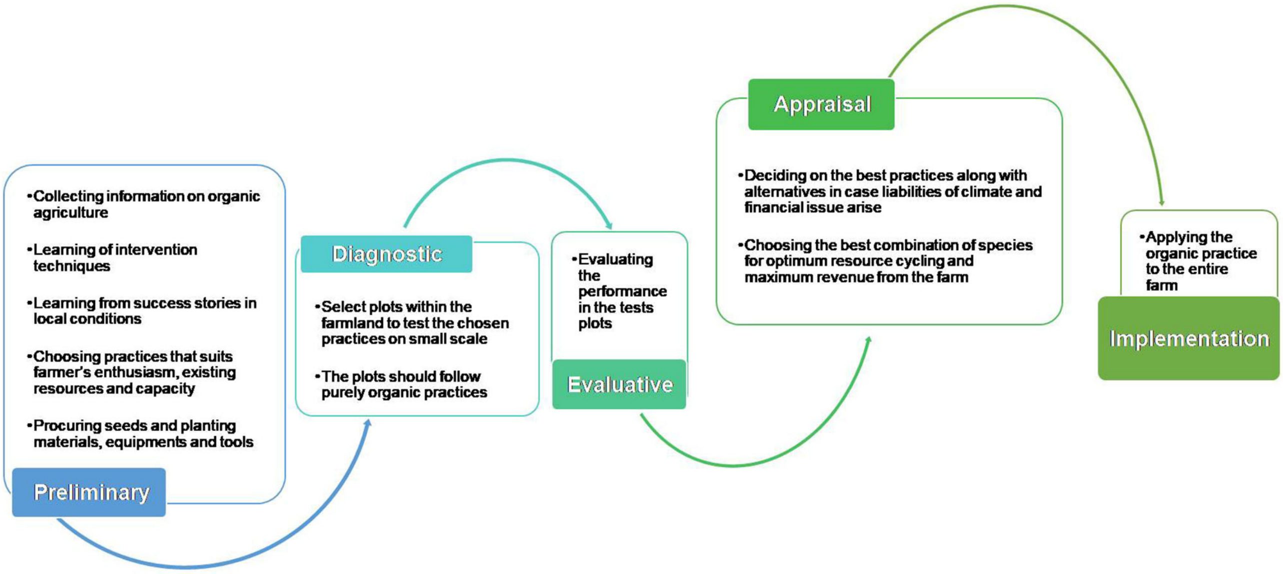

FIGURE 4
Steps in conversion of food production systems to Circular-Organo-Agroforestry.

region (Smolik et al., 1995). FAO (2011) has expressed concerns over adoption of integrated systems as even though incorporation of food crops, animal husbandry, and pisciculture is ordinarily considered progressive, smallholder farmers need to have sufficient proficiency, financial, technical, and labor to operate this system in ways that yield economic and environmental sustainability in the long term. These above points may make IOFS unappealing and rather be viewed as utopian efforts of food production systems for poorer countries that are facing food insecurity as the aim to increase production is of primary importance.

Conclusion and future prospects

The primary target for escalating food production deprived of consideration for the ecological aspects, or good nutrition, is responsible for the drastic degradation of the environment while at the same time causing a pandemic of non-communicable diseases (Bailey, 2017). It could also be noted that monoculture, along with conventional agriculture practices depending on few staples, has caused a reduction of crop biodiversity. It is expository to scrutinize the correlation of Circular Economy with Sustainability, which can determine any sector's inventory management, trade ideals, and innovative approaches (Geissdoerfer et al., 2017). It is impetuous to develop food production systems characterized by resilience, sustainability, and justice inept in satiating the hunger and providing healthy, nutritious food. There is a need to interlink the essence of local producer–consumer relations and the generation of farm and food waste. IFOAM (2022) realizes the actions to galvanize with market players to ardently increase the value of organic products, creates strong and fair value chains, and allows easy access to wholesome and nutritious organic food and products created from organically sourced materials for every individual. Organically managed agriculture systems are designed for the production of high-grade, nutritious food that forms the foundation for preventative health management and wellbeing (IFOAM, 2020b). Integrated Organic Farming Systems, considered equivalent to circular agriculture, aim to ameliorate rising concerns of unsustainable global food production, environmental degradation, and the peril of biodiversity loss (UNDESA, 2021). Niggli et al. (2009) emphasized that there are three strategic research priorities in agriculture food production systems (Gomiero et al., 2011), i.e., (1) feasible options in empowerment of rural economy on a local, regional, and global scale; (2) safeguarding agroecosystems employing eco-functional agricultural consolidation; and (3) high-quality foods, a foundation for healthy and balanced diets, and a requisite for uplifting quality of life. It should be noted that Organic Agriculture practice is created on the four principles of *Health*, *Ecology*, *Fairnes*s, and *Care* (IFOAM, 2020b). All agriculture food producers should prioritize the utilization of organic agriculture diligently in panoramic policy solutions to exigency in climate change, biodiversity loss and food security, and relocate public grants to sustainable farming practices (IFOAM, 2022).

Meanwhile, farmers as well as researchers may explore various other organic agriculture and agroforestry practices such as Biodynamic agriculture, Regenerative agroforestry practices such as Permaculture, and Syntropic agriculture. A systematic step-by-step process of conversion of existing agricultural land use system to IOFS is depicted in Figure 4. These practices take a closer to nature approach by mimicking natural ecological processes in the acreage. In a particular agroforestry system called the *Miyawaki*, system the plots of urban areas are converted to natural forests using native species. This system utilizes the soil's ecosystem service to the fullest by growing several species in confined spaces where the crops grow rapidly in height while competing for sunlight. Any local fruit-bearing species or crops may be used to provide fertilizer and pesticide-free food options for the urban population. In addition, the mentioned farming strategies are practiced without synthetic inputs and emphasized the proper management and cycling of residues within the farming systems, sustaining the farmers and producing food for local markets. Farmers may follow any organic practices that are suited to farmer's existing conditions, integrating crop, livestock, trees, and fishponds accordingly.

Linkages and interactions between the various component species can considerably improve the sustainability of farming systems (Kumar and Jain, 2005). IOFS is an innovative approach that offers a promising solution for sustainable food production and environmental conservation. Its adoption can succor the global challenges of food security worsened by climate change and environmental degradation. The linear system of an economy based on a take–make–consume and dispose of pattern can only be done away with the adoption of Circular-Organo-Agroforestry practices. However, its implementation requires concerted efforts from various stakeholders, including farmers, policymakers, and researchers, to overcome the existing barriers and promote its adoption at a larger scale through financial and revival of traditional organic practices with technical assistance within a framework that rewards farms as multifunctional systems (Vermeulen et al., 2018; Lipper et al., 2022). Bernal (2017) has also stated that the scientific community needs to develop treatment technologies for ensuring nutrients (N and P), enhancing environmental conservation and sustainability and ensuring food safety as improvization from directly using manures in traditional methods.

More research and efforts need to be encouraged in looking for sustainable food production systems that are simultaneously low-cost and provide food security in the local, regional, and global context while attempting to achieve carbon neutrality from the food production systems. A more inclusive form of agriculture system that caters to local requirements and has a shorter supply chain, as well as producing minimum waste, is what the world requires at the moment. Integrated Organic Farming Systems seem to be the right choice of land use that could fulfill all these requirements.

Author contributions

TS and LP: conceptualization, investigation, analysis, writing original draft, and project administration. TS, LP, KM, VG, KR, DB, CT, DKa, PS, RD, DKu, and HD: methodology, writing review, and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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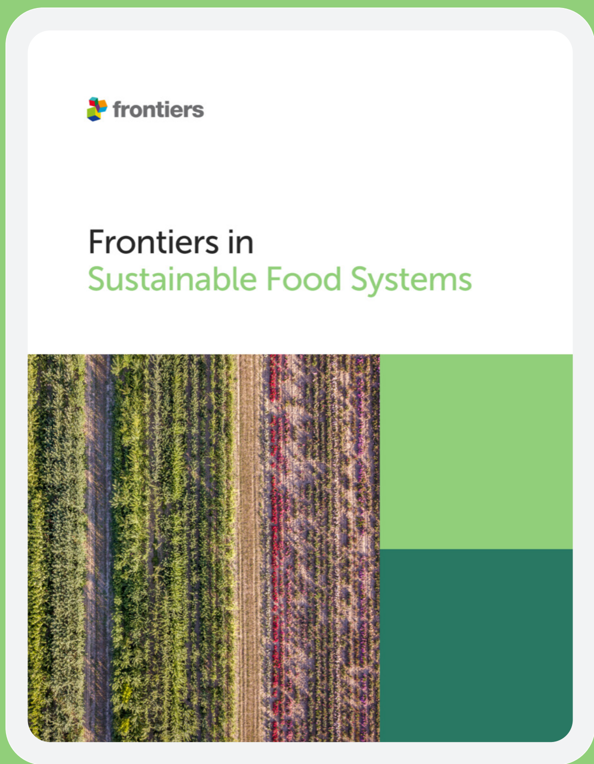

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