

Review

Artificial intelligence in soil microbiome analysis: a potential application in predicting and enhancing soil health—a review

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Abstract

Soil is a depletable and non-renewable resource essential for food production, crop growth, and supporting ecosystem services, such as the retaining and cycling of various elements, including water. Therefore characterization and preservation of soil biological health is a key point for the development of sustainable agriculture. We conducted a comprehensive review of the use of Artificial Intelligence (AI) techniques to develop forecasting models based on soil microbiota data able to monitor and predict soil health. We also investigated the potentiality of AI-based Decision Support Systems (DSSs) for improving the use of microorganisms to enhance soil health and fertility. While available studies are limited, potential applications of AI seem relevant to develop predictive models for soil fertility, based on its biological properties and activities, and implement sustainable precision agriculture, safeguarding ecosystems, bolstering soil resilience, and ensuring the production of high-quality food.

Article Highlights

- Maintaining healthy soil is crucial for sustainable agriculture to ensure long-term food production and environmental protection.
- Soil microbiota plays a main role in preserving soil health, therefore a correct analysis of soil microorganisms will help farmers understand and improve soil quality.
- Although research is limited, integrating AI technologies to monitor soil health holds great promise for sustainable precision agriculture.

Keywords Soil health · Microbial soil community · Artificial Intelligence · Forecasting models · Decision Support Systems

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1 Introduction

Soil is a depletable and not renewable resource. Soil degradation, erosion, and contamination cause soil loss and can rapidly occur and significantly impact ecosystem services. Whereas, the formation of new layers of soil occurs at an often slower rate than soil loss, resulting in a net reduction of soil availability over time, and thus compromising agricultural productivity, biodiversity, water, and nutrient cycles [1, 2]. This highlights the importance of soil conservation and sustainable soil management practices, essential for maintaining soil biological health and ensuring its continued ability to support life on Earth [3].

The current systems of food production are among the main causes of the depletion of natural resources and environmental degradation. Notably, they affect the quality and resilience of the soil, making agriculture unsustainable [4, 5]. Even if soil quality and soil health are terms often used interchangeably [6], here we will use the term soil health. Soil quality is often associated with specific functions that soil performs, such as supporting plant growth, filtering water, and cycling nutrients. It focuses on the soil characteristics that contribute to these functions, assessing how well the soil can perform its roles in the ecosystem [7]. Soil quality is influenced by both natural and anthropogenic factors, including land management practices [8]. On the other hand, soil health is a broader concept that encompasses soil quality and is not just about current physical and chemical qualities but also about the soil overall fertility and sustainability over time [9].

Soil health recognizes that the soil is a living resource, harboring a diverse community of micro and macro-organisms [10–12]. These communities are part of the soil biodiversity and their differences in quality and quantity directly affect soil processes, such as the decomposition of organic matter, the solubilization of chemical elements, and the absorption and degradation of heavy metals and other pollutants [13, 14].

Intensive farming, monoculture, and limited crop rotations are the primary causes of loss of biodiversity, a main indicator of soil biological health [15, 16]. Sustainable land management practices, soil conservation techniques, and efforts to reduce soil pollution when farming are therefore crucial for preserving soil health and biodiversity and ensuring the long-term productivity of agriculture [17].

Nowadays, governments and international agencies are offering more support for sustainable agriculture through policies and research funding, recognizing the role of agriculture in achieving sustainability and development goals. For instance, under the EU's new policy framework "Food 2030," the European Commission advocates a novel development approach, aligned with the 17 Sustainable Development Goals of the United Nations, where sustainable land management is a pillar [18, 19].

Soil health also supports plant health [20–23] and this is the basis of the current EU "One Health" policies (https://health.ec.europa.eu/one-health_en). In agricultural soils, the dynamic interplay among biological, physical, and chemical properties is crucial. The microbial biomass plays a pivotal role in this process, influencing soil health, nutrient cycling, and overall agricultural productivity [24, 25].

In the recent past, agriculture has neglected soil multi-functionality and ecosystem services, leading to practices that have negatively impacted soil health [4]. Conventional agriculture, which uses chemical fertilization, pesticides, and intensive cultivation, can boost short-term productivity but damage soil by eroding, depleting nutrients, and reducing biodiversity [26, 27].

Although since 1970 the theoretical approach to agriculture has always had a focus on agronomic practices deemed sustainable, only from 2010 began to appear a better consciousness of the importance of agricultural management aimed at restoring ecosystem balance [28]. The intention now is to preserve soil fertility avoiding the use of synthetic agrochemicals, and minimizing the environmental impact of human activities with practices such as soft tillage, integrated management of the crops, and other environmentally friendly practices aimed to improve ecosystem services. These practices are preferred over conventional systems to maintain the optimum level of agroecosystem health status [8, 29–31]. Despite the growing attention on soil science, studying soils can be quite difficult due to their functional complexity [30–32], and finding the right indicators to assess whether the soil has suitable levels of quality and health is challenging [33–35].

The role of microorganisms in improving ecosystem soil health and promoting optimal plant development was largely discussed and deeply studied [36–41], and soil metagenome is now considered a "second genome" of plants [42]. The use of beneficial soil microorganisms, as biological pesticides or as biostimulants, for example, can be considered a sustainable agronomic practice to be implemented together with integrated management [43]. Augmentation of beneficial microorganisms to increase soil fertility can consequently be instrumental for improved crops and for other successful agricultural practices [44]. An ideal agricultural ecosystem should support microbial biodiversity with practices aimed at

maintaining local biological and ecological cycles increasing soil fertility, improving soil structure (e.g. by crop rotation), and preferring integrated pest management (IPM) with a limited use of chemicals [45–47].

1.1 Soil microbiota

Microbial soil communities have a fundamental role in establishing a network of positive interaction belowground [48, 49]. There are thousands of species of soil microorganisms that interact with each other and compete for nutrients, each of them with a specific role [50–52]. Through many of the enzymes and other substances they produce, microorganisms, especially bacteria and fungi, can stimulate plant growth, protect them from the attacks of pathogens and pests, and promote the mineralization of organic matter releasing nutrients necessary for plant development [53–55]. Many microorganisms form symbiotic associations with plants at the root level, playing an essential role in plant development by providing numerous benefits such as producing antibiotics, modulating the plant immune system, and acting as biostimulants [23, 56, 57], but the mechanisms that take place within the soil after the inoculum of such microorganisms is still unclear.

For hundreds of millions of years, a co-evolutionary phenomenon occurred, in which plants and microorganisms established strong symbiotic relationships [58] and influenced each other: plants can produce organic substances, especially under biotic or abiotic stresses, changing the surrounding chemical and physical environment, activating some groups of microorganisms, and removing others, and vice versa. Understanding and harnessing the complex interactions between soil, microorganisms and plants is essential for sustainable agriculture, ecosystem restoration, and environmental conservation efforts. It underscores the importance of maintaining healthy soil ecosystems for the well-being of agricultural systems. Using beneficial microbes, modern agriculture is not in contradiction with co-evolution: it is exploiting knowledge of the co-evolutionary phenomenon to optimize crop health and productivity under current conditions. We aim to integrate these principles, along with new technologies, to advance the development of management systems and soil treatment methods in open field conditions.

1.2 Methods for studying soil microorganisms

Microorganisms and their communities in soil can be assessed, both in terms of diversity and abundance, through different laboratory techniques: from the traditional techniques as culture on selective media to the most recent applications of multi-omics approaches [59], the latter largely improving precise and accurate overviews [7] and detection of microbial changes under different conditions and management practices, which may in turn affect soil properties [48]. Combining different techniques (the “multimethod” or “mixed-methods” approach) may also help cross-verify results, mitigating the limitations of individual methods, and gaining a more comprehensive understanding of the dynamics in the soil [60]. Although individual microorganisms are tiny and work within microenvironments, such as around a single root or within soil aggregates, their processes, such as nutrient cycling, organic matter breakdown, and pathogen suppression, affect larger, visible aspects of the environment like plant health, soil fertility, and carbon storage. We should better understand how to enhance soil bacterial processes, scaling them up from the microscale to levels that are significant for soil management. Sample size, along with replication, sampling design, DNA extraction, and molecular analysis methods, significantly influence measures of community structure under field conditions [61]. Scaling up results, from small sample size to represent a large field, requires careful consideration of these factors to avoid misrepresentation of microbial diversity. For this reason, it is important to consider standardized protocols like ISO 11063, which aim to optimize soil DNA extraction for reproducibility and comparability, though variations in methods and sample sizes can still impact the assessment of microbial abundance and diversity [62]. Also, modeling the dynamics of a microbial soil community can help understand belowground interactions that have repercussions on the soil ecosystem [63]. Among approaches based on soil total DNA analysis to provide taxonomic profiles of microbial communities, one of the most common methods is the metabarcoding: it is based on the amplification of specific DNA regions, such as 16S rDNA (ribosomal DNA) for bacteria and ITS (Internal Transcribed Spacer) for fungi, using PCR (Polymerase Chain Reaction), followed by high-throughput sequencing, e.g. with NGS (Next Generation Sequencing) methods [64]. Although this approach has the undeniable advantage of enabling the simultaneous analysis of many samples with a good level of precision and in a relatively short time, it does have some limitations that can affect the accuracy of taxonomic assignments. These drawbacks include potential biases introduced during DNA extraction and amplification [65], difficulties in distinguishing between live and dead organisms [66], resolution usually limited to family or genus level that does not permit differentiation between closely related species or sub-species [67], and challenges associated with incomplete

reference databases [65]. Additionally, the presence of PCR inhibitors in soil samples can complicate the process and lead to the underrepresentation of certain taxa. To address these limitations, researchers are exploring and developing alternative approaches and technologies. For example, to enhance taxonomic resolution, multiple genetic markers could be combined, and thanks to sequencing technologies developed by PacBio (Pacific Bioscience) and Oxford Nanopore, longer reads compared to traditional NGS methods are provided, enabling a better assembly of genomes and an accurate characterization of complex microbial communities [68]. To explore the genetic diversity and functional potential of complex microbial communities in a wide range of environments, metagenomics provides a powerful tool [69]. Instead of targeting specific genetic markers, Metagenomic Shotgun Sequencing involves the sequencing of all DNAs present in a sample providing a more comprehensive view, allowing the discovery of new species and functional genes.

Investigating the genera or the species of microorganisms present in soil can unveil valuable perspectives about the environment, as specific microorganisms can serve as “indicator species” or “bioindicators” [70, 71].

At a community level, the physiological profiling method performed using the Biolog® system is based on patterns of single carbon source utilization by microorganisms present in the soil. This analysis permits obtaining information on how microbial communities respond to changes of the environment over both spatial and temporal scales [72].

Enzymes produced by microorganisms are key factors in soil processes such as decomposing organic matter, cycling nutrients, and enhancing soil structure [73]. Measuring enzymatic activities of microorganisms in soil provides insights into microbial activity, and can serve as indirect indicators of soil health. Higher enzymatic activity levels often indicate greater microbial diversity, especially in the active soil microorganisms, essential for maintaining soil health [74–76]. An example of a soil sensor able to give information on biological enzyme activity was developed by DIGIT-soil [77]. This biosensor works through a customizable membrane targeting several soil enzymes, providing in-situ measurements and automated analysis of soil enzyme activity in few minutes. These sensors can be integrated into soil monitoring systems, allowing for continuous monitoring and timely interventions to maintain, restore, or improve soil fertility and health, providing real-time or near-real-time data on soil biological parameters. All the methods mentioned are summarized in Table 1.

1.3 Harnessing technologies for smarter agriculture

Agriculture 4.0 represented a significant transformation driven by the integration of cutting-edge technologies [85] to optimize crop yields while minimizing inputs such as water, fertilizer, and pesticides.

Big data analytics involves the examination of large and diverse datasets to identify hidden patterns, correlations, trends, and valuable insights, enabling farmers to make informed decisions based on a comprehensive understanding of factors influencing farming operations [86, 87]. Analysis of large datasets collected from sensors located proximally or distally from the cultivated area (e.g. drones or satellites), through the new computational technologies, can help farmers optimize resource allocation, predict crop yields, manage risks, and identify areas for improvement of farming practices [88].

Furthermore, devices, such as soil moisture sensors, weather stations, and automated irrigation systems, allow farmers to monitor and control agricultural processes in real-time. Connected sensors provide continuous data on environmental conditions and crop status, enabling precise management and timely interventions.

By integrating these innovations, farmers can advance beyond conventional methods, adopting a more data-driven and efficient approach that enables real-time informed decisions [89, 90]. Today, the decision-making process is facilitated by Decision Support Systems (DSSs) that gradually evolved to incorporate more sophisticated tools and techniques [91, 92]. With the incorporation of machine learning (ML) algorithms and predictive models, indeed, DSSs could analyze complex datasets, offering insights and recommendations for optimizing outcomes [93]. One of the most important questions in agriculture is to predict soil suitability for specific crops before planting [94]. In this context, a balanced microbiota supports optimal production yield while maintaining the ability of the soil to sustainably produce, which is a relevant entry of the DSS algorithm [95, 96], DSS capable of monitoring soil health must utilize many data inputs including soil nutrient levels, moisture content, temperature, microbial diversity, to feed ML algorithms aiming at predicting crop performances. DSSs integrating soil microbiology monitoring can also ensure that farmers can take action to restore microbial balance when necessary, by using beneficial microorganisms or compost amendments. This approach supports sustainable crop productivity and the maintenance and enrichment of soil health.

Data that allow DSS elaboration often need to be examined by Artificial Intelligence (AI). AI technologies, such as Machine Learning (ML) and Deep Learning (DL), have found numerous applications in agriculture, offering solutions to various challenges [97–103], and could result fundamental to analyze and model large amounts of data detected

Table 1 A glossary of the main methods mentioned in the paper used for studying soil microorganisms

| Method | Basic principle | References |
|----------------------------------|---|------------|
| Metabarcoding | is a molecular biology technique used to identify and quantify species within a complex mixture of DNA, including environmental samples. It combines DNA barcoding, which involves sequencing a short, standardized region of DNA (a "barcode") that is unique to a species, with high-throughput sequencing (HTS) technologies, allowing for the simultaneous analysis of multiple species from a single sample | [78] |
| High-Throughput Sequencing (HTS) | refers to a set of advanced technologies that allow the rapid and large-scale sequencing of DNA or RNA. Unlike traditional Sanger sequencing, which sequences DNA one fragment at a time, HTS can sequence millions to billions of DNA fragments simultaneously, making it highly efficient and capable of generating vast amount of data in a relatively short time | [79] |
| Polymerase Chain Reaction (PCR) | is a widely used molecular biology technique that allows the amplification of specific DNA sequence, through key components: two primers, one forward and one reverse, short single-stranded DNA sequences that bind to opposite strands of the DNA template; an enzyme that synthesizes new DNA strands by adding nucleotides to the primers (polymerase); Deoxynucleotide Triphosphates (dNTPs), the building blocks (adenine [A], cytosine [C], guanine [G], and thymine [T]) used by DNA polymerase to synthesize the new DNA strands; a buffer solution that maintains the optimal pH and ionic conditions for the PCR reaction, ensuring that the DNA polymerase functions efficiently; the template, the DNA sequence that will be amplified. PCR involves three main steps: each cycle of denaturation, annealing, and extension doubles the amount of the target DNA sequence. After 20–40 cycles, the target DNA can be amplified millions to billions of times | [80] |
| Next Generation Sequencing (NGS) | is a collection of advanced sequencing technologies that enables the rapid and comprehensive sequencing of DNA or RNA. Unlike traditional Sanger sequencing, which sequences DNA one fragment at a time, NGS can sequence millions to billions of DNA fragments simultaneously, providing a massive amount of genetic information quickly and at a lower cost per base | [81] |
| PacBio sequencing | is an advanced DNA and RNA sequencing technology developed by Pacific Biosciences known as Single-Molecule Real-Time (SMRT) Sequencing. This technology is distinct from other sequencing methods due to its ability to produce extremely long reads, providing more comprehensive and accurate insights into genetic sequences, including complex regions that are difficult to sequence with other technologies | [81] |
| Oxford Nanopore sequencing | is an advanced DNA and RNA sequencing technology developed by Oxford Nanopore Technologies. This technology is known for its ability to produce ultra-long reads by directly sequencing nucleic acids as they pass through a nanopore, a tiny hole, embedded in a membrane. It is unique among sequencing technologies because it can generate real-time data, sequence very long fragments of DNA or RNA, and analyze a wide range of molecule types | [81] |
| Metagenomics | is the study of genetic material recovered directly from complex samples, rather than a single organism. This field allows scientists to analyze the collective genomes of entire communities of microorganisms, such as bacteria, archaea, viruses, and fungi, that inhabit a particular environment. The key advantage of metagenomics is that it enables the study of organisms that are difficult or impossible to culture in the lab, thus providing a more comprehensive understanding of microbial diversity, ecology, and function | [82] |
| Metagenomic Shotgun Sequencing | is a comprehensive and unbiased approach to study the genetic material within entire communities (including microbiota) directly from environmental samples. Unlike targeted sequencing methods, which focus on specific genes, shotgun metagenomics sequences all the DNA in a sample, capturing the full diversity of organisms and their functional potential | [83] |
| Biolog® | is a technology used for the identification and characterization of microorganisms based on their metabolic activities. It is commonly used in microbiology laboratories for the identification of bacteria, yeast, and fungi, as well as for assessing their metabolic profiles. It assesses the metabolic capabilities of microorganisms by measuring their ability to utilize various carbon sources and other substrates. It profiles different species or strains based on their metabolic fingerprints | [71] |
| Biosensors | are sophisticated analytical tools that integrate biological recognition elements with physical or chemical detectors to measure specific substances. Their applications span across medical diagnostics, environmental monitoring, food safety, biotechnology, and more, providing valuable insights and real-time data for various fields | [84] |

in the field. AI-driven DSSs are instrumental for precision farming and its applications for sustainable agricultural practices [104], aimed at preserving ecosystems, increasing the resilience of soils, and ensuring the quality and quantity of food production (Agriculture 5.0) [105]. AI technologies are increasingly being utilized in agriculture to enhance productivity, efficiency, and sustainability of crops, and play a leading role in this context by enabling machines to perform tasks related to human intelligence [106]. Moreover, the integration of Internet of Things (IoT), Decision Support Systems (DSSs), Cloud Computing, Big data analytics and Remote Sensing has indeed brought significant advancements in agriculture [107, 108]. The complementation with automation and digitization systems enables both the collection of high-throughput data and the optimization of field-level practices such as planting [109–111], fertilization [112–114], irrigation [115–119], and pest control [120–122] based on real-time data acquisition and analysis, and often are also used for features such as data visualization, modeling, and simulation [123].

Numerous publications on generic applications of AI techniques for agricultural sustainability are available, which will not be reviewed here. Bhagat et al. (2022) made a bibliometric analysis, finding 465 papers only in the period 2000–2021 on this subject [124]. However, models developed using AI techniques to differentiate soil health status based on soil microbial composition, which serves as a fundamental input for sustainable agriculture, are scant in the literature.

1.4 AI empowers microbial community studies

For this review, we intended to consider only papers dealing with the prediction of soil health metrics through microbiome data as input, but this search was very restricting. So, we added studies that utilized AI techniques to predict outcomes based on microbiome composition, as well as those that employed AI to determine microbiome composition as an output. We consulted publications from “Web of Science”, “SCOPUS” and “Google Scholar” databases until 1st October 2023, using the following keywords: “artificial intelligence”, “soil microbial community”, “soil health prediction”, “soil microbiome monitoring”, “soil health assessment”, “soil microbiome”, “soil microbial indicators”, alone and using the Boolean operator “AND” to refine the research (e.g. “soil microbial community AND soil health prediction”), in all possible combination, excluding the redundant ones. Results are shown in Table S1. Out of 3508 papers found, only 12 encountered the requested criteria (Table 2), and their classification based on the AI approach applied is shown in Fig. 1.

As already mentioned, only a few studies have been published on how to predict soil health through information on microbial soil communities using AI techniques and forecasting models, despite the high potential of these applications [125]. In the context of studying soil microbiomes, researchers have proposed microbial-based metrics to evaluate soil health [76]. Indeed, Correa-Garcia et al. (2022) [125] claim that considering the ability of microorganisms to reflect past and present conditions of the habitats in which they are found, and are directly involved in the ecosystem processes, they also have some predictive capacity for future processes. Microbiomes are ideal for modeling and predicting the evolution of ecosystem processes as they participate directly in them. De Andrade et al. (2021) [105] propose guidelines for developing an Artificially Intelligent Soil Quality Index, incorporating input data for the ML model that includes recent land management practices, current genomic features, present and projected plant cover, as well as current soil biological, chemical, and physical parameters. Wilhelm et al. (2022) [126] aimed to develop a predictive model using soil bacterial community composition (in terms of 16S DNA sequences) to estimate 12 key soil health metrics, including biological, chemical, and physical properties. The study employed supervised machine learning algorithms, specifically Random Forest (RF) and Support Vector Machines (SVM), to classify and predict soil health metrics based on microbiome data collected from 949 soil samples from North American farmlands. Biological health metrics were the most accurately predicted, while the models struggled more with chemical and physical metrics, such as aggregate stability. However, the study suggests that larger and more region-specific datasets are needed to improve accuracy further. Hermans et al. (2020) [127] applied RF models to assess the degree to which bacterial communities in cultivated soils can be used to forecast physio-chemical soil characteristics: they verified a robust correlation between the composition of bacterial communities and land use, asserting that bacteria sensitivity to changes in specific soil parameters highlights the potential of bacterial DNA analysis as a tool for assessing soil quality. Chang et al. (2017) [128] applied a RF machine learning approach to predict crop productivity based on microbiome composition, revealing that differences in crop productivity were linked to the bulk soil microbiome composition and highlighting that several taxa are associated with nitrogen utilization.

Other publications were found on the use of AI techniques to study the relationship between soil microbial characteristics and the physical and chemical properties of soils [129, 130], to predict soil microbial biomass [131], to predict soil enzyme activity [132], and to assess soil microbiological characteristics or bacterial community structure [133–135].

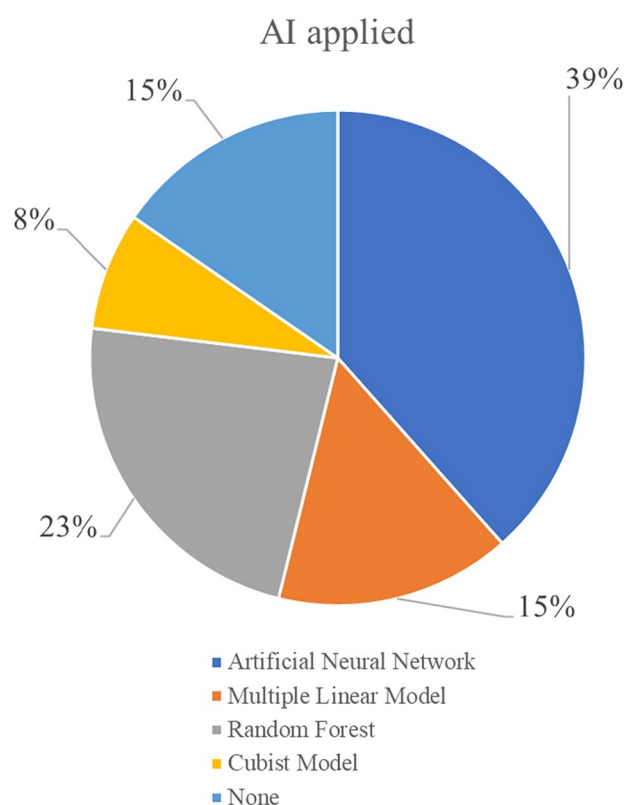
Table 2 Reviewed papers

| Research reviewed | | | | | | |
|-------------------|------|----------------------|--|-------------------------|------------------------------------|---|
| Ref | Year | Authors | Principle aspect | Category | Principle input | AI techniques applied |
| [105] | 2021 | de Andrade et al. | the authors propose guidelines for assembling an Artificially Intelligent Soil Quality Index, giving as input to the ML model data on recent land managements, current genomic features, current and projected plant-cover, time and current soil biological, chemical and physical parameters | Rewiev/Opinion paper | Microbiome data | None |
| [125] | 2022 | Correa-Garcia et al. | the authors discuss key criteria that differentiate the various statistical learning approaches and propose Supervised and Unsupervised learning methods to unlock forecasting potential of the microbiome for ecosystem processes | Rewiev/Opinion paper | Microbiome data | None |
| [126] | 2022 | Wilhelm et al. | the authors use 16S rRNA gene information to predict 12 measures of soil health through the application of supervised Machine Learning techniques | Original research paper | Microbiome data | Random Forest and Support Vector Machine regression and classification models |
| [127] | 2020 | Hermans et al. | the authors assess the degree to which bacterial communities in cultivated soils can forecast soil physico-chemical characteristics | Original research paper | Microbiome data | Random Forest model |
| [128] | 2017 | Chang et al. | the authors show that crop productivity differences were associated with bulk soil microbiome composition and highlighted several nitrogen utility-related taxa | Original research paper | Microbiome data | Random Forest model |
| [129] | 2023 | Sadeghi et al. | the authors used soil physical and chemical properties and total phospholipid fatty acid (PLFA) analysis to predict soil biological properties, and found a relationship between soil microbial characteristics and the physical and chemical properties of soil | Original research paper | Soil physical and chemical metrics | Cubist model algorithm |
| [130] | 2017 | Ebrahimi et al. | the authors evaluate the population of Azotobacter in soils with different land uses | Original research paper | Soil physical and chemical metrics | Artificial Neural Networks and Multivariate Linear Regression approaches |

Table 2 (continued)

| Research reviewed | | | | | | |
|-------------------|------|-------------------|--|-------------------------|---|--|
| Ref | Year | Authors | Principle aspect | Category | Principle input | AI techniques applied |
| [131] | 2021 | Pellegrini et al. | the authors predict soil microbial biomass from a range of physical and chemical properties | Original research paper | Soil biological physical and chemical metrics | Artificial Neural Network model approach |
| [132] | 2012 | Tajik et al. | the authors use soil and topographic attributes to predict the activity of three soil enzymes, L-asparaginase, L-glutaminase, and urease | Original research paper | Digital Terrain Analysis | Artificial Neural Networks and Multiple Linear Regression approaches |
| [133] | 2012 | Larsen et al. | the authors describe a bioclimatic modeling approach to predict microbial community structure as a function of environmental parameters and microbial interactions | Original research paper | Environmental parameters and microbial interactions | Artificial Neural Network model approach |
| [134] | 2014 | Santos et al. | the authors identify patterns in soil chemical variables and microbial community structures across forest soils in three regions of Brazil's Atlantic Forest | Original research paper | Soil physical and chemical metrics | Artificial Neural Network model approach |
| [135] | 2018 | Ramirez et al. | the authors merge 30 independent bacterial data sets comprising 1998 soil samples from 21 countries and show that disparate amplicon sequence data can be combined at the taxonomy-based level to assess bacterial community structure | Original research paper | Bacterial community | Random Forest models |

Fig. 1 Classification of the papers based on the AI techniques applied



All the above-mentioned papers are summarized in Table 2, with additional explanations of the main terms used in this paper in Table S2.

1.5 AI techniques applicable to soil microbiome analysis

The analysis of microbiome features can encompass a range of computational methods, from conventional statistical techniques to more sophisticated algorithms employed by AI. The first applications of AI to microbiota studies were used to investigate the role of human gut microbiota in human health: AI techniques such as ML and DL algorithms were applied for the diagnosis and therapy of critical diseases, with significant potential for improving public health [136–140]. An overview of ML methods for human microbiome analysis is given in Namkung (2020) [141].

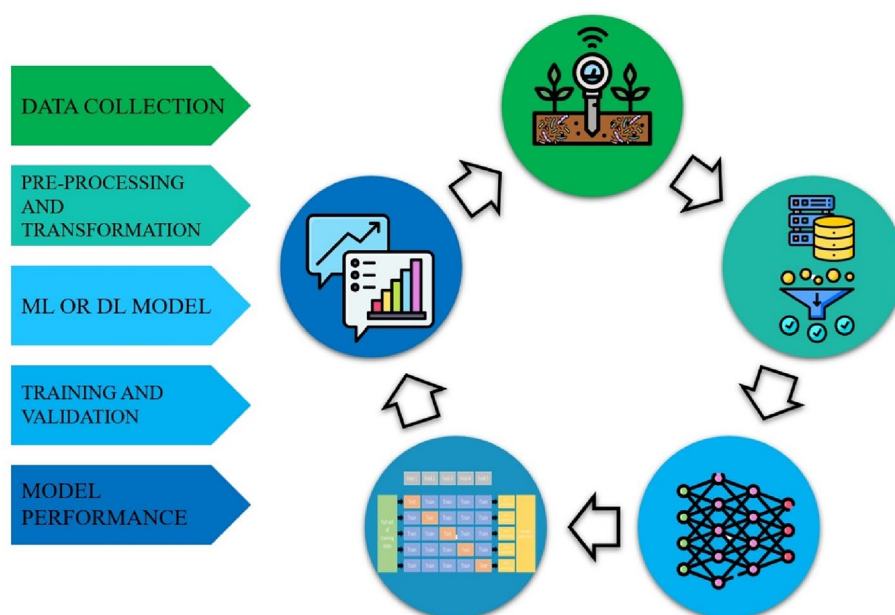
Oh and Zhang (2020) [137] used autoencoders to convert microbiome data into low-dimensional representations, thereby enhancing the ability to predict human diseases. Curry et al. (2021) [142] also investigated ML techniques for disease detection from the gut microbiome, highlighting further computational and biological challenges. Morton et al. (2019) [143] presented a training process that aims to estimate the likelihood of detecting a metabolite given the provided microbe sequence through an Artificial Neural Network (ANN) approach. Tataru and David (2020) [144] used word-embedding techniques to analyze microbiome data and to better understand microbial interactions in human diseases. Costello and Martin (2018) [145] demonstrated how ML can predict metabolic pathways from time-series multi-omics data, thereby eliminating the need for traditional kinetic models. Metwally et al. (2019) [146] used longitudinal microbiome data to demonstrate the advantages of whole genome shotgun sequencing over amplicon sequencing, providing more insight into the microbiome's impact over time. Iadanza et al. (2020) [136] explored the use of AI in the identification and management of diseases by analyzing the gut microbiota. They emphasized the importance of AI in interpreting complex data sets. Liu et al. (2022) [147] created a ML framework to identify gut microbiome biomarkers across multiple geographic regions, utilizing ensemble learning and reinforcement learning techniques for personalized modulation analysis. Li et al. (2022) [148] investigated the use of multi-omics data to improve the accuracy of disease prediction models. They used ML techniques to study the gut microbiota and its effects on host metabolism. In summary, autoencoders and other ANN architectures have proven to be efficient in handling the complex and diverse

characteristics of microbiome data in medical science. This is critical for accurately predicting phenotypes and identifying biomarkers, as demonstrated by Hernández Medina et al. (2022) [149].

Expanding the use of ML techniques from medical to agricultural microbiome analysis presents numerous challenges, including data collection, sparsity, and biomarker identification. Soil samples differ significantly from controlled medical settings due to many factors, e.g. weather, topography, and host organism diversity. Furthermore, soil microbiome datasets are frequently smaller and less dense than human ones, thus needing more sophisticated data augmentation and handling techniques to avoid overfitting. While many studies on the human microbiome focus on disease prediction, the goal of agricultural applications often is the identification of biomarkers associated with soil fertility or crop yield. Nevertheless, the aforementioned medical examples can serve as a reference for applying AI techniques to study the soil microbiome. ML algorithms can significantly contribute to the advancement of our knowledge about soil health by analyzing data on microbial communities and making predictions about different environmental indicators [150].

The procedure for analyzing soil microbiome data using AI techniques adheres to a systematic sequence of steps commonly employed in bioinformatics and computational biology research (Fig. 2). Data pre-processing is an essential step in microbiome analysis to adequately prepare the input data for accurate ML and DL modeling. Normalization of microbiome data is necessary to adjust for differences in sequencing depths among samples. Various techniques such as rarefaction, total-sum scaling, or more advanced methods can be employed for this purpose [151]. To address skewness, logarithmic or other transformations such as square root can be employed. Missing values can be managed through techniques like k-nearest neighbors (k-NN), multiple imputation, or by assigning zero or minimal values, depending on the specific circumstances. In addition, when analyzing data that encompasses different taxa, creating new features might help in capturing more complex relationships that may not be apparent from the original data alone. Techniques such as Principal Component Analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE), and Uniform Manifold Approximation and Projection (UMAP) can be used to reduce the number of features and uncover structure in the data that may be related to the phenotype of interest. Dimensionality reduction is accomplished by using autoencoders to address the issue of high dimensionality in microbiome data. This method greatly accelerates model training and hyperparameter optimization [137]. After pre-processing, the next step is to choose and train an appropriate ML or DL model based on the hypothesis and nature of the data. Random Forests, Gradient Boosting Machines, and Support Vector Machines are common models for microbiome studies, as well as ANN architectures such as Convolutional Neural Networks (CNN), Recurrent Neural Networks (RNN), and Autoencoders for DL tasks. DL models, particularly CNNs and RNNs, are used to analyze complex microbiome datasets and predict diseases or phenotypic traits based on microbiome composition. These models are specifically intended to handle the massive amounts of data generated by high-throughput sequencing technologies [137]. Implementing cross-validation is fundamental because it helps ensure that the model does not just perform well on the training data but also generalizes well to new, unseen data, using techniques such as k-fold cross-validation, grid search, random search, or Bayesian optimization, that can help find the optimal model

Fig. 2 Principal phases of developing a Machine Learning (ML) or Deep Learning (DL) model



settings [152]. Additionally, overfitting can be avoided by training the model on the training dataset and evaluating its performance on a validation set. The outcomes of the model evaluation, which occurs after it has been trained, can be interpreted within the framework of microbiome research. Metrics such as accuracy, precision, recall, F1-score for classification tasks, and mean squared error (MSE) or root mean squared error (RMSE) for regression tasks are computed to evaluate the performance of the model, depending on the specific task at hand. Models such as feature importance from tree-based models, SHAP values (Shapley Additive exPlanations), or permutation importance are among the commonly used to ascertain the taxa, features, or functions that are influencing the predictions of the model. By integrating diverse omics data, including metabolomics and metagenomics, and applying ML methodologies to this information, predictive models become more accurate. By adopting this workflow researchers can gain comprehensive insights into how microorganisms interact with one another and how these interactions influence their broader environment, whether this is a specific ecosystem, agricultural setting, or human body [137]. An overview of ML approaches useful for analyzing microbiome data is available in the paper by Papoutsoglou et al. (2023) [153]. A comprehensive review of utilizing DL techniques for analyzing environmental microbiome data is also provided by Deng et al. (2021) [154]. This last review outlines the advancements in DL techniques and elucidates their advantages over classical ML methods. The insights gained can be applied to predict outcomes or classify new samples in environmental settings. This workflow must be modified to reflect specific project goals, available data, and computational resources. Each step should be carefully planned and carried out to ensure the conclusions are reliable and valid.

A significant step forward would be the creation of AI-based forecasting models that integrate physical, chemical, and biological soil characteristics as well as agrometeorological data, allowing for the correlation of variation in microbial community composition with different levels of soil health. Most of the bases are in place to complete this step, but much work remains to be done on collecting data, soil parameter monitoring, parameter correlation, and mathematical model training for prediction. The initial step involves systematically collecting detailed information over time at regular intervals, focusing on soil properties such as pH, nutrient levels, organic matter content, and microbial composition. Scheduling and tailoring the samplings at consistent intervals (e.g., monthly, seasonally) to capture temporal variations, collecting them from multiple locations within the study area to account for spatial variability. This information could be obtained through classical soil sampling and analysis, and with the help of remote sensing technologies. Also, it is important to use a centralized database to store all collected data and record contextual information about sampling methods, locations, and conditions to provide context for data interpretation. Understanding the interactions between soil microorganisms and their surroundings is critical: the roles of various microbial species in soil health, their functions, and how they interact with plants can provide useful information. Using advanced molecular techniques such as metagenomics, researchers can identify beneficial microorganisms found in healthy soils, such as nitrogen-fixing bacteria, mycorrhizal fungi, and other symbiotic microbes that improve soil fertility and plant growth. Algorithms can be developed based on the collected data and understanding of microbial ecology to recommend specific microbial inoculants or management practices that are tailored to the soil's unique characteristics and the crops grown. By incorporating these elements into a DSS, farmers will be able to make more informed decisions that promote sustainable agriculture and productivity. Furthermore, farmers' feedback on the effectiveness of recommended practices and observed changes in soil health can be used to fine-tune the algorithms over time, which is critical for the DSS ongoing improvement. This entire process will take a long time to develop, but the results will be significant for farmers and the global agricultural sector.

The use of AI in microbiome research could revolutionize our understanding and control over microbial communities.

2 Concluding remarks

Globally, soils face severe degradation due to unsustainable agricultural practices, deforestation, and climate change impacts, leading to declines in soil fertility, erosion, and reductions in biodiversity (FAO on soil recarbonization practices: The Nature Conservancy <https://www.nature.org/en-us>).

The use of AI to predict soil health through microbiome information could represent a significant advancement in soil science. Microbiome data is inherently complex, with numerous microbial species interacting in intricate ways. Traditional analysis methods can be time-consuming and may overlook the full depth of these interactions. Therefore, microbiome data manipulation relies on key techniques to manage, analyze, and interpret the complex, high-dimensional datasets produced by sequencing technologies.

AI models can efficiently process vast amounts of data and identify patterns that may be difficult for humans to discern. Currently, the main examples of data manipulation on the microbiome are in medicine, while soil science

appears to be a new and still open field for AI applications. ML techniques, including RFs, SVMs, and ANNs, are widely used in microbial community classification, clustering, and prediction modeling. CNNs excel at interpreting microbial abundance data by transforming it into structured formats, whereas RNNs are especially effective at analyzing time-series data, such as modeling microbial succession dynamics. One significant advantage of AI models is their ability to constantly learn and adapt as new data becomes available. This adaptability is critical for studying soil microbiomes, which live in constantly changing environments. As new information becomes available, models can be refined to provide more accurate predictions of soil health. Furthermore, as discussed by Cuomo et al. (2024) [155], novel techniques such as Physics-Informed Neural Networks (PINNs) show promise in modeling the growth of microbial populations while accounting for environmental factors, providing a powerful tool for studying microbial dynamics.

Certainly, the advent of AI-based tools has the potential to significantly reduce the time and human resources needed. Future developments should prioritize collaboration between researchers, farmers, and industries for developing standardized datasets and protocols. This can improve the reliability and interoperability of AI models across different regions and agricultural systems, ensuring broader applicability and adoption. Understanding how well AI predicts soil health is essential for gaining the trust of farmers and other stakeholders, and is crucial for the widespread acceptance and adoption of AI-driven technologies.

In conclusion, the integration of AI for the prediction of soil health through microbiome information holds great promise for sustainable agriculture. Continued research, technological advancements, and collaborative efforts are essential to unlock the full potential of AI in optimizing soil management practices for a more resilient and productive agricultural future.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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