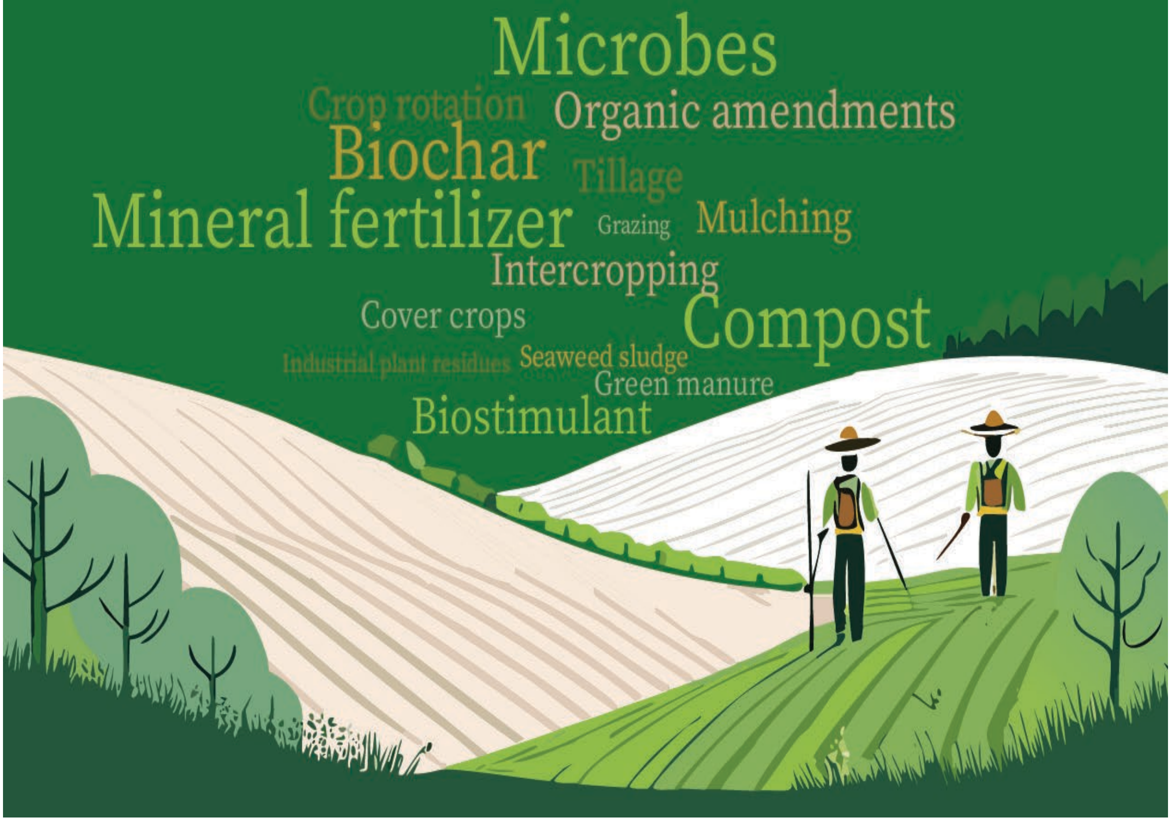
university of groningen

fascinating

El Mujtar, Prack McCormick, Falcao Salles, Tiedge, Tittonell, and van Veen

The Effects of Regenerative Agriculture on the Quality of Soil and Plant/Animal Produce

A systematic review



The effects of Regenerative Agriculture on the quality of soil and plant/animal produce: a systematic review

Authors: Verónica El Mujtar[1], Bárbara Prack McCormick[2], Joana Falcao Salles[3], Kira Tiedge[4], Pablo Tittonell[5], Kees van Veen[6]

For: FASCINATING – 100 hectare experiment
Josien van Harten en Mathijs Niehaus

Date: 09-09-2024

[1] Dr. Verónica El Mujtar is a researcher of the Consejo Nacional de Investigaciones Científicas y Técnica (CONICET) at the Instituto de Investigaciones Forestales y Agropecuarias Bariloche (IFAB, CONICET-INTA).
[2] Dr. Bárbara Prack McCormick is a postdoctoral researcher at Resilient Landscapes Group in the Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen.
[3] Dr. Joana Falcao Salles is a professor in Microbial Community Ecology at the Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen.
[4] Dr. Kira Tiedge is an Assistant Professor for Functional Genetics and Genomics at the Groningen Institute for Evolutionary Life Sciences (GELIFES).
[5] Dr. Pablo Tittonell is professor of Agroecology and Sustainable Landscapes at the Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, and is senior associate researcher at the French International Cooperation Centre for Agricultural Research and Development (CIRAD).
[6] Dr. Kees van Veen is an Associate Professor at the UG (Faculties of Behavioral and Social Sciences + Economics and Business). He is co-initiator of the Sustainable Food Systems research group within the UG, and initiator of the "Oogst van Groningen" initiative in the region.

Content

Index of Figures

Index of Tables

Management summary:

1. By now, **Regenerative Agriculture** (RA) is a broad umbrella concept with different interpretations for different audiences. Momentarily, there is **no coherent idea that shows systematically how principles and agricultural management practices fit together** and what the **overall outcomes** would be on soil and produces. Indeed, most of the research on RA is focused on what RA is and on the drivers/barriers conditioning its adoption, while only a few studies focus on a robust and integrated assessment of its effects.
2. Regenerative Agriculture distinguishes itself from conventional agricultural approaches by a **set of different principles** (such as "minimize synthetic inputs") **that subsequently translates into a varied list of agricultural management practices** (such as the use of manure, biochar, mulching, microbes, etc.). Different practices are subsequently used in different combinations in different studies This makes it difficult to properly summarize its effects on a detailed level.
3. To understand/grasp the effects of RA, the **focus of this systematic literature review is on the known effects of different management practices that fall under the umbrella of RA**. Considering the complexity of reported practices, the total set has been reduced by developing a set of six classes of practices.
4. In the last decade, the **number of studies that focus on these practices is growing rapidly**. Additionally, the number of different practices studied is on the rise, as is **the number of crops studied** in terms of numbers and varieties.
5. **The effects of practices on the plant produce quality** (**macro-nutrients**): different practices or practice arrangements have a positive, but varying, effect on plant quality and particularly in terms of macro-nutrients. In terms of indicators, carbohydrates and mineral elements are most often included.
6. **The effects practices on the plant produce quality** (**taste**): taste is **scarcely considered** as a relevant indicator of practice effects.
7. **The effects of practices on plant and human health**: If indicators are included in different studies, a large variety of direct and indirect indicators can be observed. Among the most frequent indicators used are antioxidant capacity, photosynthetic efficiency and the abundance of bioactive compounds.
8. The **effects of practices on the soil quality and plant quality:** research supports the use of practices usually considered in RA to improve soil quality and, consequently, in plant quality. However, the effect differs and less than half of the selected studies integrates both aspects at the same time.
9. **In general, our literature review – and related reviews – supports the effects of practices usually considered in regenerative agriculture to improve soil and plant quality. Effects on taste are hardly studied.**

Remaining open questions:

Simultaneously**, a set of open questions remains**:

- Systematic **comparison of the effects of different (combinations of) practices on different outcomes is lacking**. Present knowledge is mainly related to one or two practices with a limited set of isolated outcome indicators. More integrated approaches are necessary (with combinations of soil health, plant health and produce nutrients indicators)
- Systematic **comparison of the effects of different practices on different produces is lacking**. Only a few produces are studied and focus only a few characteristics.
- Systematic **analyses of practice effects on taste of produces is very rare** in this field.
- Systematic analyses of the **consequences for human health is lacking** although the available literature indicates the changes on soil and plant quality could have consequences in this respect
- A conceptual **framework** based on existing scientific RA knowledge **to classify or categorize regenerative agriculture initiatives is lacking**

Opportunities for the 100 hectare experiment of FASCINATING: some first ideas.

The 100 hectare experiment of FASCINATING offers an new opportunity to work on a few of the open questions suggested. The close collaboration with farmers offer the opportunity to assist them in their personal steps, and at the same time unlock the results for other farmers and related stakeholders. The 'teeltplannen' are an interesting starting point in that respect and offer possibilities. Considering the highlighted research gaps:

- Experiments could be designed considering a categorization system of regenerative agriculture. The categorization system should be based on how practices are implemented and combined.
- Experiments should combine regenerative systems on different level of transition as determined by the categorization system.
- Assessment of effects could integrate soil and plant quality indicators, including macro-nutrients, health and taste indicators, depending on the practices used and crops chosen.
- Assessment of the effects could be further extended to integrate human health indicators.
- Interrelationship of RA effects on soil and plant quality could be incorporated on the assessments.
- Integrate different produces (e.g. animal and crops), including relevant but scarcely studied produces (e.g. potato, carrot, beans)
- Integrate short- and long-term assessment of indicators with the right controls and sampling design.

Chapter 1: Introduction

Regenerative Agriculture (RA) aims to improve the environmental and economic viability of farmland (Tittonell et al. 2022). The term RA has evolved over time, gaining popularity in different sectors and initiating movements among consumers, producers, industry, academia, non-governmental organizations, and policymakers (Giller et al. 2021, O'Donoghue et al. 2024). During its evolution, a growing heterogeneity emerged in the principles, practices, and outcomes considered or prioritized amongst RA-supporting sectors.

a) **Principles** refer to the guiding truths or reasons behind practices (e.g. minimize soil disturbance, fostering plant diversity, integrating livestock and cropping)
b) **Practices** are applications of principles which are essentially flexible and adaptable to varying circumstances. For example, practices such as diversified rotation, leys, and growing legumes and cover crops could be implemented to foster plant diversity. Therefore, even RA systems under the same principles could have different arrangements of practices.
c) Similarly, **outcomes** refer to the environmental, social or economic foci of the improvements (e.g. soil health, carbon sequestration, crop health, profit, land productivity, human welfare, social justice) and can be related to different practice arrangements and principles.

The concept of RA turned out to be flexible in different contexts. At the same time, the lack of a common definition of RA constraints a global understanding of what regenerative agriculture is about (Newton et al. 2020, Schreefel et al. 2020). The heterogeneity in terms of principles and outcomes that guide RA approaches has led to a wide range of practices, used alone or in different combinations. Besides the claims of the environmental crises that RA can address (e.g. soil deterioration, biodiversity loss, climate change), recent reports highlight that its outcomes vary between agroecosystems, climate conditions, soil types and the practices involved (e.g. Montgomery et al. 2022, Manzake-Kangara et al. 2023, Khangura et al. 2023, Rehberger et al. 2023). Therefore, there is an increasing and urgent need to shed light on the diversity of current approaches and their effects to further guide work that focuses on the added value of RA. How to design long-term farming system trials is a growing question both for farmers and academia. To develop such designs, a robust overview of the known effects of practices on the expected outcomes is necessary.

In this context, we were approached by FASCINATING to execute a literature review to systematically map the outcomes of Regenerative Agricultural initiatives. This way, a state-of-the-art overview emerges which serves as scientific input for the design of their upcoming RA field experiments on 100 hectares in the North of the Netherlands. Considering the regional experiences, and if possible, there will be special attention to wheat, potatoes, field beans, carrots and milk. The **main goal** was to develop an overview of the effects of different practices currently used on RA initiatives on the plant and food produce quality in terms of nutrients, taste and health, as well as on soil quality and the relationship between soil, plant and food produce.

Research strategy

Considering the specific questions that should be addressed for this report a three-step workflow was defined (Figure 1.1).

This stepwise approach allowed:

i) defining the strategy of the systematic review (Step 1),
ii) collection and selection of relevant articles (Step 2) and
iii) Extraction, organization, and analysis of the collected data (Step 3).

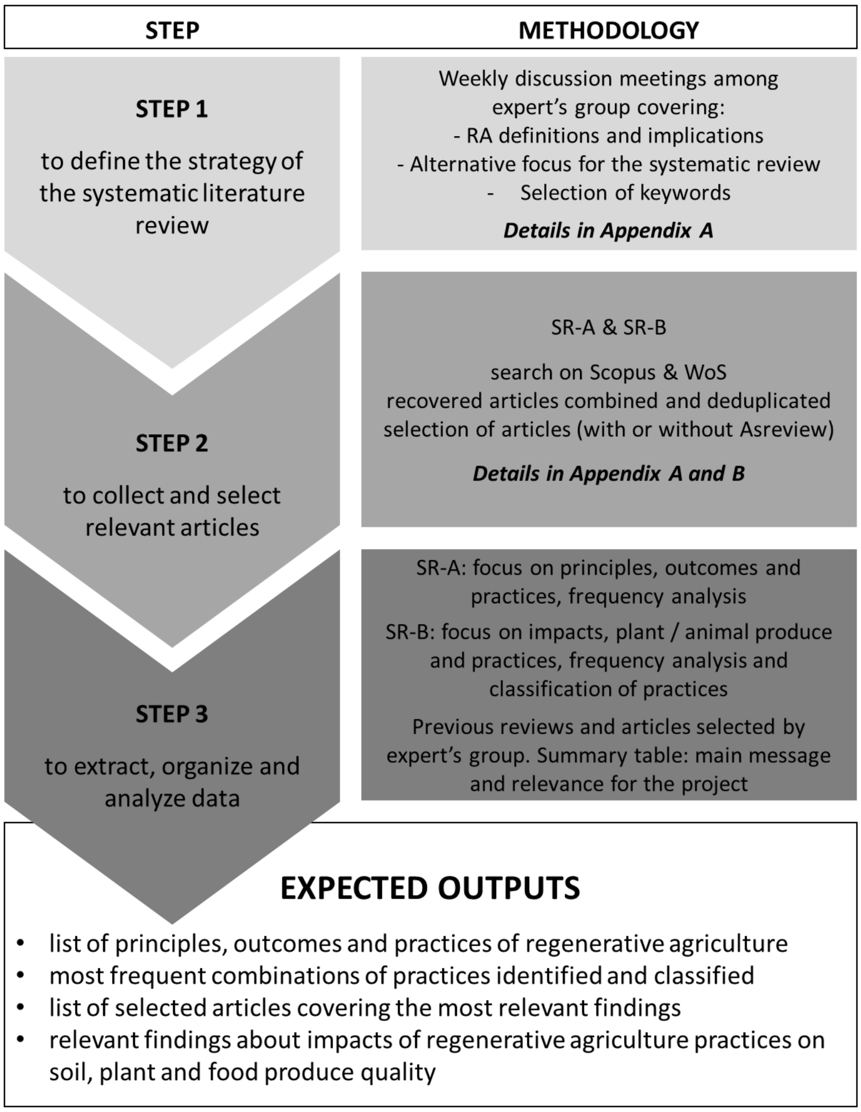


Figure 1.1. Stepwise approach used for the two related systematic literature reviews

Based on the weekly discussions of Step 1 (Appendix A), the expert group decided to perform the literature review on Scopus and WoS databases, splitting the collection of articles in two parts (Figure 1.1). The first part of the review was focused on the concept of "regenerative agriculture" (i.e. SR-A) as such. The SR-A was performed using the keywords "*regenerat* agricult**" OR "*regenerat* farm**", considering only the *Title* field for the search. A total of 212 articles were recovered from this search, which after deduplication were considered as input for the selection of articles that define regenerative agriculture based on principles, practices and/or outcomes, yielding a total of 26 articles for the final database of SR-A. The results of the SR-A are presented in the first part of Chapter 2.

The wide range of definitions of RA and the variation on the practices used in different RA initiatives, indicated that an alternative search strategy should be used to collect articles assessing effects on plant and soil quality. Therefore, to understand what is known about the effects of regenerative farming on plant and soil quality, the expert team decided to perform a second systematic review. This review focuses on agricultural "management practices" (i.e. SR-B). The expert's group chose agricultural management practices as entry point of the search because they are indeed the backbone of experimental design and to avoid missing studies that evaluate these practices (e.g. no tillage) but do not use the term 'RA' to refer to them. This allowed us to retrieve a large number of usable articles covering many of the major crops worldwide. One possible consequence of this search strategy is that studies not explicitly labelled as "RA"-related on e.g. dairy may not be retrieved, for example because no-tillage is not a relevant practice in these systems.

SR-B allows for the identification of the most frequent combinations of practices assessed on the literature and the most relevant findings about their effects on soil, plant, and plant/animal produce quality. For the SR-B, three blocks of keywords were defined, namely (a) the farming management practice, (b) the quality of the plant/animal produce, and (c) the target plant/animal produce of the search. For each of the three different blocks an associated list of keywords was defined (Appendix B). The search was performed considering the *Title* field and separately for plant and animal produce. For the animal produce search, only 46 articles were collected, after deduplication 26 articles were retained and used for the selection of relevant articles. After selection, 10 articles with available pdf were retained in the final database. For the plant produce search, a total of 785 articles were recovered, after deduplication they were used as input for the selection of relevant articles assisted by ASreview. After the selection, articles with available full text pdf were retained, excluding articles in Chinese language. Then, after reading, some articles were excluded because plant quality was not evaluated, practices applied were not adequate for regenerative agriculture (e.g. plastic mulching) or the articles considered only soilless systems. The final database of SR-B for plant produce was composed of 134 articles. The results of SR-B are presented in (the second part of) Chapter 2, and in the chapters 3, 4 and 5.

Finally, a series of related literature reviews were found when selecting relevant scientific articles. These review articles had to be excluded from SR-A and SR-B to avoid contamination of the results (when included, the results from empirical articles could show up twice in our results). However, these review articles are a helpful additional source to the findings of SR-A and SR-B. So, an overview of the main findings and conclusions of these articles was developed and summarized. This list of review articles was extended with a set of reference articles selected by the expert group to provide guidelines or discuss specific topics. The results are presented in Chapter 6. This summarizing tables (Tables 6.1 and 6.2) can also be used as a guideline for further reading depending on the topic one has in mind.

Outline

The main results of this systematic literature review are presented in the following chapters targeting the above-mentioned specific questions. Based on a first systematic review (SR-A), Chapter 2 provides an overview of the diversity of regenerative agriculture definitions, the associated principles, practices and outcomes (Chapter 2.1). Based on the second systematic review (SR-B), Chapter 2 also presents a bibliometric analysis and a categorisation of articles based on species and crop types, management practices, experimental design and indicators used to assess effects of management practices on plant/animal produce and soil quality (Chapter 2.2). This part of Chapter 2 also presents a clustering of management practices based on a lexical analysis. The resulting classes were used as categories throughout the rest of the chapters. The subsequent chapters analyse the existing evidence on the effects of agricultural management practices on nutrient density and taste of plant and animal produces (Chapter 3), on indicators of plant and human health (Chapter 4) and on soil quality and their relation to plant quality (Chapter 5). Chapter 6 summarises additional information provided by earlier systematic reviews and reference articles selected by the expert's group published in the scientific literature that were excluded of the systematic reviews A and B. Chapter 7 presents a general discussion and main conclusions of the study.

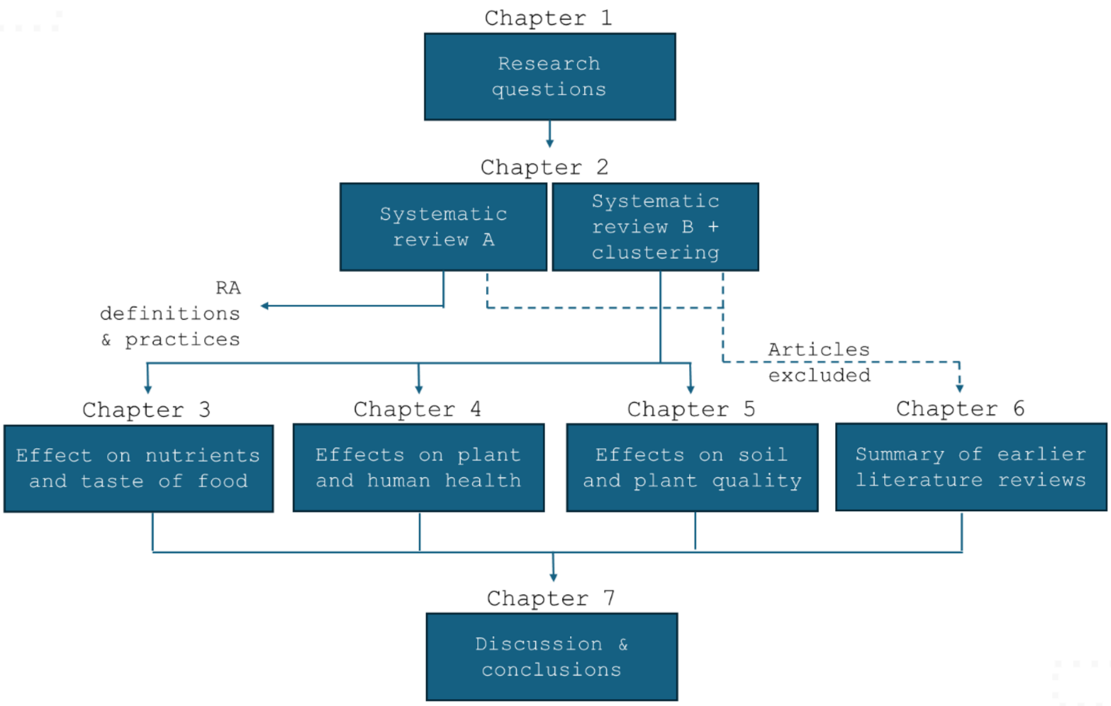


Figure 1.2. Outline of chapters and information flows within the report. The dotted line from Chapter 2 to Chapter 6 indicates the review articles that were excluded from the clustering to avoid replication. These earlier reviews were analysed in Chapter 6.

In each of the chapters, there is also attention for produces that play an important role in the North-of-the-Netherlands (wheat, potato, beans and milk). As turned out during the analyses, not much literature related to these plant and animal produces has been traced in the selection of articles, except for wheat. The limited research results that have been found for these specific plant and animal produces will be described in the specific chapters.

Chapter 2: Definitions of regenerative agriculture and overview of the management practices and their effects on the quality of plant/animal produce and soil

To start our systematic literature review, we first focus on the question what the concept of RA entails. We present the principles, practices and outcomes used to define regenerative agriculture in the literature (SR-A). As will turn out, the combination of these three different levels makes the field very complex and branches out in multiple ways. The second part (SR-B) summarizes the characteristics of the studies that assess the effects of management practices on plant/animal produce and soil quality. For the search performed on animal produce a global summary is presented in this section as the number of articles was too low to implement other types of analysis. For the search performed on plant produce, this section considered the plant species, crop types, management practices, the experimental design and the indicators used to assess the effects of the management practices.
Additionally, based on the data collected for the agricultural management practices a framework of 7 different classes of practices were identified and used to provide an overview of the effects of management practices on plant and soil quality and to present the temporal trends of crops and management practices reported in the literature.

Research question of the chapter

1. What is known in the scientific literature about the principles, practices and/or outcomes considered to define regenerative agriculture?
2. What is known in the scientific literature about the plant/animal species, crop types, management practices and experimental design considered to assess the effects of regenerative agriculture practices on plant/animal produce and soil quality?

2.1 Results of the systematic literature review A: overviewing definitions

A total of 212 articles were retrieved from the systematic literature review A (SR-A) on the topic of regenerative agriculture (Figure 2.1). After de-duplication, 122 unique articles remained, all focused on regenerative agriculture. Finally, 26 articles that define regenerative agriculture using principles, outcomes and/or practices were identified.

Among these 26 articles, a total of 10, 18 and 19 articles enumerated principles, practices and outcomes of regenerative agriculture, respectively. A total of 21 principles were mentioned across the articles (Figure 2.2) with most studies emphasizing the importance of contextual factors in designing regenerative agricultural systems. A high number of practices were reported, which were distributed in 14 groups based on their similarities (Figure 2.2, Appendix C). Three main groups of outcomes were identified covering environmental (17 articles), economic (10 articles) and social (10 articles) outcomes (Figure 2.2).

It is important to mention that there is neither an agreed list of principles, practices and outcomes reported in the literature, nor a common definition of regenerative agriculture. While some definitions focused primarily on principles (7 articles), practices (12 articles) or outcomes (10 articles), others combined practices and outcomes (3 articles) or principles and outcomes (1 article).

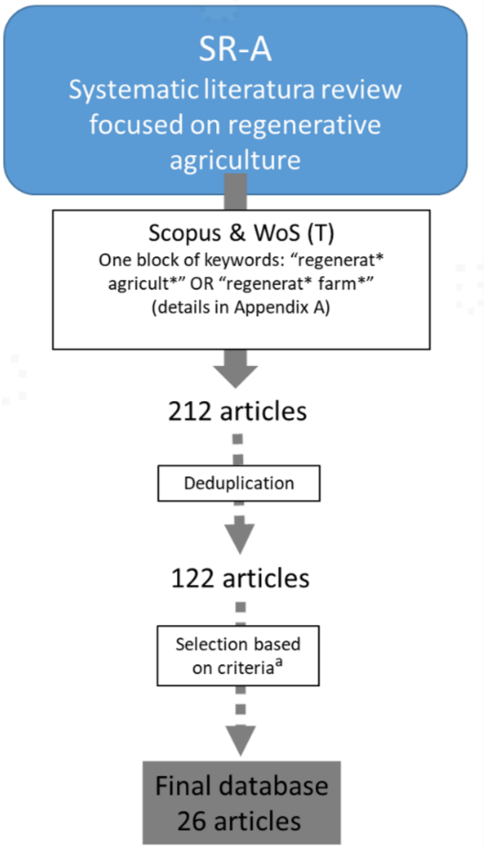


Figure 2.1. Workflow used to generate the database of the systematic literature review SR-A [a] Only articles defining regenerative agriculture based on principles, outcomes and/or practices were retained.

Figure 2.2. List of principles, practices and outcomes reported in the literature. Numbers between parentheses indicate the ratio between the number of articles reporting a specific principle, practice or outcome and the total number of articles reporting principles, practices or outcomes.

2.2 First results of the systematic literature review B

2.2.1 Overview of SR-B for animal produce

This search was performed considering three blocks of keywords, namely 'management practices', 'animal produce quality' and 'targeted animal produce' (Appendix B). According to the specific interests of the project, the 'target animal produce' block considered only the keyword "milk". Through this review (SR-B animal produce) a total of 46 articles were recovered from Scopus and WoS (Figure 2.3). After deduplication 26 articles remained and, among them, 10 articles were selected by reading the title and the abstract. Considering the low number of articles recovered only main results are presented here and will not be included in the following chapters because the low amount of data constrains more specific analyses.

Selected articles covered the period 2001-2024 and mainly assessed the effects of different types of feed diets and production systems on milk quality of dairy cows, yaks or buffaloes. Effects of different diets on milk quality were reported in cows. For example, feeding metabolizable protein (MP) deficient diets without supplementation decreased milk protein content, while not change was observed when MP were supplemented with rumen-protected lysine and rumen-protected methionine (Lee et al. 2012). Other comparative studies revealed that compared with grass/clover/maize silage, cows grazing grass/clover pasture produced milk 70% higher in beneficial omega-3 fatty acids, which increased by an additional 15% when grazing more diverse pasture (Loza et al. 2023). Milk from grazing also had less omega-6 fatty acids compared with silage diets, and their ratio with omega-3 fatty acid fell from 2.5:1 on silage to 1.2:1 when grazing grass/clover and 1.1:1 on diverse pasture. Also, the increase level of crude protein in non-protein nitrogen-based compounds decreased total milk production, as well as fat and milk protein, while increasing fat content and feed efficiency (Oliveira et al. 2001). The use of total mixed ration briquettes with higher crude protein and energy concentrations than the conventional diet including fresh-cut Guinea grass and commercial cattle pellet, tended to increase milk yield, milk protein yield and decreased milk urea nitrogen (Karunanayaka et al. 2022).

Arvidsson et al. (2012) evaluated the effects of grass silages subjected to different N-fertilisation regimes fed to dairy cows on the fatty acid (FA) composition of their milk. Nitrogen-fertilisation regimes included 30, 90 and 120 kg N/ha, designated G-30, G-90 and G-120, respectively. There were differences in concentrations of both individual and total FAs amongst silages. The daily milk production did not significantly differ between treatments, but G-30 silage resulted in higher concentrations of specific fatty acids in the milk compared with the other two grass silages. A higher recovery when red clover is included in the diet confirms previous reports. With the rates and types of concentrates used in this study, the achieved differences in FA composition among the silages were not enough to influence the concentrations of unsaturated FAs in milk. For the other side, urea molasses treated wheat straw fermented with cattle manure can replace 30% of dietary concentrate without affecting the milk yield and its quality (Nisa et al. 2007).

Regarding the effects of production systems, a study revealed that raw Murrah buffalo milk from mixed crop-livestock farming system meets the Indonesian National Standard for milk quality (Ratni et al. 2024). The same study also reported a positive correlation between water content and total bacterial colony count. On the other hand, in cows, a comparative study of compost-bedded pack barns and cubicle barns revealed a smaller number of bacteriologically positive quarters and lower prevalence for minor pathogens in compost compared to cubicle (Wagner et al. 2021). The study also reported for pathogen prevalence a quite constant proportion of bacteriologically negative udder quarters across milk yield levels in compost, but a slight increase with increasing milk yield in cubicles. Cell fraction responses in both systems differed in relation to the overall bacteriological infection status and farming system particularities. In dairy herds housed on compost bedding, a study demonstrated that bedding wet density was positively associated with all cleanliness scores and bulk milk concentration of total bacteria (Fávero et al. 2015). Results suggest that managing bedding to remain dry and loose will result in cleaner animals with a decreased risk of mastitis and improved milk quality.

Additionally, Sun et al. (2019) evaluated the differences in concentrations of branched chain fatty acids (BCFA) in yak milk and manure between lactation periods and evaluated gene expression levels of certain genes involved in the biosynthesis and elongation of fatty acids. The study revealed that half-lactation yak milk contained higher levels of BCFA than the full-lactation milk. ELOVL1 enzyme involved in the elongation of saturated C18 to C26 acyl-CoA substrates and MCAT enzyme involved in the transfer of a malonyl group to the mitochondrial acyl carrier protein were upregulated in full-lactation milk.

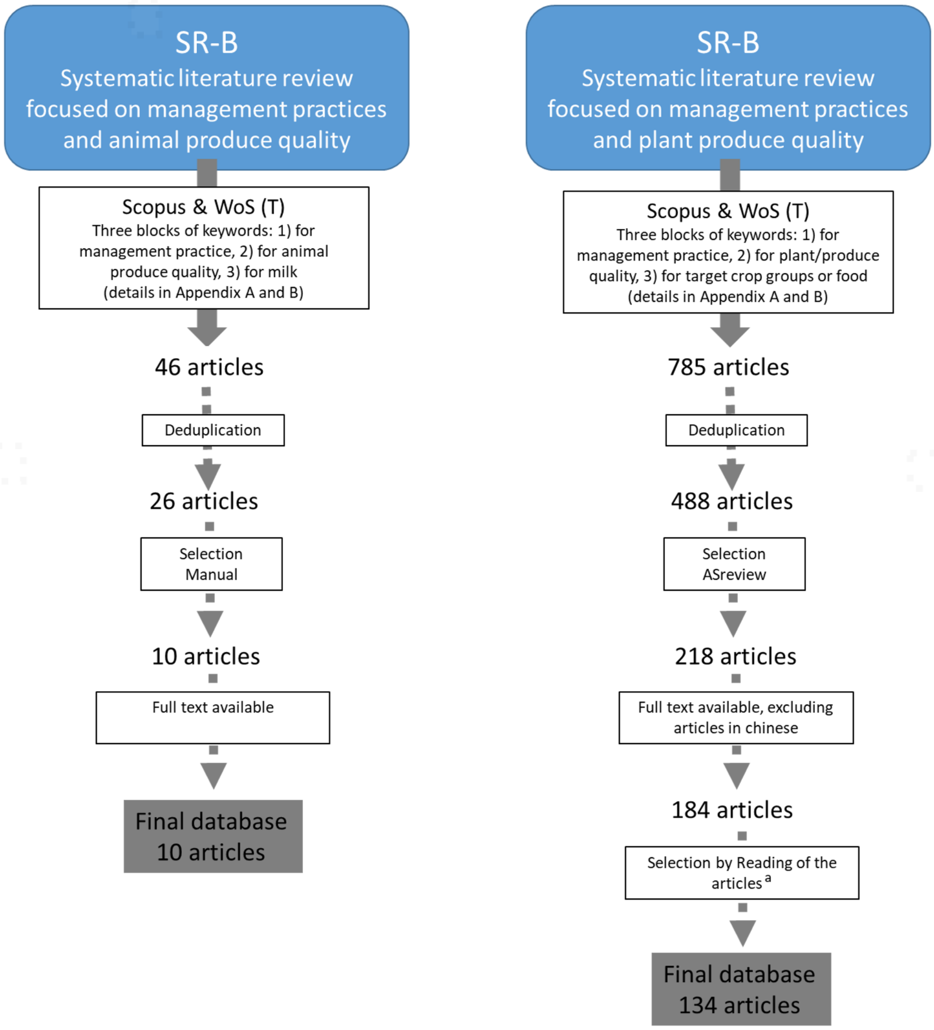


Figure 2.3. Workflow used to generate the database of the systematic literature review SR-B for animal and plant produce. [a] Articles were excluded if plant quality was not evaluated, practices applied were not adequate for regenerative agriculture (e.g. plastic mulching) or articles considered only soilless systems.

2.2.2 Overview of SR-B for plant produce and systematic categorisation of agricultural management practices

This search was performed considering three blocks of keywords, namely 'management practices', 'animal produce quality' and 'targeted animal produce' (Appendix B). According to the specific interests of the project, the target plant produce block considered the keywords "food* OR fruit* OR vegetable* OR grain*". Through this review (SR-B plant produce), 785 articles were recovered from Scopus and WoS (Figure 2.3). A total of 488 articles remained after deduplication and 218 after the selection process in ASreview. The full text of the article was available for 184 of them (84.4%). After reading, some articles were excluded because plant quality was not evaluated, practices applied were not adequate for regenerative agriculture (e.g. plastic mulching) or the articles considered only soilless systems. The final database was then composed of 134 articles. All these articles assessed plant quality variables, 111 (82.8%) also assessed growth and yield variables, and 64 (47.8%) added soil quality variables in the assessment. Only 21 (15.7%) articles assessed the relationships between soil quality and plant quality.

Considering the purpose of this report, this set of articles has been described and analysed with a specific focus on:

- Plant species and crop types
- Experimental designs and practices assessed
- Assessment of indicators of management practices and classification of practices based on descriptions provided in the articles
- Clustering of management practices
- General trends

- **Plant species and crop types**

Among the 134 articles reviewed, a total of 64 plant species were studied, with 34 (53.1%) of them reported only in one article. Wheat was the most frequently studied species, followed by tomato and maize (Figure 2.4 A). However, among crop types, *vegetables & melons* was the most frequent, followed by *fruits & nuts*, while *cereals* and *legumes* were the least frequent crop types examined in the studies (Figure 2.4 B).

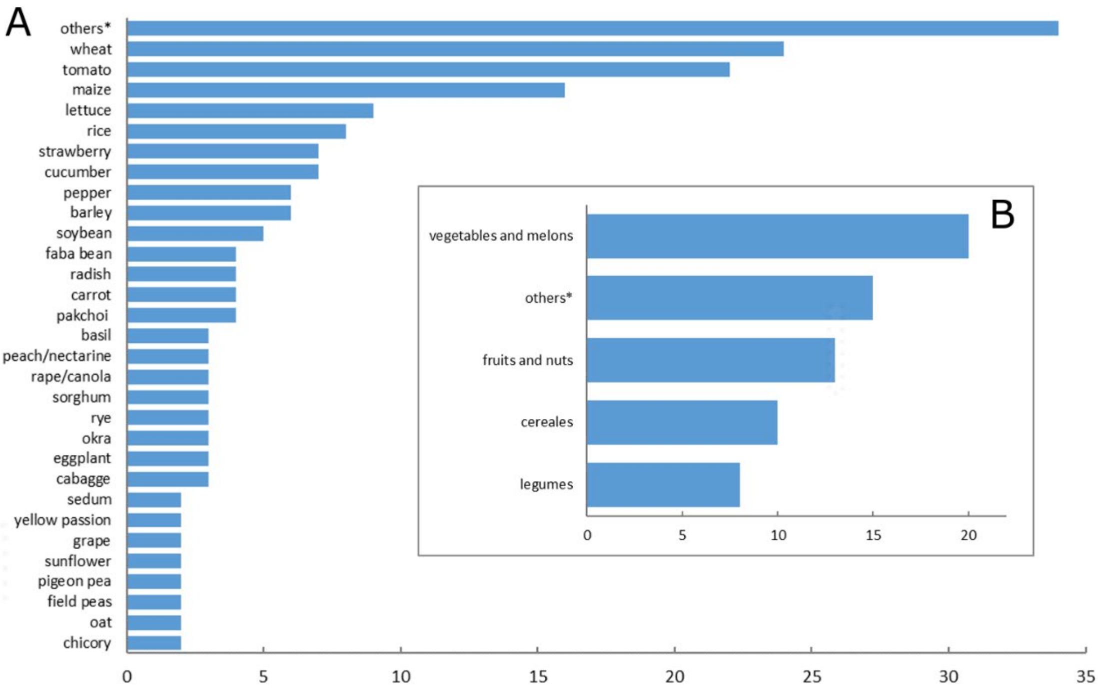


Figure 2.4. Number of species (A) and crop types (B) assessed in the reviewed articles. * Others include species with frequency lower than 2 in graph A and other crop types such as stimulant, spices and aromatic crops, oilseed crops and oleaginous fruits, and fibre crops in graph B.

- **Experimental designs and practices assessed**

Among the articles reviewed, field assays (63.4 %) and pot experiments (32.1 %) were the most frequent types of experimental design used. A wide range of practices were retrieved, which were reported with confusing, alternative or mixed names, especially in the case of soil management practices. Therefore, practices retrieved from the systematic literature review (frequency $\geq$ 2) were classified in 16 management practices. The definition of each management practice is presented in Table 2.1.

The 51.5 % of the articles assessed the use of combined practices. The number of practices assessed by article varied between 1 and 5, with an average value of 1.75; indicating that most articles assessed only one or two practices. Articles with the highest number of practices, in general, assessed different types of soil amendments simultaneously.

Table 2.1. Definition of the practices assessed on articles of the systematic literature review. The terms highlighted in **bold** and *italics* indicate the short name of the practice as used throughout the text.

Practices	Definition used for the classification of practices
Use of ***animal manure***	Non-chemical fertilizers mostly derived from animal faeces and urine, but normally also containing plant material (often straw), which has been used as bedding for animals and has absorbed the faeces and urine; includes solid manure, liquid manure and slurry. This group includes fresh and stored animal manure. Composted animal manure without clear details about the aerobic or anaerobic conditions and method use for composting were included also in this group.
Use *of* ***green manure***	Non-chemical fertilizers derived from plants or plant residues, used fresh.
Use of ***compost***	Non-chemical fertilizers derived from plant, animal and food wastes obtained by composting.
Use of ***biochar*** and derived by-products	Non-chemical fertilizers derived from plant, animal and food wastes obtained through thermochemical conversion of biomass with presence of little or no oxygen to produce a black, carbon-rich and porous solid material (like charcoal). This group also includes by-products of biochar production (e.g. wood vinegar).
Use of ***industrial plant residues***	Non-chemical fertilizers based on plant residues from industry such as oil palm mill, sugar cane press muds. Composted industrial plant residues without clear details about the aerobic or anaerobic conditions and method use for composting were included also in this group.
Use of ***sewage sludge***	Residues from wastewater treatment.
Use of plant ***biostimulants***	A wide range of products that stimulate plant nutrition processes. This group includes products such as aqueous extracts derived from plant, algae, yeast, or cyanobacteria and amino-acid solutions, enzymatic hydrolysate or other related compounds derived from plant or animal protein. This group exclude products based on isolated microbes.
Use of isolated ***microbes***	Isolated and characterized microorganisms used as biofertilizers, biostimulant, biofungicides.
Use of ***other*** organic ***amendments***	Commercial organic fertilizers and biofertilizers (including microbial communities but not isolates microbes) reported without providing clear details about the process, methods and material sources used for production, or other organic or organo-mineral fertilizer such as biodynamic preparations or derived products. This group also includes amendments reported without a clear description that can be not therefore assigned to previous groups, also includes amendments with description but that could not be assigned to the other groups.
Reduction or combination of ***mineral fertilizers***	Traditional and commercial chemical mineral fertilizers. This groups only included mineral fertilizers used at reduced doses and/or combined with different amendments such as composts, animal manure, etc. The group also includes the use of inorganic amendments such as zeolites or mineral waste.
Incorporation of ***mulching***	This group consider the use of crop residues to cover the soil surface, normally around the plants, to create favourable conditions for the plant growth and proficient crop production. Articles focusing only on the use of synthetic mulch to cover soil were not considered.
Use of ***cover crops***	Crops typically grown between main crops to cover and keep living plants on the soil during non-cash-cropping periods.
Use of ***intercropping***	Crops grown together on the same field in alternate rows or in the same row. This groups includes different strategies such as row intercropping, strip intercropping, mixed intercropping, and others.
Use of ***crop rotation***	At least two crops in different years.
Grazing in crop-livestock systems	This groups includes articles assessing the effects of grazing in crop-livestock systems.
Conservation ***tillage***	A management approach to minimize the frequency or intensity of tillage to leave plant residues on the soil surface to protect soil. This group included several forms such as strip tillage, no-tillage, and others.
Use of ***other practices***	This groups includes all the practices retrieved with low frequency such as crop load, row spacing, agroforestry.

Practices related to different principles of regenerative agriculture were recovered in the systematic review, although the practices most frequently assessed (e.g. mineral fertilizer, animal manure and microbes) were related to the principle focused on minimize synthetic inputs (Figure 2.5).

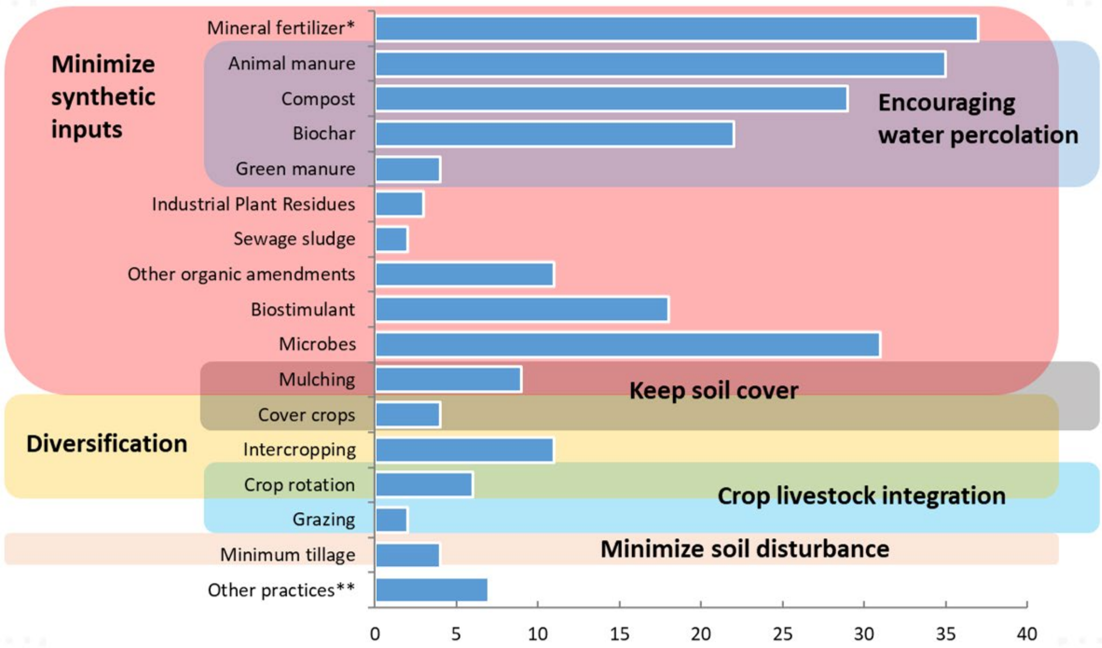


Figure 2.5. Number of articles assessing each management practice identified in the review. The association of each practice with principles of regenerative agriculture is indicated by coloured boxes. *Mineral fertilizer: refers to the reduction or combined used of mineral fertilizers not to the use of mineral fertilizer as a regenerative agriculture practice. ** Other practices: refers to a group of practices that appeared in low frequency, such as agroforestry, crop load, sown density, row spacing. See table 2.1 for more details.

- **Indicators of management practices effects**

Articles assessing the effects of management practices used a wide range of indicators covering plant and soil quality indicators (Figure 2.6). Indicators used to assess effects of management practices on plant and plant produce quality covered different types of nutrients, organic compounds, and activity or efficiency assessments. Considering the specific questions that should be addressed for this report, these indicators were classified in three groups:

- **Nutrients**: including macro-nutrients and mineral element content and composition
- **Taste**: including the content of compounds that contribute to taste
- **Health**: including micro-nutrients (e.g. vitamins) and secondary compounds (e.g. polyphenols, pigments) that contribute to plant health and can contribute to human health. This group also includes the evaluation of specific plant activity or efficiency assessments.

This classification aimed to organize the data in a simple way. We recognize that some indicators could be assigned to different groups (e.g. vitamins that could be considered as micro-nutrients and as health compounds). However, we prioritized the assignation of each indicator to the group in which it has the most relevant contribution, taking into account the aim of their use in the reviewed articles. This classification will be also used in Chapters 3 and Chapter 4.

Among indicators of the nutrient group, elemental content (e.g. nitrogen, phosphorus, potassium, heavy metals) was the most frequently reported, while the nutrient content or composition in terms of fats, carbohydrates, and proteins were less frequently considered (Figure 2.6). Taste indicators were considered even less frequently and were mostly focused on the assessment of nutrient content that contributes to sweetness (e.g. total soluble solids) and acidity (e.g. citric acid, pH) (Figure 2.6). Only in some cases these indicators were used to estimate an index taste. On the other side, health indicators considered both direct and indirect indicators: Direct indicators focused on the evaluation of antioxidant (e.g. ferric reducing antioxidant power (FRAP), DPPH (radical scavenging assessment), enzymes (peroxidase, superoxide dismutase)), defence (e.g. pathogen presence, number of diseased plant, microorganisms colonization) or osmoprotectant (e.g. free proline) activity, and on photosynthetic efficiency (e.g. content of photosynthetic pigments, stomatal conductance, net photosynthesis), while indirect indicators focused on the assessment of the content and/or composition of secondary compounds (e.g. polyphenols, flavonoids, carotenoids, vitamins) and phytohormones (e.g. abscisic acid, cytokinins, gibberellic acid).

Additionally, although the search was focused on practices and plant quality keywords, the retrieved articles also assessed the effects of practices on growth and yield and on soil quality (82.8% and 47.8% of the articles respectively). Growth and yield indicators included fresh and dry weight, plant size, number of fruits per plant, number of grains perplant, and weight of 1000 grains, among others (Figure 2.6). Assessment of plant indicators were performed on one or several plant parts in the same article (e.g. grain, fruit, shoot, root, leaf). Soil indicators were mostly focused on physicochemical properties, while the assessment of biodiversity indicators was less frequent (Figure 2.6).

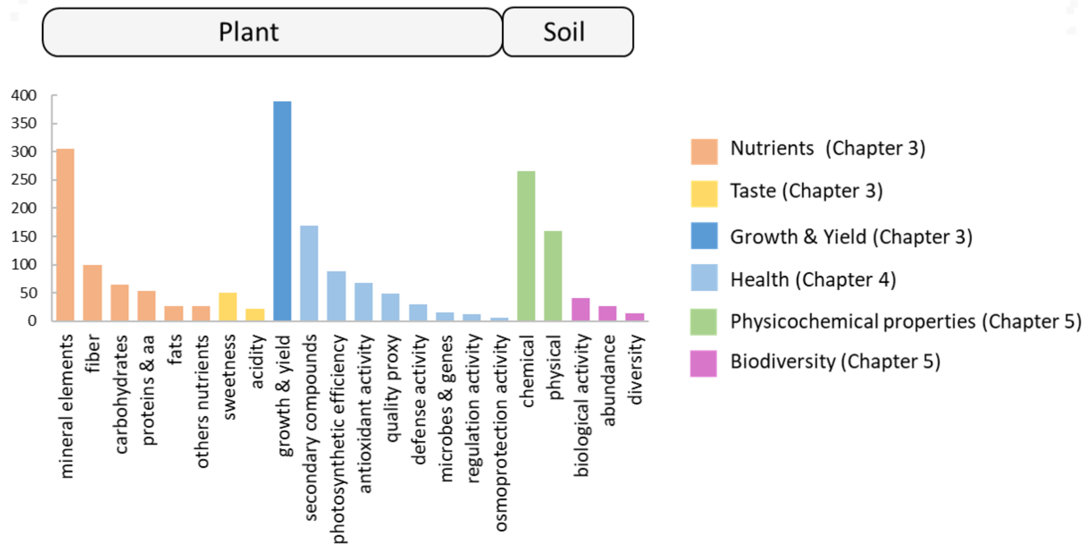


Figure 2.6. Type of indicators used for the assessment of management practices effects, organized in six major groups.

- **Clustering of management practices assessed in the literature**

Using lexical analysis (i.e. descendent hierarchical classification by Reinert's method) to analyse the binary matrix of management practices assessed in the articles (TableS1, Supplementary Tables), six classes were identified (Table 2.2). Each class was associated with a different arrangement of practices. In general, the use of ***Biostimulants*** and ***Compost*** defined Class1 and Class5 respectively, while the use of ***Biochar & Microbes*** defined Class3. The rest of the classes were defined by the use of ***Animal manure & Mineral fertilizer*** (Class2), ***Microbes*** (Class4) and ***Intercropping***, ***Mulching & Other practices*** (Class6), and could involve a combination of evaluation strategies (practices assessed alone or combined). In Table 2.2, the last class (Unclassified) represents unrelated articles using different combinations of practices with low frequency in the database. The description of each class is presented below.

Table 2.2. Classification of management practices reported in the 134 articles of the systematic literature review including for each class the practice, article and plant species associated. See table 2.1 for the definition of practices. Author name, date of publication, title and abstract of each article are reported in TableS1.

Class	Defined by the use of	Article ID	Plant species*
Class1	Biostimulants	82, 92, 39, 124, 34, 120, 89, 88, 94, 65, 61, 60, 69, 57, 91, 42	tobacco, coriander, melon
Class2	Animal manure, Mineral fertilizer	[29, 130, 135, 38, 35, 32, 84, 81, 104, 127, 90, 14, 108, 129, 33, 131, 119, 116, 113, 76, 71, 112, 103, 106, 44, 4, 5, 110, 97, 83]	lettuce, pigeon pea, rape/canola, yellow passion fruit, carrot, radish [millet, kiwi, snake fruit, african eggplant, celery, purslane, quinoa]
Class3	Biochar, Microbes	21, 24, 56, 132, 98, 85, 122, 99, 16, 17, 19, 13, 68, 117, 80, 107, 45, 63,	olive, blueberry [maize]
Class4	Microbes	22, 27, 31, 95, 10, 62, 64, 58, 70, 101, 102, 50, 51, 52	[tomato, rye, sorghum]
Class5	Compost	96, 36, 37, 121, 125, 86, 93, 100, 75, 114, 47, 49, 55, 53	potato, tanner grass, spinach, japan knotweed, calafate [pepper, tomato, wheat]
Class6	Intercropping, Mulching, Other practices	126, 3, 136, 137, 73, 105, 30, 78, 79, 12, 11, 109, 134, 66, 77, 72, 2, 1, 7, 8, 115, 54	sedum, paiaguas plisadegrass, sunflower, black nigthshade, alfalfa, lingonberry, apple, citrus, oat, cowpea [wheat, pakchoi]
Unclassified	Diverse practices	*6, 9, 15, 18, 20, 23, 25, 28, 40, 43, 46, 48, 59, 67, 74, 87, 111, 118, 128, 133*	-

*Plant species with significant higher frequency in the class. Plant species between brackets showed higher frequency in the class but the difference was not significant (p-value > 0.05).

Biostimulants (Class1): Among the 18 articles evaluating the use of biostimulants 16 (88.9 %) were associated with Class1. Twelve articles assessed the use of biostimulants alone, while the others evaluated its use alone and in combination with other types of amendments. Assessed biostimulants included henna leaf extracts in wheat plants, protein hydrolysates, amino acids and chitosan solutions in tomato, humic acid in maize, palm pollen grain extracts in basil, amino acids solutions in barley, algae and yeast extracts in peach, spermine and spermidine in strawberry, spirulina in soybean, cucumber and wheat, aqueous extracts of *Acacia saligna* fruit in coriander, iodine solutions in melon, seaweed extracts in cucumber, saffron by-products in eggplant, and hydrolysable tannins from sweet chestnut in tobacco.

Animal manure & Mineral fertilizer (Class2): Among the 35 articles assessing the use of animal manure, 30 (85.7%) were associated with Class2, while the others were not classified. Fourteen articles assessed the use of animal manure alone, for the others, animal manure was assessed in combination with mineral fertilizer (10 articles) or other amendments (6 articles). Among the 37 articles assessing the use of mineral fertilizer, 10 (28.6%) were associated with Class2, while the others were distributed among classes 1, 3, 4, 6 or were not classified. The ten articles in Class2 also included the assessment of animal manure, reflecting the assessment of their combined used. Assessed animal manure included cattle/dairy and poultry/chicken manure, but sheep, swine, goat, horse manure, pig slurry, and animal manure without clear description were also assessed. The use of animal manure was assessed in grains (e.g. wheat, maize, rice, quinoa, rye, barley, millet), vegetables (e.g. tomato, strawberry, lettuce, carrot), fruits (e.g. kiwi, passion fruit, snake fruit) and other crops (e.g. pepper).

Biochar & Microbes (Class3): Among the 22 articles evaluating the use of biochar 18 (81.8%) were associated with Class3. Only two of these studies evaluated the single used of biochar, in the other articles biochar was assessed in combination with other amendments such as compost, biostimulant or mineral fertilizer. Biochar was produced from different materials, including wheat, maize, cassava straw, rice, tea and bamboo residues, willow, pine, holm oak and other tree branches, blended wood waste and empty fruit bunch. Among the 31 articles evaluating the use of isolated microbes 11 (35.5%) were associated with Class3, and all of them were included in the assessment with biochar and with other amendments. Microbes assessed included, plant growth promoting bacteria (e.g. *Bacillus* spp. and *Pseudomonas* spp.), nitrogen-fixing bacteria (e.g. *Stenotrophomonas* spp.), wheat-derived endophytic bacteria (cadmium (Cd)-immobilizing endophytic *Pseudomonas paralactis* and *Priestia megaterium*), metal-immobilizing bacteria (*Bacillus megaterium* and *Serratia liquefaciens*), arbuscular mycorrhizal fungi (e.g. *Glomus intraradices*, *Funneliformis mosseae*, *Rhizophagus aggregatus*, *Claroideoglomus etunicatum* and *Rhizophagus intraradices*), phosphate-solubilizing fungi (e.g. *Acremonium*, *Aspergillus*, *Hymenella* and *Neosartorya*).

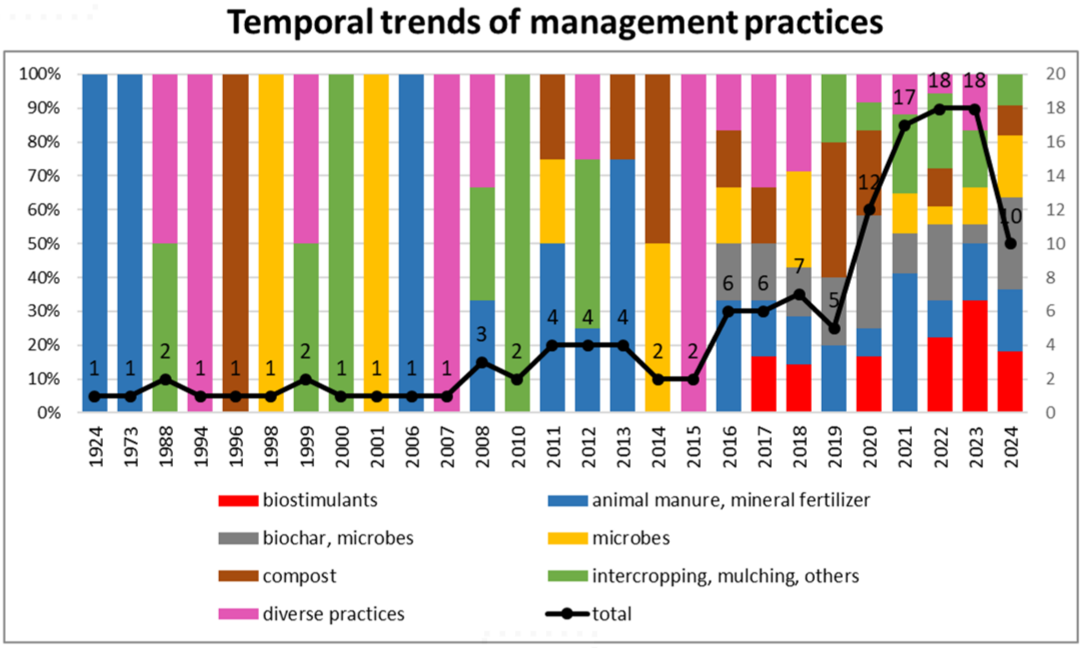
Microbes (Class4): Among the 31 articles evaluating the use of isolated microbes 11 (35.5%) were associated with Class4. Five of the articles assessed the use of microbes alone, were for the other articles microbes were combined with other amendments such as compost, industrial plant residues or mineral fertilizers. Microbes assessed in these articles included plant growth-promoting bacteria (e.g. *Arthobacter* sp., *Bacillus* sp., *Lysinibacillus* sp., *Paenibacillus* sp. and *Sinomonas* sp., *Kosakonia radicincitans*, *Azospirillum brasilense*, *Azotobacter vinelandii*, and *Beijerinckia mobilis*, *Actinomycete* bacteria, *Bacillus subtilis*, *Bacillus licheniformis*) recognized by their potentials for nitrogen fixation, phosphorus solubilisation, siderophore and phytohormone production, arbuscular mycorrhizal fungi (e.g. *Glomus mosseae*, *G. caledonium*, *G. viscosum*, *G. intraradices* and *G. coronatum*, *Rhizoglomus irregular*, *Funneliformis geosporum*, *F. mosseae*, *Glomus versiforme*, *Acaulospora scrobiculata*, *Rhizophagus intraradices*, and *Gigaspora margarita*, *Glomus etunicatum*, *Glomus clarum*), dark septate endophytic fungi, *Penicillium vinaceum* and *Eupenicillium hirayama* isolated from a mangrove habitat and selected based on their activity against five phytopathogenic fungi, their plant-growth promotion ability, and their phosphate solubilisation ability, *Fusarium*, *Trichoderma* and bacteria isolated from suppressive compost and tested for control of plant pathogens. *Azotobacter chroococcum*, *Azotobacter vinelandii*, actinomycetes and rhizobacteria were also evaluated.

Compost (Class5): Among the 29 articles evaluating the use of compost 12 (41.4%) were associated with Class5. Only one of these articles assessed compost in combination with cover crops and grazing. Composts assessed were based on poultry-mortality waste, chicken or beef manure and straw, chicken manure, juice lees and stems and sawdust, pulp and paper mill sludge, fruit-vegetable waste, mushroom spent substrate and rye straw, suppressive composts, peat and pumice, and cow manure, food processing liquid slurry including fruit juices, milk and milk-based products, municipal biowastes, poultry litter and chicken feathers.

Intercropping, mulching and other practices (Class6): The 11, 9 and 7 articles evaluating the application of intercropping, mulching and other practices were associated with Class6. Intercropping included the combination of wheat with pea, bean or forage legumes, barley with alfalfa, rice with solanum, eggplant with sedum, packchoi with sedum, tomato with sunflower, lettuce with chicory, paiaguas palisadegrass with sorghum. Mulching assessed the use of different plant derived mulch on wheat, maize, wheat and forage legumes, strawberry, citrus, lingonberry and apple. Other practices, including for example sown density, crop load, direct seeding or agroforestry, were assessed on wheat, wheat and bean or barley, nectarine, brasicaceas and vegetable cropping.

- **General trends**

Temporal trend analysis revealed an increase in the number of articles assessing management practices since 2016, which was accompanied also with an increase on the number of practices and crops assessed (Figure 2.7). This analysis also revealed that the assessment of ***Biochar & Microbes*** and ***Biostimulants*** started in the last decade (2016 and 2017 respectively), while the other practices have been assessed for several decades.



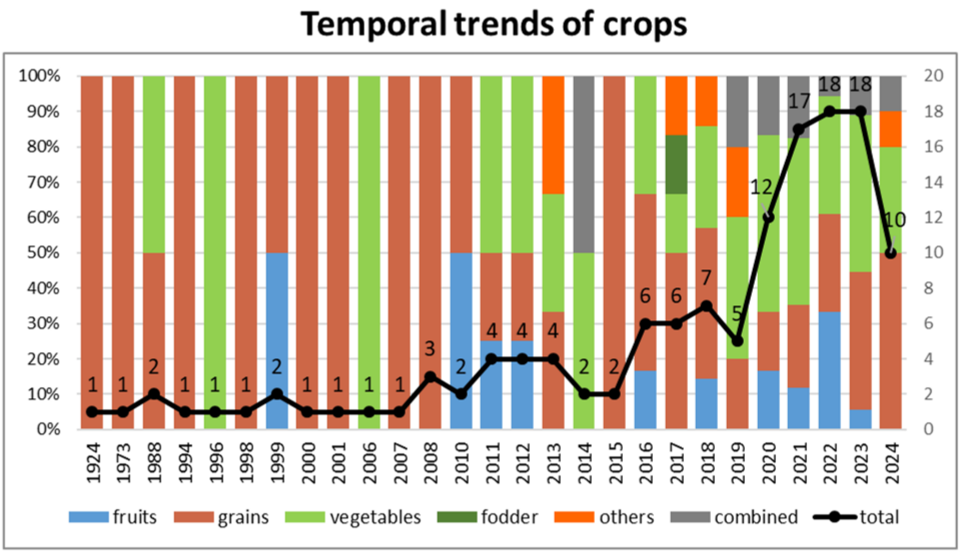


Figure 2.7. Temporal trends of the number of management practices and crops reported in the 134 articles recovered in the systematic review. Solid line represents total number of articles.

The number of articles associated to each class varied between 14 and 30. Most plant species were associated to one class (Table 2.2), and particular tendencies were also detected for crop groups (Figure 2.7). The use of ***Compost*** and ***Intercropping*, *Mulching* & *Other practices*** involved the highest number of crop types, while the use of ***Microbes*** the lowest. On the other side, in terms of the type of crops, the use of ***Biostimulants*** and ***Compost*** was most frequently assessed on V*egetables* (58.8 % and 56.3 % respectively), the use of ***Biochar* & *Microbes*** on G*rains* (61.1 %), and the use of ***Animal Manure* & *Mineral Fertilizer***, ***Microbes*** and ***Intercropping*, *Mulching* & *Other practices*** on both V*egetables* and *Grains* (43.3% and 40.0 %, 53.3% and 46.7 %, 24.1 % and 37.9 % respectively). The unclassified articles were more frequently focused on *Grains* (70%).

The report of positive, neutral, negative and ambiguous effects of management practices on quality variables varied among classes (TableS2, Supplementary Tables). Globally, the use of ***Biochar* & *Microbes*** and ***Biostimulants*** showed the highest percentage of positive effects (78.2% and 73.7%); while the use of ***Intercropping*, *Mulching* & *Other practices*** and ***Compost*** showed the lowest percentage (36.3%, 44.1% respectively). Negative effects were lower than 20%. Neutral effects were generally lower than 20%, although higher values were detected for the use of ***Intercropping*, *Mulching* & *Other practices*** (32.5%) and ***Microbes*** (21.6%). Similarly, ambiguous effects were in general lower than 20%, although higher values were detected for the use of ***Compost*** (32.4%) and ***Animal manure & Mineral fertilizer*** (26.0%). Within each class, however, some differences were also detected among plant quality, yield & growth, soil & environment variables (Figure 2.8).

Specific crops also showed different patterns. For example for wheat, no reports were found for the use of compost, while for the use of biostimulants no reports were detected for the effects of the practice on soil quality indicators (Figure 2.9). Higher level of ambiguous data was detected for the use of microbes (Class4) and intercropping, mulching and other practices (Class6) than for the other classes. For grain legumes, no reports were detected for biochar & microbes (Class3) and biostimulants (Class1) (Figure 2.10). In the case of compost (Class5) and microbes (Class4), data was missing for yield & growth and/or soil & environment indicators. Ambiguous effects were more frequent for Class6 and Class2. The low number of articles in this case could influence the detection of trends. For tomato, no reports were detected for soil & environment for Class 4 and for the unclassified articles (Figure 2.11). Ambiguous effects were more frequent for Class6 and Class2. For root and tubers, a low number of articles was recovered, so clear trends could be not detected (Figure 2.12).

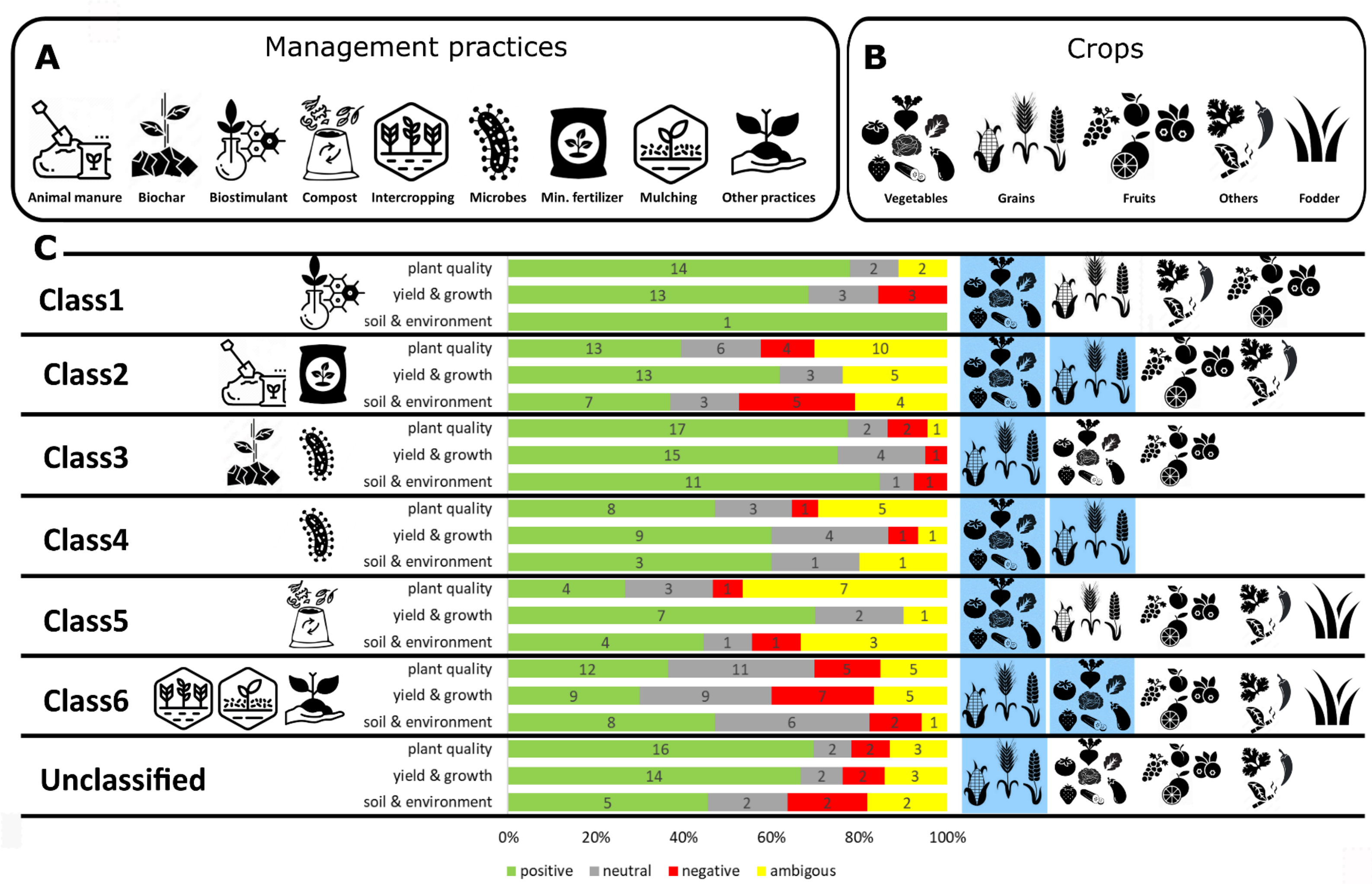


Figure 2.8. Main results considering all crops. A) Practices assessed in the articles of the systematic literature review. B) Percentages of positive, neutral, negative and ambiguous effects reported in the 134 articles of the systematic literature review organized according with the classification of practices. Icons in each class represent the practices that define it. Main crop types of each class are highlighted in blue.

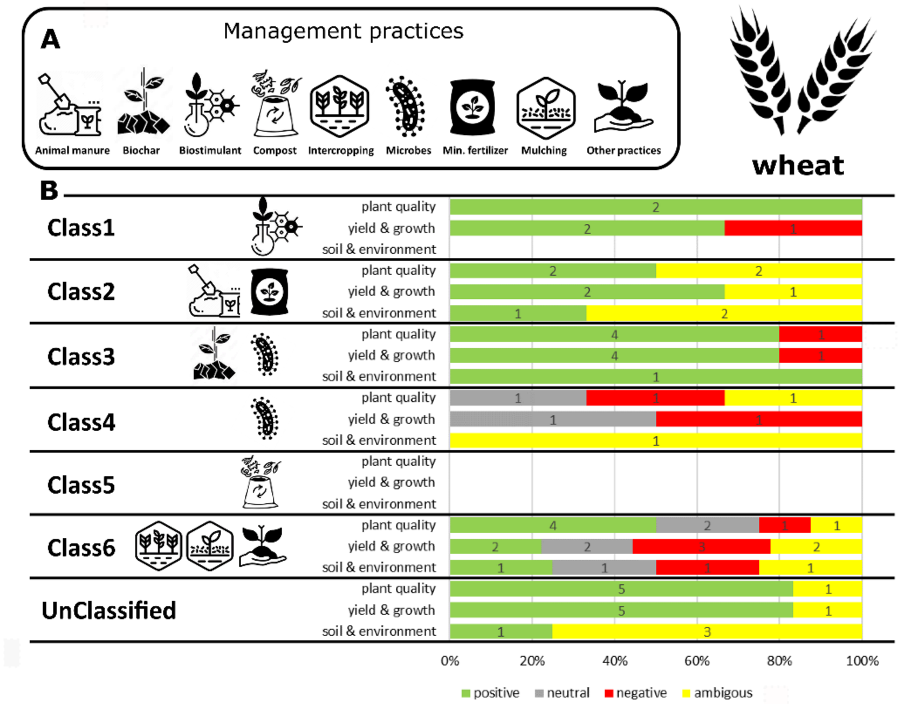


Figure 2.9. Main results for wheat. A) Practices assessed in the articles of the systematic literature review. B) Percentage of positive, neutral, negative and ambiguous effects of practices on plant quality, yield & growth and soil & environment. Results are presented considering the classification of practices. Icons in each class represent the practices that define it.

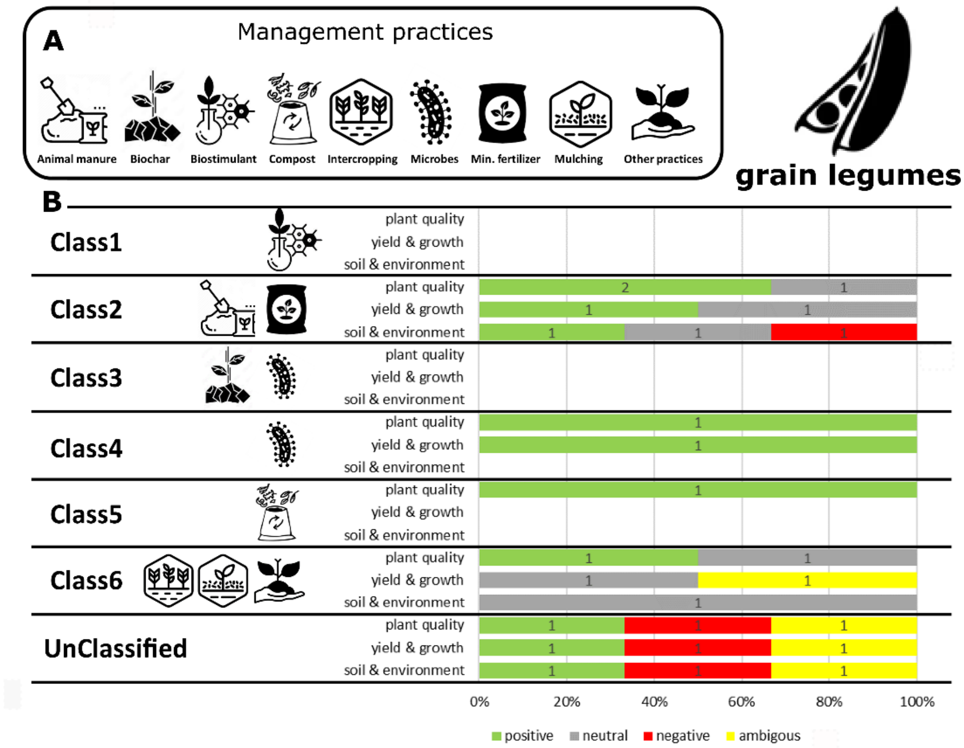


Figure 2.10. Main results for grain legumes. A) Practices assessed in the articles of the systematic literature review. B) Percentage of positive, neutral, negative and ambiguous effects of practices on plant quality, yield & growth and soil & environment. Results are presented considering the classification of practices. Icons in each class represent the practices that define it.

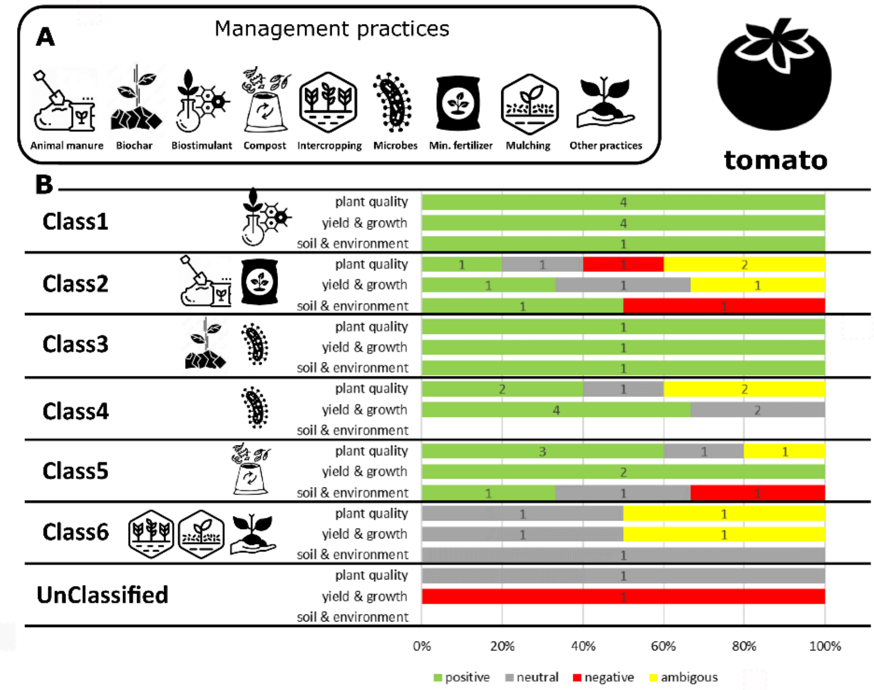


Figure 2.11. Main results for tomato. A) Practices assessed in the articles of the systematic literature review. B) Percentage of positive, negative, neutral and ambiguous effects of practices on plant quality, yield & growth and soil & environment. Results are presented considering the classification of practices. Icons in each class represent the practices that define it.

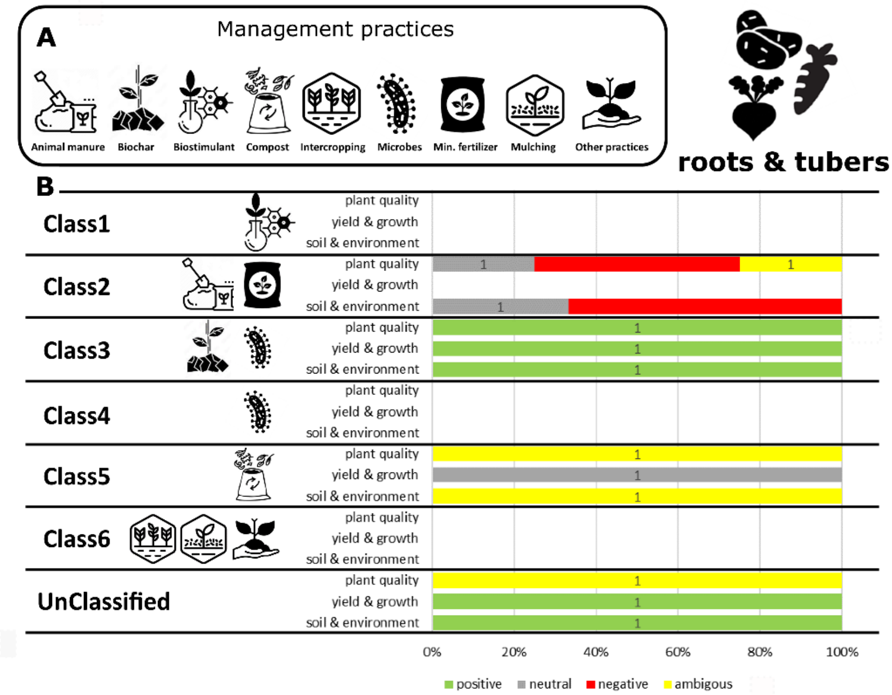


Figure 2.12. Main results for roots and tubers. A) Practices assessed in the articles of the systematic literature review. B) Percentages of positive, neutral, negative and ambiguous effects of practices on plant quality, yield & growth and soil & environment. Results are presented considering the classification of practices. Icons in each class represent the practices that define it.

Conclusions

Considering the first research question *("What is known in the scientific literature about the principles, practices and/or outcomes considered to define regenerative agriculture?")* data extracted from the articles retrieved in the systematic literature review SR-A indicate that:

- a wide range of principles, practices or outcomes are considered when defining regenerative agriculture, and that only in some cases the definitions include a combination of principles and practices or outcomes
- the relationships between principles, practices and outcomes are generally not established
- an agreed list of principles, practices and outcomes of regenerative agriculture is not yet available.

Definitions of regenerative agriculture are so diverse that different arrangements of practices are considered in different systems. This result indicates that assessment of regenerative agriculture versus conventional agriculture could be constrained due to the wide range of definitions of regenerative agriculture. With this in mind, and for this literature review, it was decided to start systematizing the links between principles, practices and outcomes.

Considering the second research question *("What is known in the scientific literature about the plant/animal species, crop types, management practices and experimental design considered to assess the effects of regenerative agriculture practices on plant/animal and soil quality?")* data extracted from the articles retrieved in the systematic literature review SR-B indicate that:

- research on management practices effects on product quality was more frequent for plant produce than for milk
- many articles focused on the effects of management practices on animal produce quality reported changes on proteins and fats, with a special focus on fatty acids composition
- in general, articles focused on the effects of management practices on plant quality also include the assessment of growth and yield, while less than half consider the effects on soil quality
- information about the effects of practices is available for wheat, tomato and maize while they are scarce for potato, carrots and beans
- field assays are frequently used to assess the effects of practices on plant and soil quality
- half of the studies focus on one practice only, while the other half assesses a combinations of practices
- most of the studies focus on practices aiming to minimize synthetic inputs. Other principles of regenerative agriculture such as crop-livestock integration, minimize soil disturbance, keep soil covered or foster species diversity, are less frequently considered
- indicators used to assess the effects of practices on plant and soil quality mostly focus on mineral elements for nutrient quality, secondary compounds for plant health and physicochemical parameters for soil quality
- reviewed articles provide knowledge of the effects of practices on plant and soil quality, although the percentage of positive, neutral, negative and ambiguous effects on plant quality, yield and growth and soil quality varied among classes and crops
- reports of positive impacts were higher than 50%, while for negative, neutral and ambiguous impacts were in general lower than 20%

Based on these results, we conclude that:

- a protocol to classify systems/practices according to their level of transition to regenerative agriculture is required for the assessment of practices effects on animal produce, plant and soil quality. This protocol should be based on the relationships of principles, practices and outcomes of regenerative agriculture and will allow to disentangle different practice arrangement used on regenerative agriculture

- field experiments should incorporate a higher number of indicators of animal and plants (e.g. nutrients, health and taste indicators) and soil quality (e.g. biodiversity indicators).

Chapter 3: Effects of management practices on nutrients and taste of plant produce

In Chapter 2, a general overview of the practices assessed in the literature was presented along with the global results of the practice effects on plant/plant produce and soil quality. Chapter 3 presents the main results of the effect of the assessed practices on plant nutrients and taste, considering the six classes identified in Chapter 2 (Table 2.2). As mentioned in the previous chapter, especially macro-nutrients and mineral elements were considered, while micro-nutrients such as vitamins will be included as indicators of plant health and will be therefore presented in Chapter 4.

Research question

What is known in the scientific literature about the effects of agricultural management practices on the plant produce quality in terms of macro-nutrients (carbohydrates, proteins, fats, mineral elements) and taste (flavour changes, flavour strength)?

Results

Positive effects of management practices were reported in the literature reviewed. Differences were however detected among classes of practices in terms of percentage of positive effects and also in terms of the indicators reported (Table 3.1). In general, mineral elements and carbohydrates were assessed in all classes. Proteins were assessed in all classes, with the exception of Class5. Fats and taste indicators were reported in four and three classes respectively, while fiber was the least reported indicator. For some classes positive effects on soluble solids and titratable acidity were reported suggesting an effect on taste as index taste is normally estimated as the ratio between soluble solids and titratable acidity.

Globally, **Biostimulants (Class1)** and **Biochar & Microbes (Class3)** and the unclassified articles showed the highest percentage of positive effects on plant quality indicators (Table 3.1, see also Figure 2.5). Recovered articles indicate that these could be good management practices to stimulate plant nutrition processes. The assessment of these practices has been increased since 2016 (Figure 2.7) suggesting that they are good candidates for new experiments. For **Microbes (Class4)**, **Animal manure & Mineral fertilizer** (Class2) and **Intercropping, mulching and other practices (Class6)** the report of positive effects of management practices on plant quality were moderate (Table 3.1, see also Figure 2.5), suggesting that more studies are required. **Compost (Class5)** was the class that showed the lowest percentage of positive effects, that were mostly focus on carbohydrates and mineral elements (Table 3.1, see also Figure 2.5).

Ambiguous data were reported in some articles for all classes, but in higher frequency for classes 5 (46.7%), 2 (30.3%) and 4 (29.4%). In general, ambiguity was due to differences detected among different types of treatments compared but also among crops, seasons/years or phenological stages. Complex experimental designs with more than two factors, including seasons/years, were also associated with ambiguity. However, this type of experimental design is important for robust assessment of practice effects. Some articles compared nutrients indicators among manure treatments, however the absence of a control (e.g. non-fertilization or mineral fertilization) constrained a clear conclusion about the effects of manure use as an alternative practice. Negative values were reported for some indicators; however, the reported frequency was lower than 20% for all classes. The highest report of negative effects was reported for Class6 (15.2%).

Table 3.1. Percentage of positive effects on plant quality, and content of nutrients and taste indicators reported for each class of practices. Bold and italics refers to indicators in which the positive effects were associated with a decrease of the content, in all other cases the positive effects were associated with an increase of the indicator content. Green and white cells indicate soil indicators with and without positive effect reported.

Practice class	Positive effects on plant quality	Carbo-hydrates	Proteins	Fats	Fiber	Mineral elements	Acidity
Class 1 (Biostimulants)	**77.80%**	soluble sugar, starch, soluble solids	gluten, soluble protein, total protein	essential oil		Ca, Na, Fe	pH, titratable acidity
Class 2 (Animal Manure, Mineral Fertilizer)	**39.40%**	soluble sugars, reducing sugars, starch, soluble carbohydrates, total sugars, total soluble solids	crude protein, total protein and amino acids	oil, phospholipids, fatty acid		N, P, K, Zn, Na, ***Se, Cr, Co, Ni***	
Class 3 (Biochar / Microbes)	**77.30%**	hexoses, pentoses, total sugars, soluble sugars, individual sugars	amino acids, protein and gluten, soluble protein	oil, fatty acid		N, K, P, Fe, Zn, Cu, Mn, **Pb, Al, Ni, Cd, Cr**	
Class 4 (Microbes)	**47.10%**	glucose, fructose, carbohydrate	protein		fiber	N, P, K, Zn, Ni, Fe, Mn, Cu	citrate
Class 5 (Compost)	**26.70%**	carbohydrate content and total soluble solids				P, K, Ca, Mg, Cu, Zn, Fe, Mn, Na, B, S	
Class 6 (Intercropping / Mulching / Other practices)	**36.40%**	sugar, soluble solids	protein, amino acids			N, P, K, Cd, Mg, Ca,	acidity
Unclassified (Several practices)	**69.60%**	carbohydrates	protein	oil		N, P, K, Zn, B, Ca, Mg, Zn, Cu, Fe, Mn, Cu, ***Sr, Cd, Pb, Co, Ba, Cr***	

Ca: calcium, Na: sodium, Fe: iron, N: nitrogen, P: phosphorus, K: potassium, Zn: zinc, Se: selenium, Cr: chromium, Co: cobalt, Ni: nickel, Mn: manganese, Pb: lead, Al: aluminium, Mg: magnesium, B: boron, S: sulfur, Sr: strontium, Ba: barium.

Positive effects of management practices on the nutrient content of crops of potential interest
Positive effect of practices on plant quality has been reported for **wheat**. For example, one of the two articles that assessed effects of biostimulants reported changes on nutrients (Maksoud et al. 2023). Based on a field experiment, the use of henna leaf extracts improved quality of grains in terms of increase of soluble sugars (23%), starch (19%), gluten (50%), soluble proteins (37%), calcium 184%), sodium and iron (10%), although the effects depended on the biostimulant dose. Improvement of grain quality was accompanied by an increase on yield and growth parameters (e.g. grain yield (kg/blot, number of spikes/plant, number of grains/plant, weight of grains/plant, weight of 1000 grains, shoot height, root length, and fresh and dry weights of shoots and roots).

An increase in grain protein was also reported in rice-wheat cropping system by the use of farmyard manure at the higher dose (30 t ha-1), while plant biomass and grain and straw yield were higher for the mineral fertilization treatment, followed by farmyard manure at the higher dose (Ahlawat et al. 2023). In maize-wheat cropping system, the use of organic fertilizer in combination with mineral fertilizer increased nutrient (nitrogen, phosphorus, potassium), protein grain and yield in maize and wheat in comparison with no-fertilization or organic fertilization alone (Basak et al. 2013). Although differences were detected among the different types of organic fertilizer used, value added manures reduced mineral fertilizers doses and increased grain yield and quality.

Use of organic amendments can also alleviate the deleterious effects of seleniferous soils in wheat and rape grains, decreasing selenium grain concentration and increasing sulfur concentration in grain (Sharma et al. 2011). Effects on other grains nutrients (e.g. reducing sugars, starch, free amino acids, protein) varied with the crop and the type of organic amendment applied. In general, wheat showed an increase of reducing sugars and starch, while rape showed increase of starch and free amino acids. Oil concentration in rape grains increase, and the proportion of phospholipids and free fatty acids increased, the total sterol content decreased, and triglycerides increased by organic amendments. In general, organic amendments resulted in lower concentrations of palmitic, linolenic, and arachidic acids, whereas the concentrations of oleic and erucic acids increased. The relative proportion of major globulin subunits in rape grain were affected by organic fertilization, but differences were observed among amendments.

The study of Weber et al. (2024) in wheat-canola cropping system revealed that effects of cattle fed biochar and manure, in different combinations, on grain quality was modelled by the amendment treatment and the climatic conditions. In wheat, the use of biochar increased amino acid contents of plants in saline soils (Sun et al. 2019). Effects depended on dose, with 5–30 t/ha biochar showing relatively higher (by 5.2–19.1%) total amino acid contents. Also increase on the content of protein and gluten and decrease of lead and aluminium accumulation in grain were reported for the use of biochar combined with sewage sludge (Rozylo et al. 2017a). The use of either the arbuscular mycorrhizal fungi inoculant or the biochar alone resulted in decreased nickel uptake (Xiong et al. 2025). Only one article assessed the effect of isolated microbes on wheat, revealing that arbuscular mycorrhizal fungi have different effects on nitrous oxide (N_2O) emission and nitrogen translocation between sandy and clay soils (Zhai et al. 2021).

Regarding other crops of potential interest, the report of positive effects was scarce.

- In **pigeon pea leaves**, organic fertilizers alone or combined with mineral fertilization improved food quality (e.g. crude protein, crude fibre, soluble carbohydrate, ash) compared to control or mineral fertilization, although better results were generally reported for the combined fertilization (Das et al. 2017).

- Comparing organic with conventional farms, one article assessed the use of compost on **potato and carrot** (Warman and Havard 1996). The study revealed that phosphorus, magnesium, sodium and boron were higher in organically-grown potatoes and leaves, while tuber Mn was higher in conventionally-grown potatoes. In the case of carrots, the organically-grown carrots were higher in sulfur in both edible and leaf tissue, and higher in root boron and leaf sodium, while conventionally-grown carrots were higher in nitrogen, manganese and copper.

Conclusions

Considering the research question of this chapter (*"What is known in the scientific literature about the effects of agricultural management practices on the plant produce quality in terms of macro-nutrients and taste?"*) data extracted from the articles retrieved in the systematic literature review SR-B indicate that:

- different practices or practice arrangement varied in the positive effects on plant quality and particularly in terms of macro-nutrients and taste.
- mineral elements and carbohydrates are the most common indicators used for nutrient quality.
- taste is scarcely considered as a relevant indicator of practice effects. And when it is assessed, reports focus only on the ratio between total soluble solids and titratable acidity without considering indicators related to flavour changes, flavour strength, etc.
- there is some variation in the indicators used in the scientific literature to assess the effects of practices on plant nutrients and taste.
- ambiguous data reported were associated with experimental designs with more than two factors, in particular when different genotypes, varieties, crops, seasons or years are considered.

Based on these results we conclude that:

- data from this systematic literature review supports the use of practices usually considered in regenerative agriculture to improve plant nutrients, while the support for the improvement of taste is not studied a lot and seems not so robust.
- new experiments to assess the effects of practices on plant nutrients and taste should consider the identification of the main factors of the target system to allow a solid validation of the results (e.g. seasons/years, genotypes/varieties).
- new experiments to assess the effects of practices on plant nutrients and taste should include more indicators to cover adequately all types of macronutrients. Additionally, it should consider the estimation of at least one taste index.

Chapter 4: Effects of management practices on indicators of plant and human health

Chapter 4 presents the main results about the effects of the management practices on direct or indirect indicators that contribute to plant health and can contribute in some cases to human health. Results are presented considering the six classes identified in Chapter 2 based on the arrangement of practices assessed in the articles retrieved from the systematic literature review SR-B.

Research question

What is known in the scientific literature about the effects of agricultural management practices on plant quality in terms of direct and indirect indicators that can contribute to plant and human health (e.g. secondary compounds, response to biotic or abiotic stress or shelf life)?

Results

Positive effects of management practices on direct and indirect indicators of plant or human health were reported in the literature reviewed, although differences were detected among classes and indicators (Table 4.1). Reported indicators were grouped in Table 4.1 considering the focus of the article research to simplify data presentation. This classification should not be considered as rigid, as some indicators could be related with different outcomes (e.g. carotenoids could be considered as bioactive compounds and also as an indicator of plant efficiency).

Among direct indicators, positive effects on the antioxidant capacity have been reported for all classes of practices, both by assessment of enzyme activities or different methodologies such as radical scavenging (ABTS, DPPH), ferric reducing antioxidant power (FRAP), malonaldehyde peroxidation (MAD), etc. Positive effects on plant efficiency indicators were also reported for all classes, including both photosynthetic pigments (chlorophyll a, b, total, carotenoids) or physiological parameters (stomatal conductance, net photosynthetic rate, transpiration rate). Positive effects were less reported for osmoprotection and defence. Osmoprotection activity was associated with the assessment of proline; while defence activity was based mostly on the assessment of resistance to pathogens.

Among indirect health indicators, bioactive compounds were reported for all classes, while phytohormones were reported only for class1. The bioactive compounds reported included more frequently the assessment of total content and/or composition of polyphenols, flavonoids, lycopene and vitamin C. Other indicators in these groups were also considered in some articles, such as tannins, anthocyanins, carotenes, other vitamins. Reports of shelf life were scarce and related to class1, class3 and class5. Indicators associated with human health were the least reported, and were based only on in-vitro antioxidant, cytotoxicity or viability assays in few studies.

It is important to point out that a common set of indicators for the assessment of the effect of practices on plant health was not detected in the literature reviewed. Articles varied in the combination of methodologies used and in general not justified the use of the indicators selected. For example, antioxidant activity was most frequently evaluated by DPPH method, but FRAP, ATBS, MAD were also used and, in several cases, combined.

Table 4.1. Health indicators with positive effects reported in the literature for each class of practices. Bold and italics refers to indicators in which the positive effects were associated with a decrease of the content. In all other cases the positive effects were associated with an increase of the indicator content, except in the case of proline where the positive effect could be associated with an increase or decrease of content according to the characteristic of the study.

Practice classes	Direct Indicators of plant health			Indirect indicators of plant health		Shel life	Human health
	Antioxidant or Osmoprotectant activity	Defence capacity	Plant efficiency	Bioactive compounds *	Phytohormones		
Class 1 (Biostimulants)	catalase, superoxide dismutase, guaiacol peroxidase, ABTS, DPPH, FRAP, proline		chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phytomelanine, photosystem II (PSII) activity (Fv/Fm) and stomatal conductance (gs)	total phenolics, total flavonoids, total tannins, lycopene, glutathione, phenolic acids, anthocyanin, flavan-3-ol, carotenoids, vitamin C, vitamin E	abscisic acid, indole acetic acid, gibberellic acid, cytokinin	***weight loss***, fruit firmness, ***fungal contamination***	In-vitro cellular antioxidant activity
Class 2 (Animal Manure, Mineral Fertilizer)	DPPH	antibacterial activity	chlorophyll b, total chlorophyll, carotenoids	tannins, oxalate, flavonols,total phenolics, total flavonoids, carlinoside, lycopene, vitamin C, vitamin B			
Class 3 (Biochar / Microbes)	catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, dehydoascorbate reductase, DPPH, reducing power, total antioxidant activity, chelating power, ***MDA***, ***H_2O_2***, ***generation rate of oxygen radical***, proline	resistance to pathogenes	chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b ratio, carotenoids, transpiration rate, stomatal conductance, photosynthetic rate, ***intercellular CO2 concentration***	total phenolics, total flavonoids, lycopene, anthocyanins, vitamin C		fruit hardness	In-vitro cell viability and cytotoxicity assay

Class 4 (Microbes)	proline	resistance to pathogenes	total chlorophyll, osmotic potential, phothosynthetic rate, stomatal conductance, transpiration	lycopene, β-carotene, luteine, total phenolics, carotenoids, vitamin C, vitamin E			
Class 5 (Compost)	DPPH, ATBS	resistance to pathogenes	total chlorophyll	total phenolics, total flavonoids, lycopene, β-carotene, vitamin C		fruit firmness	
Class 6 (Intercropping / Mulching / Other practices)	DPPH	resistance to pathogenes	total chlorophyll, net photosynthetic rate, nitrate reductase activity	total phenolics, total flavonoids, anthocyanins, proanthocyanidins, flavonols, catechin, β-carotene, lutein, vitamin C			
Unclassified (Several practices)	catalase, superoxide dismutase, MDA, DPPH, FRAP, Relative Antioxidant Capacity Index (RACI), ABTS, reducing power, chelation power, antioxidant capacity index		chlorophyll a, chlorophyll b, total chlorophyll, carotenoids	total phenolics, total flavonoides, hydrolyzable tannins, condensed tannins, vitamin C			

ABTS and DPPH are two methodologies for radical scavenging assessment. FRAP is a method for ferric reducing antioxidant power. MAD is a method for the assessment of malonaldehyde peroxidation. Green and white cells indicate soil indicators with and without positive effect reported.

Although soil borne pathogens and antimicrobial resistance in plants are clearly associated with **human health,** there were no reports about positive effects of management practices on these indicators in the literature reviewed. However, some reports were identified that consider the human health risk of some practices. Based on a microcosm experiment, a study explored the effects of manure fertilization on the abundance of endophytic pathogens (Li et al. 2002). Results from radish, lettuce and pakchoi showed that manure fertilization increased the abundance of pathogens in the plant endosphere, highlighting that soil is a vital source of both bacterial communities and human pathogens for the plant endosphere. Similarly, a study in southern Benin assessed the effects of poultry manure in eggplant, tomato, and carrot, revealing that the abundance of mean fecal bacteria count per g of fresh vegetables were variable but higher than AFNOR criteria (Atidégla et al. 2016). These reports contrast with the results of other studies. For example, a field study examined the appropriateness of Australian guidelines (i.e. withholding period of 90 days between manure application and harvest for high-risk products such as leafy salad greens) to reduce the risk of contamination (Ekman et al. 2021). Under conditions replicating those on a commercial vegetable farm, this study revealed that 90-day withholding period between application of manure and harvest significantly reduces risk from enteric pathogens. Other study focused on the presence of foodborne pathogens and survival of generic *Escherichia coli* in an organic integrated crop-livestock system in a maize/tomato rotation (Cheong et al. 2024). The study revealed that the effect of sheep grazing on foodborne pathogen contamination in integrated crop-livestock system is minimal, but that further studies should be performed to distinguish the source of foodborne pathogen contamination (soil or animal feces).

Regarding antibiotic resistance, a field study explored the abundance of antibiotic-resistant bacteria and the presence of antibiotic resistance determinants on vegetables often eaten raw such as tomato, cucumber, pepper, carrot, radish, lettuce (Marti et al. 2013). This study revealed that abundance increase of resistant bacteria showed no coherent pattern, and that the antibiotic resistance determinants were also detected in plants from unmanured soils. Other study based on a glasshouse pot experiment highlighted the potential risks of plant resistome migration to the human food chain (Zhang et al. 2019). The impacts of poultry and cattle manure application on the patterns of resistome in lettuce microbiome including rhizosphere, root endosphere, leaf endosphere and phyllosphere, revealed that poultry manure may have a stronger impact on lettuce resistomes than cattle manure. The study reported that 90-day post-application cattle manure increased the abundance of antibiotic resistance genes (ARGs) in root endophyte, while poultry manure application increased ARGs in rhizosphere, root endophyte and phyllosphere. The impact of chicken litter pre-application treatment on the abundance, field persistence, and transfer of antibiotic-resistant bacteria and antibiotic resistance genes to vegetables was also evaluated by Subirats et al. (2021). This study assessed the impact of raw and composted chicken litter applied to field plots that were cropped with carrots, lettuce and radishes, revealing that under field conditions there was limited data about transfer of antibiotic resistance genes from raw or composted manure to vegetables that then persisted through washing.

Positive effects of management practices on health indicators of crops of potential interest

For **wheat,** positive effects of practices on indicators of plant health have been reported. For example, the application of a biostimulant determined changes in photosynthetic pigments of wheat leaves in response to treatments with different concentrations of henna leaf extracts (Maksoud et al. 2023). This response showed a concentration dependent pattern at any stage of growth evaluated. The optimum dose increased the level of chlorophyll a, chlorophyll b and carotenoids for tillering, elongation and grain filling stages, respectively. Maksoud et al. (2023) also reported an increase of phytohormones and total phenolics, flavonoids and tannins, and changes in the composition of polyphenols and flavonoids compounds with the application of the biostimulant.

The evaluation of the effect of fertilization with sewage sludge (SL) with varying rates of biochar (BC, 2.5, 5 and 10% of dry weight) revealed that the application of SL+5%BC increased the total polyphenolics content compared to all other fertilization treatments, however the use of SL+10BC reduced it (Rozylo et al. 2017a). A significant increase was also reported for total flavonoids and antioxidant capacity for both SL+5%BC and SL+10%BC. In wheat plants stressed by soil Ni content, foliar pigment contents (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids) and physiological indicators (e.g. photosynthetic efficiency, stomatal conductance) were increased with the application of activated carbon biochar (ACB) and arbuscular mycorrhizal fungi (AMF), while bioactive compounds (anthocyanin, flavonoids, phenolics) were reduced (Rehman et al. 2022). These results indicate that the combined application of ACB and AMF was able to reduce the negative effects of the stress by Ni content.

Intercroping increased the chlorophyll content in wheat and faba bean in comparison with sole crops in full and half density (Sammama et al. 2021). The chlorophyll increase was even higher for crops without nitrogen fertilization. Increase in chlorophyll content were associated with increase in the nitrate reductase activity, indicating a N fixing activity in the intercropped wheat and faba bean compared to monocropping. The application of biogas digestate (BD) and mining waste (carboniferous mudstones from coal mine, MS), alone or in combination, indicated that BD and MS+BD increased the content of polyphenols in wheat grains compared to NPK and no fertilization in the three analysed years, while the antioxidant capacity index increased only in the first year (Rozylo et al. 2017b). The combination of Zinc oxide-nanoparticles (ZnO-NPs) with a Zinc-biofertilizer, prepared with Zinc-solubilizing bacteria with multiple plant growth-promoting traits, revealed that the amendment with ZnO-NPs increased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content compared to the application of biofertilizer alone (Saleem et al. 2023).

For **faba bean**, a study revealed that the application of fungi and bacteria isolated from a suppressive compost were able to control *Rhizoctonia solani* disease (Pugliese et al. 2014). Isolated microorganisms were also able to control *Pythium ultimum* in cucumber, and *Fusarium oxysporum f. sp. basilica* in basil. Results reported by Pugliese et al. (2014) indicate that among isolated microorganisms, bacterial strains showed to control the pathogens better than *Trichoderma* and other fungi. For **pigeon pea**, the application of vermicompost and farmyard manure increased leaves antibacterial properties and total flavonoid content, and total phenols and chlorophyll respectively (Das et al. 2017). In **turnip**, organic amendments such as sewage sludge, chicken manure, horse manure, vermicompost, and organic fertilizer, increased the glucosinolates in roots of different varieties compared with native soil without amendment (Antonious et al. 2023).

Conclusions

Considering the research question of this chapter ("Wh*at is known in the scientific literature about the effects of agricultural management practices on plant quality in terms of direct and indirect indicators that can contribute to plant and human health (e.g. secondary compounds, response to biotic or abiotic stress or shelf life?")* data extracted from the articles retrieved in the systematic literature review SR-B indicates that:

- antioxidant capacity, plant efficiency and bioactive compounds are the most frequent indicators used.
- defence capacity, phytohormones, shelf life and human health indicators are the least frequent indicators used.

- indicators used varied among studies.
- a common set of indicators for the assessment of effects of practices on plant or human health is not yet available.

Based on these results we conclude that:

- data from systematic literature review supports the use of practices usually considered in regenerative agriculture to improve the plant quality in terms of indicators that can contribute to plant and human health.
- new experiments should consider a common set of methodologies allowing to cover direct and indirect indicators of plant and human health.
- new experiments should consider the assessment of indicators directly associated with human health such as soil borne pathogens or determinants of antibiotic resistance.

Chapter 5: Effects of management practices on soil quality and their relationship to plant quality

Chapter 5 summarize the main results about the effects of the management practices on soil quality indicators and the interrelationships between soil quality and plant quality indicators. As in the previous chapters, results are presented considering the six classes of practices identified in Chapter 2.

Research question

What is known in the scientific literature about the effects of agricultural management practices on the soil quality?
What is known in the scientific literature about the relationships between soil quality and plant quality changes?

Results

Less than 50% (64 out of 134) of the articles reviewed in the systematic literature review B (SR-B plant produce) assessed plant quality along with soil quality indicators. Among them, positive effects of management practices on soil quality were reported, with some differences among classes (Table 5.1). Positive effects of management practices on soil physicochemical indicators were reported in all classes, except for Class1. Among biodiversity indicators, positive effects of management practices for biological activity indicators were reported in classes 2, 3, 5 and among unclassified articles. Positive effects of management practices on abundance indicators were reported for classes 2, 3 and 4; while for α-diversity they were reported only for Class2. Despite some particular differences, physicochemical indicators were similarly used among classes and were mostly focused on macro- and micro-nutrients, organic matter, and electric conductivity. Physical indicators were less reported, focussing on soil bulk density, moisture and temperature (Table 5.1). Among biodiversity indicators, biological activity indicators focused mostly on soil enzymes activities, with a few reports of root microbial colonization. For the other side, abundance indicators included microbial biomass carbon, microbial counts and earthworm density. Diversity indices were considered as indicators for α-diversity. It is important to mention that β-diversity was also considered in some studies, reporting changes on the relative abundance of microorganisms among treatments. In these cases, amplicon sequencing methodology was used to characterize microbial communities and to analyse how practices affect composition of microbial communities (e.g. Zhang et al. 2019, De Tender et al. 2021, Milkereit et al. 2021, Li et al. 2022, Xiong et al. 2025).

Positive effects of management practices on soil quality of crop systems of potential interest

Positive effects of practices on soil quality indicators were reported for **wheat** among the articles reviewed. For example, higher values of soil quality indicators were reported for farmyard manure compared with the recommended dose of nitrogen, phosphorus, potassium and control. Changes on physicochemical indicators varied with the farmyard dose, highest values of nitrogen, phosphorus, potassium, soil organic carbon and organic matter were determined for manure at 30 t ha^{-1}, while highest values of electric conductivity, zinc and copper were determined for manure at 20 t ha^{-1} (Ahlawat et al. 2023). This study also demonstrated that farmyard manure affects the diversity of soil bacteria and bacterial gene expression, revealing a specific pattern for each treatment (Ahlawat et al. 2023). Increase of soil dehydrogenase and catalase activity, microbial biomass carbon and microbial biomass nitrogen with the application of organic amendments was also reported (Basak et al. 2013). Nitrogen fertiliser rates, crop rotation and soil tillage affected soil quality indicators (Agenbag 2012). For example, higher soil pH was determined from the crop rotation system, while conventionally tilled soil showed slightly higher values compared to less intensive methods of soil tillage and no-tilled plots.

Table 5.1. Soil quality indicators with positive effects reported in the literature for each class of practices. Bold and italics refers to indicators in which the positive effects were associated with a decrease of the content. In all other cases the positive effects were associated with an increase of the indicator content. Green cells indicate soil indicators with reported positive effect. White cells indicators without reported positive effects.

Practice classes	Physicochemical indicators	Biodiversity indicators Abundance	Biological activity	α-diversity
Class 1 (Biostimulants)				
Class 2 (Animal Manure, Mineral Fertilizer)	OC, OM, total C, total N, available N-P-K, Zn, Cu, Mn, nitrate, amonium, dissolved organic C-N, CEC, EC, pH, bulk density	MBC, nematode biomass, pathogen CFU	dehydrogenase, MBN, AMF colonization	OTUs of top 30 bacterial classes, genera, plant growth promoting bacteria, lignocellulose degrading bacteria, shannon diversity index, nematode indices
Class 3 (Biochar / Microbes)	OC, OM, total N, available P-K, exchangeable K, Mg, Cd, Cr, As, Pb, Cu, nitrate, ammonium, phosphate, pH, bulk density, CEC, EC, moisture, total and fractions of heavy metals (Cd, Cr, Cu, Pb), alkali-hydrolyzed nitrogen, amorphous Fe oxides	MBC, bacteria, fungi, actinomycetes, N2-fixing bacteria, Fe/Mn-oxidising Leptothrix species	acid phosphatase, alkaline phosphatase, β-glucosidase, catalase, cellulase, dehydrogenase, fluorescein di-acetate, peroxidase, phosphomonoesterase, sucrase, urease, metabolic quotient, beneficial bacteria colonization, AMF colonization, TGSP, MBN	
Class 4 (Microbes)	OM, total N, available P, exchangable K-Ca-Mg, nitrate, exchange acidity, C/N ratio, bulk density	earthworm density		
Class 5 (Compost)	OM, extractable P-K-Zn, exchangable K-Ca-S-Mg, nitrate, phenols, dissolved organic C, CEC, EC, aggregate stability		alkaline phosphatase, amylase, catalase, deshidrogenase, protease	
Class 6 (Intercropping / Mulching / Other practices)	OM, total N, available N-P-K-Mg-Ca, nitrate, ***DTPA-extractable Cd, pH, temperature***, moisture			
Unclassified (Several practices)	OC, OM, available N-P-K-Cd		acid phosphatase, alkaline phosphatase, invertase, urease	

OC: organic carbon, organic matter, C: carbon, N: nitrogen, P: phosphorus, Zn: zinc, Cu: copper, Mn: manganese, Mg: magnesium, Cd: cadmium, Cr: chromium, As: arsenic, Pb: lead, Fe: iron, Ca: calcium, S: sulfur, CEC: cation exchange capacity, EC: electric conductivity, MBC: microbial biomass carbon, CFU: Colony-forming unit, MBN: microbial biomass nitrogen, AMF: arbuscular mycorrhizal fungi. TGSP: total glomalin related soil protein, OTUs: operational taxonomic units.

Phosphorus and potassium showed a tendency of increase when less intensive methods of soil tillage were used. For organic carbon, higher values were found in crop rotation system and less intensive methods of tillage and decrease with soil depth. In monoculture system, calcium and magnesium tended to increase and sulfur to decrease with less intensive tillage, but all tended to decrease in crop rotation system.

A study of **wheat-winter pea** intercropping revealed that apparent available nitrogen depended greatly on the preceding crops and on the differences in their nitrogen treatments, experimental nitrogen fertilization, nitrogen fertilizer efficiency, soil nitrogen mineralization, initial nitrogen mineral content and weather conditions (Bedoussac and Justes 2010). The effect of forage legume–winter wheat strip tillage intercropping on soil nitrate nitrogen (N-NO_3) content in various sequences of rotation in organic production systems has been also reported (Arlauskiene et al. 2021). This study revealed that conventional tillage increased N-NO_3 content cultivating winter wheat after forage legumes. Based on a cropping system experiment (2004– 2010) with three 3-year rotations of different number of grain legumes (GL0, GL1 and GL2, none, one and two grain legumes, respectively) with (CC) or without (BF, bare fallow) cover crops, a field study revealed that as an average cumulative nitrogen leaching increased when increasing the number of grain legumes in the rotation without cover crops (Plaza-Bonilla et al. 2015). However, the use of cover crops reduced nitrogen leaching. A comparative study of winter wheat and barley growing in an agroforestry system with walnut trees and as cereal monocrops demonstrated that soil mineral nitrogen was lower in the agroforestry than in the monocrop system before walnut budburst (Arenas Corraliza et al. 2022). After budburst the availability of mineral nitrogen or potassium, on average, was similar among systems, but phosphorus availability was significantly higher in agroforestry than in monocrop (Arenas Corraliza et al. 2022). Barley plots showed lower mineral nitrogen in agroforestry while no difference was found for wheat (Arenas Corraliza et al. 2022). The use of biochar and two wheat-derived cadmium-immobilizing endophytic bacteria was evaluated alone and in combination, revealing the reduction of cadmium availability in the soil and cadmium translocation from the roots to grains, a process regulated by changes on soil microbial communities (Xiong et al. 2025).

Regarding **other crops of potential interest**, a field experiment investigated the effect of six **leguminous** and **non-leguminous** grain crops on soil fertility over a year revealing that all crop had negative nitrogen balance during growing season, and that nitrogen removed from soil was greater for barley, rape and lupins than for beans, field peas or lentils (Francis et al. 1994). The study also showed that nitrogen mineralization was greater following leguminous than non-leguminous crops, with exception of lupins, that cumulative apparent leaching losses largely reflected the mineral nitrogen content and that these changes affected the wheat growth in the following period. In **pigeon pea**, at the end of the 2-year experiment vermicompost and farmyard manure resulted in maximum change in the soil quality with respect to its initial values, showing a decrease of pH and an increase of total organic carbon and total nitrogen, and available phosphorus and potassium (Das et al. 2017). A potted plant growth experiment was conducted to characterize cattle manure P mineralization as modified by iron amendments and uptake by pigeon pea and soybean, revealing that phosphorus solubility and plant uptake were reduced when manure was amended with iron at 1:3 molar ratio, in spite of the legumes' reported ability to secrete siderophores (Rao and Dao 2008). The study reported that the iron amendment did not affect plant dry matter production at rates up to 3 mol of iron to each mol of manure, but it affected plant uptake over the growing season and reduced final phosphorus content in soybean.

In **potato**, a comparative study of conventional vs organic crops revealed that Mehlich-3 extractable soil magnesium was greater for organic than for conventional crops, a change that affected the magnesium content of the potatoes (Warman and Havard 1996). Field plots cropped with **carrots**, lettuce and radishes revealed that manure amended soil showed higher abundances of antibiotic resistance genes compared to unmanured soil (Subirats et al. 2021). At harvest, those genes that were detected in soil samples before the application of manure showed a larger number of gene targets detected on the radishes than in the carrots or lettuce. Moreover, an increase of antibiotic resistance genes on radishes produced in soil receiving raw manure may be due to changes to soil microbial communities following manure application, rather than transfer of enteric bacteria to the radishes. A field assay focused on the assessment of bacteria resistant to various antibiotics on vegetables often eaten raw (tomato, cucumber, pepper, carrot, radish, lettuce) and on how this might vary with growth in soil fertilized inorganically or with dairy or swine manure, also revealed that soil receiving manure was enriched in antibiotic-resistant bacteria and various antibiotic resistance determinants (Marti et al. 2013). The effect of manure fertilization on soil and vegetables (including radish) revealed that manure altered soil microbiomes, whereas have less influence on endophytic microbial communities, and that the abundance of pathogens increased both in soils and endosphere under manure fertilization (Li et al. 2022). An experiment combining biochar and metal-immobilizing bacteria revealed the reduction of edible tissue metal uptake in vegetables due to the increase of amorphous iron oxides and abundance of iron- and manganese-oxidising *Leptothrix* species in soil (Cheng et al. 2020).

Interrelationships between plant quality and soil quality indicators

Relationships between plant quality and soil quality indicators were reported only in 15.7% of the reviewed articles (21 out of 134). Among them, significant correlations have been reported (positive and/or negative) between plant quality and soil quality indicators. In general, correlations between physicochemical soil indicators and plant nutrient and yield have been reported among practices classes, while relationships with biodiversity indicators are less reported.

For wheat, grain yield varied with soil nitrate ($N-NO_3$) content (Arlauskiene et al. 2021), protein content showed negative correlation with soil microbial biomass carbon, microbial biomass nitrogen, electric conductivity and temperature (Weber et al. 2024). In canola, protein content showed negative correlation with soil nitrogen, and biomass yield positive correlation with microbial biomass carbon (Weber et al. 2024). In maize total amino acid content showed positive correlations with total soil nitrogen (Khan et al. 2023), yield showed positive correlation with $N-NO_3$ (Vyn et al. 1999), pH, soil organic carbon, cation exchange capacity, deshidrogenase activity and metabolic quotient and negative correlation with bulk density (Phares et al. 2022) and grain protein showed positive correlation with soil nitrogen content (Ngone et al. 2023). In rice total sugar released showed positive correlation with soil enzyme activity (Ali et al. 2022a), Cd in the rice grains showed positive correlation with available cadmium and negative correlation with pH, available phosphorus and total carbon (Jin et al. 2021), arsC gene abundance, that affects total arsenic in roots, stems and husk, and As(II) in grain and roots, showed positive correlations with pH, soil organic matter, total nitrogen and phosphorus (Tang et al. 2021).

Among vegetables, in spinach, total chlorophyll and phenols showed negative correlations with soil nitrates, phenols, dissolved organic carbon, electric conductivity and soil indices; while in lettuce leaf fresh weight showed positive correlation (Tavarini et al. 2011). In tomato, total phenols, lycopene and antioxidant activity (FRAP assay) showed negative, positive and negative correlation with nitrogen dose under biofertilization (Ochoa Velasco et al. 2016). In eggplant, cadmium concentration showed positive correlation with pH, total and available cadmium, and negative correlation with available phosphorus and potassium, while dry weight showed positive correlation with available phosphorus (Ma et al. 2022). On the other side in sedum, cadmium concentration and dry weight showed positive correlation with available cadmium, nitrogen, phosphorus and potassium, and negative correlation with pH and total cadmium.

Considering fruits, in citrus, significant correlations between fruit quality indices with the abundance of dominant soil phyla. For example, total soluble solids and vitamin C showed positive correlations with *Chloroflexi* at the phylum level, while at the genus level total soluble solids showed positive correlation with Gp2 and total soluble solids, titratable acidity and vitamin C showed negative correlation with Gp13 (Yang et al. 2023). In blueberry, vitamin C content was positively correlated with soil organic matter, pH, available potassium, magnesium, zinc and copper, nitrate ($N\text{-}NO_3$) and ammonium ($N\text{-}NH_4$) (Zhang et al. 2020).

Conclusions

Considering the research questions of this chapter (*"What is known in the scientific literature about the effects of agricultural management practices on the soil quality?"* and *"What is known in the scientific literature about the relationships between soil quality and plant quality changes?"*) data extracted from the articles retrieve in the systematic literature review SR-B indicates that:

- less than half of the articles assessing the effects of management practices on plant quality integrated the assessment of soil quality indicators, and only 15.7% of them assessed also the interrelationships between plant and soil quality indicators.
- physicochemical indicators are more frequently used than biodiversity indicators.
- different practices used similar physicochemical indicators, that mostly focused on nutrients, organic matter and electric conductivity.
- the assessment of enzyme activities was frequent and the main focus of biodiversity assessment.
- assessed interrelationships mostly considered soil physicochemical and plant nutrient and yield.

Based on these results we conclude that:

- data from the systematic literature review supports the use of practices usually considered in regenerative agriculture to improve soil quality and, in consequently, plant quality.
- new experiments should be performed to increase the knowledge about management practices effects on soil quality and the interrelationships between soil and plant quality.
- these experiments should integrate physicochemical and biodiversity indicators.

Chapter 6: Summary of earlier systematic reviews and reference articles selected by expert's

Chapter 6 presents a summary of the main findings of regenerative agriculture considering definitions, effects of associated management practice on plant, plant/animal produce and soil quality and potential useful data for the identification of RA typologies and/or indicators for monitoring. This summary was based on review articles recovered from the systematic literature analysis (SR-A and SR-B) which were combined with reference articles based on participant expert's knowledge.

Research question

What can previous reviews articles and reference articles add to our research of regenerative agriculture?

Results

Review articles recovered from SR-A and SR-B were considered for the generation of the summary table. Among them a selection was performed retaining articles that focus on the effects of practices on plant, plant/animal produce quality (nutrients, taste, health) and/or soil quality, or that can contribute with the identification of RA typologies and/or monitoring indicators. After selection, 15 articles from SR-A and 3 articles from SR-B were retained. For each article, data were extracted considering focus and main message, recommendations and relevance for the project (Table 6.1). A color code was added to indicate the main contributions of each article in terms of plant quality, soil quality, identification of RA typologies and/or monitoring indicators (Table 6.1). This table also includes details about the specific parts in which relevant information was reported in the articles, considering, in particular, the crops or topics of potential interest for the project.

Among these 18 articles, 4 reviewed the effects of management practices on plant quality (3 for nutrients and 4 for health), while no data was detected for plant quality in terms of taste. These articles revealed that practices aligned with RA can: i) improve micronutrient concentrations of edible portions crops, ii) modify bioactive and nutrients profiles in plants (e.g. using intercropping) and iii) affect the persistence of foodborne pathogens in soil (e.g. with manure addition). RA could be also relevant for the management of non-communicable chronic diseases in humans. Regarding micronutrients, information for wheat, pulses, and other crops was reported. For wheat, good evidence for positive effects was reported for biostimulants on zinc and intercropping on iron. Some inconclusive evidence was reported for positive effects of organic inputs or zero tillage on zinc and for biostimulants on iron. In the case of pulses, some inconclusive evidence was reported for organic inputs in zinc and iron. Regarding intercropping effects, an increase of grain protein and/or amino acids was reported for wheat and faba bean, spring wheat and different legumes or wheat and clover, while positive effects on bioactive compounds were not reported for wheat but for maize and other intercropped systems.

Eight articles reviewed the effects of management practices in soil quality. They covered the impact of digestates, composts and manure on foodborne pathogen fluxes, the suppression of weeds through soil microbiome management and the impacts on soil carbon, soil quality and/or crop productivity. The relationship between soil quality, plant quality and the impact on human health was also reported in some of them. Eight articles were focused or discussed alternative definitions of RA, the consequences of the lack of a common definition or of the use of the term without a definition. Some of these articles discussed principles, outcomes and practices, and proposed frameworks that could be useful for experimental design. Similarly, five articles provided information that could be useful for the identification of monitoring indicators.

Table 6.1. Summary table of existing literature reviews covering the the specific questions addressed on this report. N: nutrient quality. H: health quality. S: soil quality. RAT: regenerative agriculture typology. M: monitoring. SLR: systematic literature review. LR: literature review.

Origen	Reference	Article	Focus and main message	Recommendations	Relevance for the project	N	H	S	RAT	M
from SR-A	Manzeke-Kangara et al. (2023). Do agronomic approaches aligned to regenerative agriculture improve the micronutrient concentrations of edible portions of crops? A scoping review of evidence. Frontiers in Nutrition, 10, 1078667.	SLR	Some RA practices have a potential role on micronutrient content in the edible portions of several crop types, but this role is modelled by context specificity. Magnitude and reproducibility of effects were constrained by diversity of RA approaches, geographical conditions, and the limited number of studies.	Future research with appropriate designs, improved on-farm surveillance and nutritional diagnostics are needed for better understanding the potential role of RA in improving the quality of food, human nutrition, and health.	Data for wheat, pulses and other species (e.g. carrot, potato) in manuscript text, Table 4, Supplementary Table 2, Supplementary Table 5, Supplementary Table 7 reporting increases (and decreases) in micronutrients with respect to a specific Regenerative Agriculture practice.					
from SR-B (plant produce)	Arenas-Salazar, A. P., Schoor, M., Parra-Pacheco, B., García-Trejo, J. F., Torres-Pacheco, I., & Feregrino-Pérez, A. A. (2024). Intercropping Systems to Modify Bioactive Compounds and Nutrient Profiles in Plants: Do We Have Enough Information to Take This as a Strategy to Improve Food Quality? A Review. Plants, 13(2), 194.	LR	Review on intercropped systems introduced for the purpose of modifying nutrients and bioactive compounds in the cultivated species in order to improve human health indicates that some investigations obtained favorable results in modifying the amounts of bioactive compounds, some macronutrients, or even both; while others did not find an impact on these indicators regarding the different treatments implemented in intercropping.	Future research needed, such as adaptive responses related to crop allelopathy; plant–soil interactions due to vegetation patterns around the plants of interest; if species used enrich the microbiota; the plant environment limitations; the plant–plant interaction; where physio-agronomic parameters are sought, among others.	Summary of research where an intercropping system was introduced to modify both bioactive compounds and macronutrient content (or one of them) of one or more of the cultivated species, for the benefit of human nutrition (Table 1). This table includes information about wheat/faba bean, spring wheat and legumes, wheat and clover among other intercropping systems. In the case of wheat intercropped increase of grain protein and/or amino acids were reported.					
from SR-A	Ramkumar et al. (2024). Food for thought: Making the case for food produced via regenerative agriculture in the battle against non-communicable chronic diseases (NCDs). One Health, 100734.	LR	The potential of RA products in mitigating these diseases, considering the effects of dietary modifications on gut microbiome (associated with NCDs), and the higher food quality and nutritional value of food from RA compared to industrial agriculture.	Continued study of the effect of agricultural practices on food quality and other potential benefits including, but not limited to, carbon and water cycling, pollution, effects on habitat biodiversity, and climate change.	Brief summary and bibliografy references for the effects of soil health on crop health/food quality and comparision of RA in quality of animal source foods.					
from SR-B (plant produce)	Black, Z., Balta, I., Black, L., Naughton, P. J., Dooley, J. S., & Corcionivoschi, N. (2021). The fate of foodborne pathogens in	LR	This review summarizes information about positive and negative effects of manure use on soil health, with a main focus on foodborne pathogens and antimicrobial resistance genes, considering main	-	References for manure effects on soil health and management practice to support the sustainable use of manure. The article discusses manure use in the context of Northern Ireland.					

	manure treated soil. Frontiers in Microbiology, 12, 781357.		pathogens and practice to reduce pathogen loads.		
from SR-B (plant produce)	Piveteau, P., Druilhe, C., & Aissani, L. (2022). What on earth? The impact of digestates and composts from farm effluent management on fluxes of foodborne pathogens in agricultural lands. Science of The Total Environment, 840, 156693.	LR	Laboratory and field experiments suggest that (i) populations of microbial pathogens can survive in the soil, (ii) the results are species-specific, and (iii) the results are dose dependent. Data recovered highlight that it is important to identify exactly which environmental factors affect the survival of pathogenicmicroorganisms after application.	Authors argue that incorporating land characteristics in the management of safety issues connected with the spreading of organic fertilisers and soil improvers can improve the sustainability of biomass recycling.	Data provided about current safety rules, environmental regulations and specific regulations designed to mitigate health hazards involved in the management and recycling of farm manures and slurries in the European Union could guide experimental design. Also, information about factors affecting the fate of pathogens after land spreading could help with experimental design.
from SR-A	Khangura et al. (2023). Regenerative agriculture—A literature review on the practices and mechanisms used to improve soil health. Sustainability, 15(3), 2338.	LR	Support of the potential positive effect of minimum tillage, residue retention, and cover cropping on soil carbon, crop yield, and soil health, and of combining livestock with cropping and agroforestry on soil carbon and other co-benefits. However, indicates that benefits can vary among different agroecosystems.	To implement rigorous long-term farming system trials to compare conventional and RA practices to build knowledge on the benefit and mechanisms associated with RA on regional scales.	Discuss potential benefits of RA for soil health, focusing on main practices (minimum/no tillage, cover crops, stubble retention, crop rotation and diversity, rotational grazing) that increase soil carbon, increase of soil biodiversity and microbial function, mechanisms involved in improved microbial functions, effect of management practices on microbial activity. Focus on relevance of RA for western Australian farming systems.
from SR-A	Cheng, L., DiTommaso, A., & Kao-Kniffin, J. (2022). Opportunities for microbiome suppression of weeds using regenerative agricultural technologies. Frontiers in Soil Science, 2, 838595.	LR	Several promising approaches for weed control are based on the soil microbiome, including bioherbicides, natural products derived from microbes, and manipulation of the existing microbiome through agricultural practices. The article describe the three options, advantages and limitations.	Use of metagenomic sequencing to accelerate research on the microbial management of agricultural weeds.	Data about the use of agricultural practices to control weeds thorough soil microbiome management could be used for experimental design.
from SR-A	Rehberger, E., West, P. C., Spillane, C., & McKeown, P. C. (2023). What climate and environmental benefits of regenerative agriculture	LR	The review based on case studies from a range of regenerative agriculture systems suggest that practices can increase SOC, but regenerative agriculture studies must also consider the importance of maintaining	The carbon sequestration benefit of regenerative practices could be maximized by targeting soils that have been intensively managed and	Data recovered for effects of management practices on soil organic content.

	practices? an evidence review. Environmental Research Communications.		yield or risk the potential of offsetting mitigation through the conversion of more land for agriculture.	have a high carbon storage potential. The anticipated benefits of regenerative agriculture could be tested by furthering research on increasing the storage of stable carbon, rather than labile carbon, in soils to ensure its permanence.	
from SR-A	Jordon, M. W., Willis, K. J., Bürkner, P. C., Haddaway, N. R., Smith, P., & Petrokofsky, G. (2022). Temperate Regenerative Agriculture practices increase soil carbon but not crop yield—a meta-analysis. Environmental Research Letters, 17(9), 093001.	SLR & MA	Bayesian meta-analysis finds statistically significant increases in SOC concentration for reduced tillage intensity and ley-arable rotations compared to conventional practice over an average study duration of 15 years, but no effect of cover crops. None of these practices reduce yield during cropping years, although we find no evidence of a win–win between increasing SOC and enhanced agricultural productivity following adoption.	Future work should also evaluate the net greenhouse gas emission implications of each practice and potential for synergistic effects if RA practices are adopted in combination.	Data provided about the effects of some practices on soil quality.
from SR-A	Musto, G. A., Swanepoel, P. A., & Strauss, J. A. (2023). Regenerative agriculture v. conservation agriculture: potential effects on soil quality, crop productivity and whole-farm economics in Mediterranean-climate regions. The Journal of Agricultural Science, 161(3), 328-338.	LR	The review evaluates a set of agroecological practices which constitute a Regenerative Agriculture (RA) concept, for their potential to address these challenges from a soil quality, crop productivity and whole-farm economics perspective, and provides evidence that some of these practices offer promising perspectives.	To validate the potential of these technologies in Mediterranean small-grain systems, more long-term and context-specific research is called for.	Data recovered and summarized about management practices contribute to the understanding of management practice effects on soil quality (and on plant yield) and could help to define RA typologies and experimental design or monitoring. Context in mediterranean systems.
from SR-A	Jayasinghe, S. L., Thomas, D. T., Anderson, J. P., Chen, C., & Macdonald, B. C. (2023). Global Application of Regenerative Agriculture: A Review of Definitions and Assessment Approaches. Sustainability, 15(22), 15941.	SLR	The lack of a standardized definition and limited bioeconomic assessments of RA hinder the understanding and application of RA more broadly. Diverse definitions of RA based on outcomes, principles and/or practices. The article identified indicators, tools, and models for assessing biophysical and economic aspects of RA and discussed	Propose a working definition that integrates socioeconomic outcomes and acknowledges the significance of local knowledge and context to complement established scientific knowledge.	Data recovered about definitions of RA, indicators (biophysical, economical, and social indicators, Table 1), tools and frameworks for assessing biophysical and socioeconomic indicators (Table 2), biophysical and economic models (Table 3), advanced analytical techniques (Table 4) and

			the potential integration of advanced analytical methods into future assessments, including artificial intelligence and machine learning.		machine learning algorithms (Table 5) could be useful for RA typologies identification and for definition and/or selection of monitoring design. Australian system as case study.
from SR-A	Voisin, R., Horwitz, P., Godrich, S., Sambell, R., Cullerton, K., & Devine, A. (2024). What goes in and what comes out: a scoping review of regenerative agricultural practices. Agroecology and Sustainable Food Systems, 48(1), 124-158.	SLR	Organic amendment inputs and regenerative land management processes promote biology and improve nutrient cycling at soil, farm, and landscape scales. Regenerative agriculture overlaps with other farming practices including those associated with agroecology and conservation agriculture.	There is a need for food systems stakeholders to develop and improve circularity within food systems and move toward genuinely sustainable food production methods.	Data provided about inputs and processes that summarize output effects on yield, soil nutrients and agroecosystem (Table 2) could contribute to the definition of RA typologies and to guide indicator selection for monitoring.
from SR-A	O'donoghue, T., Minasny, B., & McBratney, A. (2022). Regenerative agriculture and its potential to improve farmscape function. Sustainability, 14(10), 5815.	SLR	Farmscape Function framework, to monitor the impact of change in our agricultural resources over time, and a mechanism to support further data-based innovation.	Propose an Intention, Principle, Practice, and Indicator (IPPI) mechanism for monitoring.	Data recovered and IPPI proposal could help to define indicators for monitoring and for RA typology identification/definition.
from SR-A	Al-Kaisi, M. M., & Lal, R. (2020). Aligning science and policy of regenerative agriculture. Soil Science Society of America Journal, 84(6), 1808-1820.	LR	Urgent need and commitment on the national and global levels for establishing a set of policies that accelerate the implementation of RA systems.	To develop and build a research agenda that advances the principles of RA in a more comprehensive and systemic approach to ensure the provision and exchange of scientific information that are critical for the acceleration of RA adoption.	Some indicators of soil health that could help organize monitoring.
from SR-A	Tittonell, P., El Mujtar, V., Felix, G., Kebede, Y., Laborda, L., Luján Soto, R., & de Vente, J. (2022). Regenerative agriculture—agroecology without politics?. Frontiers in Sustainable Food Systems, 6, 844261.	LR & SLR	Several definitions of RA coexist. RA needs a comprehensive definition that (i) rests on scientific evidence, (ii) allows informing new theories of change and (iii) avoids co-optation of the approach for green-washing purposes. Three types of RA that co-exist, namely "philosophy RA," "Development RA" and "Corporate RA," share in different degrees the ecological and social principles	A comprehensive definition, one that allows articulating RA with sustainability and resilience, needs to incorporate the social and political dimensions of agri-food transitions and transformations.	Could contribute to understanding the diverse definitions of RA, and the need to identify RA typologies.

			of agroecology, more easily at farm than at community level.		
from SR-A	Schreefel, L., Schulte, R. P., De Boer, I. J. M., Schrijver, A. P., & Van Zanten, H. H. E. (2020). Regenerative agriculture–the soil is the base. Global Food Security, 26, 100404.	SLR	A clear scientific definition of RA is lacking. Convergence is related to objectives that enhance the environment and stress the importance of socio-economic dimensions that contribute to food security. Divergence is related to socio-economic dimensions that are general and lack a framework for implementation.	This review contributes to establishing a uniform definition of RA; subsequently, indicators and benchmarks should be created to assess RA.	The core themes of regenerative agriculture (Fig. 2) could be useful as reference for the development of a framework that integrates principles, practices and outcomes of RA and considers them to identify RA typologies.
from SR-A	Newton, P., Civita, N., Frankel-Goldwater, L., Bartel, K., & Johns, C. (2020). What is regenerative agriculture? A review of scholar and practitioner definitions based on processes and outcomes. Frontiers in Sustainable Food Systems, 4, 577723.	LR	No legal or regulatory definition of the term “regenerative agriculture” exists nor has a widely accepted definition emerged in common usage. This wide variance in the definitions may lead to uncertainty about what different actors mean. Processes of RA have been used for many centuries. RA overlaps with other forms of agriculture. Without a clear, stated definition of RA, it may be difficult or impossible for researchers to test a specific claim about the benefits or outcomes of RA, consumers may be misled or confused about the significance or truth basis of a claim about food produced using, open space to greenwashing and has implications for policy and program development.	It may be helpful for individual users of the term “regenerative agriculture” to define it comprehensively for their own purpose and context.	List of processes and outcomes, recovered from journal articles and practitioner’s websites, that can contribute to the definition of RA typologies. Also, discussion about implications and risks of the absence or ambiguity of RA definition could contribute and guide identification of RA typologies.
from SR-A	Sands, B., Machado, M. R., White, A., Zent, E., & Gould, R. (2023). Moving towards an anti-colonial definition for regenerative agriculture. Agriculture and Human Values, 40(4), 1697-1716.	SLR	Provides evidence that while regenerative agriculture is often framed as a novel solution to anthropogenic environmental and socioeconomic crises, the associated practices can be traced back to Indigenous cultures and pre-colonial knowledge systems around the world.	Propose an anti-colonial definition for regenerative agriculture.	Data provided about practices and their relationship with indigenous cultures and pre-colonial knowledge could be useful for experimental design and identification of RA typologies

Additionally, some research articles were selected by the expert's group based on the information they provide (Table 6.2). In particular, the article of Montogmery et al. (2022) provide preliminary support for the conclusion that regenerative soil-building farming practices can enhance the nutritional profile (nutrient and health) of conventionally grown plant and animal foods. Measurements from paired farms across the United States indicate differences in soil health and crop nutrient density between conventional or regenerative practices. Regenerative farms that combined no-till, cover crops, and diverse rotations produced crops with higher soil organic matter levels, soil health scores, and levels of certain vitamins, minerals, and phytochemicals. Similarly, crops from two regenerative no-till vegetable farms had higher levels of phytochemicals than values reported from supermarkets, and a comparison of wheat from adjacent regenerative and conventional no-till fields found a higher density of mineral micronutrients in the regenerative crop. Regarding animal produce, a comparison of the unsaturated fatty acid profile of beef and pork raised on a regenerative farm showed higher levels of omega-3 fats and a more health-beneficial ratio of omega-6 to omega-3 fats than that of animals for a regional health-promoting brand and conventional meat from local supermarkets.

The article of Van Vliet et al. (2021) provides important data about the role of feeding diet on milk quality, revealing that livestock eating in a diverse array of plants has a higher content of phytochemicals. This result was also detected for meat, indicating that for these animal produce feeding diet is very important for phytochemical composition. The articles of Luján-Soto et al. (2020, 2021), reported the development and assessment of participatory monitoring and evaluation methodology, and proposed local and technical soil quality indicators and a visual soil assessment tool for monitoring the impacts of regenerative agriculture on ecosystem services that integrates farmers' and researcher's knowledge. These articles could be considered as a reference framework for the assessment of the experiments of this project.

Table 6.2. Summary table of reference articles covering some specific questions addressed on this report. N: nutrient quality. H: health quality. S: soil quality. RAT: regenerative agriculture typology. M: monitoring. RM: research manuscript.

Origen	Reference	Article	Focus and main message	Recommendations	Relevance for the project	N	H	SQ	RAT	M
provided by expert's	Montgomery, D. R., Biklé, A., Archuleta, R., Brown, P., & Jordan, J. (2022). Soil health and nutrient density: preliminary comparison of regenerative and conventional farming. PeerJ, 10, e12848.	RM	Preliminary support for the conclusion that regenerative soil-building farming practices can enhance the nutritional profile of conventionally grown plant and animal foods. Regenerative soil-building farming practices can enhance the quality profile (nutrient and health) of conventionally grown plant and animal foods.	-	Experimental design of paired farms (regenerative vs. conventional farms), use of soil organic matter and soil health scores (to account not only for soil organic matter but also for microbial abundance and activity) as soil quality indicators and validation or characterization of paired farms. Specific comparisons in wheat (no-tilled farms with different weed control and crop rotation treatments), carrots (no-till farms vs. conventional/market) and beef and pork (regenerative vs. conventional/market). See also data for peas (e.g. Table 2). Context United States.					
provided by expert's	Fenster, T. L., LaCanne, C. E., Pecenka, J. R., Schmid, R. B., Bredeson, M. M., Busenitz, K. M., ... & Lundgren, J. G. (2021). Defining and validating regenerative farm systems using a composite of ranked agricultural practices. *F1000Research*, *10*.	RM	Practice-based scoring systems to distinguish regenerative cropland and rangeland. The scoring system scaled positively with desired regenerative outcomes and provides the basis for predicting ecosystem responses with minimal information about the farming operation. Natural clusters in the number of regenerative practices used can be used to distinguish regenerative and conventional operations.	-	The proposed methodology could be considered for the definition of RA typologies.					
selected by expert's from SR-A	Jordon, M. W., Smith, P., Long, P. R., Bürkner, P. C., Petrokofsky, G., & Willis, K. J. (2022). Can Regenerative Agriculture increase national soil carbon stocks? Simulated country-scale adoption of reduced tillage,	RM	The study used a well-validated model of soil carbon turnover (RothC) to simulate adoption of three regenerative practices (cover cropping, reduced tillage intensity and incorporation of a grass-based ley phase into arable rotations) across arable land and	Further work could combine our approach here with data on current farm management and cropping practices, in addition to economic and behavioural models, to	Modelling framework and the use of RothC could contribute to monitoring. Context Great Britain.					

	cover cropping, and ley-arable integration using RothC. *Science of the Total Environment*, *825*, 153955.		develop a modelling framework which calibrates RothC using studies of these measures from a recent systematic review, estimating the proportional increase in carbon inputs to the soil compared to conventional practice, before simulating adoption across arable land. Results indicate that adopting RA practices could make a meaningful contribution to agriculture reaching net zero greenhouse gas emissions despite practical constraints to their uptake.	estimate the likely capacity for further adoption of these practices.	
selected by expert's from SR-A	Soto, R. L., Padilla, M. C., & de Vente, J. (2020). Participatory selection of soil quality indicators for monitoring the impacts of regenerative agriculture on ecosystem services. Ecosystem Services, 45, 101157.	RM	The combination of technical and local indicators provided complementary information, improving the feasibility of RA impact assessment. This integrated soil quality monitoring system offers a practical tool to enhance knowledge exchange and mutual learning to support the implementation of RA and optimize the delivery of ecosystem services.	-	Proposed local and technical soil quality indicators and visual soil assessment tool for monitoring the impacts of regenerative agriculture on ecosystem services that integrates farmers' and researchers' knowledge. Could be used as reference and adapted to the specific requirements of the project. Context southeast Spain.
selected by expert's from SR-A	Soto, R. L., de Vente, J., & Padilla, M. C. (2021). Learning from farmers' experiences with participatory monitoring and evaluation of regenerative agriculture based on visual soil assessment. Journal of Rural Studies, 88, 192-204.	RM	Including farmers in the design, decision-making and evaluation of research projects for agroecosystem restoration is imperative to enhance efficient, sound and inclusive transitions towards long term sustainable agroecosystems.	-	Reference for development and assessment of Participatory Monitoring and Evaluation (PM&E), the methodology could be adapted for the specific requirements of the project. Context southeast Spain.

Conclusions

Results of this chapter indicate that databases and information provided in the articles analysed complement the information presented in the previous chapters.

Based on these results we conclude that:

- available literature supports the use of practices usually considered in regenerative agriculture to improve plant and soil quality.
- available literature indicates the changes on soil and plant quality could have consequences on human health.
- wide range of definitions or the lack of definition of RA constrained the analysis of practices effects.
- experiments for the evaluation of practice effects require a conceptual framework to classify or categorize systems according with their level of regenerative transition.
- the experiments should integrate physicochemical and biodiversity indicators.

Chapter 7: General conclusions

7.1: What are the results from this systematic literature review?

The systematic literature review revealed that regenerative agriculture overlaps with other agricultural management systems, such as conservation agriculture, climate-smart agriculture, organic agriculture and agroecology, in terms of its principles, outcomes, and particularly in terms of practices. Indeed, the practices used on regenerative agriculture have been used in other agricultural systems for a long-time. This review also highlights that regenerative agriculture could have different meanings amongst different RA-supporting sectors. Although this could provide some flexibility for the use of the term, it also opens the door to several risks, as for example green-washing.

This review also highlights the wide range of practices group under the umbrella term regenerative agriculture. We provided a classification system for organizing of these practices as assessed in the literature. We noticed that there is misuse of some of the terms, or that certain terms are used without proper definition. Within the proposed classification, it is important to note that practices vary not only among classes but within classes. For example, biostimulants could be based on different types of compounds that can have different effects on soil or plant quality. Similarly, within compost, a wide range of nutrient sources could be combined, hence affecting the composition of the final product and, in consequence, its potential effects on soil and plant quality.

The number of articles focusing on the assessment of the effect of RA practices on milk quality was limited compared with that focusing on plant and plant produce. This difference could be due, in part, to the keywords used for the search, as some of the keywords could be not so relevant in terms of their effects on milk quality (e.g. tillage). In the case of plant and plant produce, a high frequency of reports was detected for wheat, tomato and maize. Although we could not rule out a bias due to the keywords defined for the search, a similar trend of species with more available data was reported by Manzake and Kangara (2023) that focused on plant micronutrients.

In general, indicators related to effects of management practices on animal produce were mostly associated with proteins and fat content, particularly on fatty acids. Plant and plant produce indicators were associated with mineral elements and carbohydrates for nutrient quality. They also included bioactive compounds and antioxidant capacity for plant health, and physicochemical parameters for soil quality. Taste was scarcely considered among studies. It was also determined that among articles that focused on the effects of management practices on plant produce a high percentage (> 82%) also evaluated growth and/or yield. A low percentage of these articles (< 50%) also assessed effects on soil quality, while an even lower percentage (< 16%) included the evaluation of the relationship between soil quality and plant quality.

Reviewed articles provide knowledge on the positive effects of practices. The percentage of positive effect reports was higher than 50% and higher than the percentage of neutral, negative and ambiguous effects (lower than 20% each of them). Ambiguous data were often detected with more complex experimental designs (e.g. more than two factors, different genotypes, varieties, crops, seasons or years) revealing the relevance of factor interactions or environmental variation.

7.2: Knowledge gaps

Besides these more general conclusions, the results of the review allowed us to determine some knowledge gaps that could be relevant for the design of the 100-hectare experiments.

New studies required to cover more produce. Most studies focus on wheat, tomato and maize. In particular, for the crops of potential interest for the 100 hectare experiments (e.g. potato, carrot and grain legumes), low number of reports were detected. Low number of articles were recovered also for milk.

New studies required to cover more practices and their combination. Regenerative practices varied across studies, but a few practices were considered in most articles. For example, practices related to the reduction in the use of mineral fertilizers (e.g. animal manure, compost, biochar) were more frequently reported that non-fertilization practices (e.g. grazing, tillage, cover crops, mulching, crop rotation, intercropping). Moreover a low number of practices were assessed in each study, although several practices were combined in the production system.

A common and integrated set of indicators is lacking. Indicators used for the assessment varied across studies. For example, studies focusing on the effects of practices on plant and plant/animal nutrients showed low frequency report on proteins, fats and fiber compared to mineral elements. Taste was almost never considered in the assessments. Similarly, indicators of plant photosynthetic efficiency, shelf life and human health were less frequent compared to bioactive compounds, and soil biodiversity parameters less often reported than physicochemical ones. The definition of a common minimal set of indicators is required.

Lack of integrated assessments. The review provides support for the use of RA management practices to improve nutrient density and health of plants and plant/animal produce. However, less than 50% of the articles retrieved evaluated at the same time soil properties, and even less (< 17%) assessed the relationship between soil and plant quality, revealing that assessment are not integrated, constraining the understanding of the mechanisms of management practices effects.

Lack of a categorization system of regenerative agriculture: the concept of RA is wide, in consequence, even for the same guiding principles/outcomes different management practices could be combined. Without a system that allows to categorize RA system based on different practice combinations, a robust assessment of RA effects is no possible.

New studies required to integrate environmental and/or genotype/varieties interactions: most studies focus on single practices, crops and seasons. Positive effects were mostly reported for this type of studies, while studies with more factors showed higher percentage of ambiguous results revealing that factor interactions should be also considered. Indeed, environmental variation such as inter-season or inter-annual climatic variation modelled effects. Therefore, experimental designs should considered factor interactions and, if possible, long-term assessments.

7.3. What could this mean for the 100-hectare experiments? Some preliminary thoughts.

The lack of a common definition of RA and the wide range of principles, outcomes and practices combined in RA initiatives represent a challenge for the assessment of regenerative agriculture effects on quality of soil, plants and plant/animal produce. These characteristics of RA should be considered for the design of the upcoming RA field experiments on 100 hectares in the North of the Netherlands. A method to categorize or characterize RA systems (for example, by assigning an RA score) would be required. This method should reveal differences among different regenerative farming systems by prioritizing certain practices. For example, if a farmer adopts the use of organic amendments and crop rotation as innovations to minimize synthetic inputs and keep the soil covered, and other farmer introduces the use of mulching and bio-stimulants as innovations for the same principles, can these two farms be consider equally regenerative? Or, if a farm uses animal manure and implements wheat-maize crop rotations, it is similar to a farm that uses compost and implements wheat-faba bean rotations? Are farming systems that use no-tillage in a wheat monoculture, equally regenerative compared to systems that use minimum tillage and intercropping?

While it may not be easy to answer the above questions, a system of prioritization and/or ranking or scoring of RA practices would help differentiating among 'levels' of RA implementation. Management practices are the backbone of experimental design, and they can be used to generate regenerative agriculture classification system and typology. The robustness of the assessment of RA effects remains limited without a proper classification method. The variation among and within classes of practices should be also considered in field experimental designs and the corresponding statistical methods to assess impacts. That is, analytical approaches to assess RA should not be based only on the number of practices combined, but also on the type of practices implemented and for how long.

Crop species and plant/animal produce to be considered on the experiments would be of course determined for the interest of the producers involved and/or the potential specific interest of FASCINATING. However, as previously presented, available data could be useful to guide the experimental design at least for the case of the most frequently reported species.

Regarding the results of the systematic review, the experimental design should also consider the temporal assessment of the practices incorporated. In particular, the design should consider periodic and long-term assessments (e.g. more than two years) to be able to capture the interaction between management practices and environmental conditions, and also the complementation of practices implemented sequentially over the year (e.g. cover crops and fertilization effects on the cash crop). Importantly, experiments should at least consider the assessment before and after the implementation of the practices. Other relevant factors should be also considered in some cases, for example different crops, genotypes, varieties, etc.

To better understand the mechanisms behind the effects of management practices on quality of plant and plant/animal produce it should be also useful to consider the jointly assessment of physicochemical and biodiversity indicators of soil health. Physicochemical indicators should include the soil type identification, and biodiversity indicators should consider not only diversity but also abundance and biological activity, the latter representing the link to the soil functions. Indicators for plant and plant/animal produces will of course depend on the focus of the research and the associated experiment, but it should be useful to combine the assessment of nutrient, taste and health indicators. Nutrient indicators should include not only mineral elements but also proximate composition. Similarly, health indicators should cover not only bioactive compounds but plant efficiency indicators and enzyme activities, and if possible, consider also indicators associate with human health such as soil borne pathogens or determinants of antibiotic resistance. The incorporation of taste indicators is also highly recommended as this type of indicators has been consider scarcely in previous studies.

Considering the magnitude of the proposal it would be highly recommended to define a **common set of indicators to be use in all experiments**. The specific indicators to be combined in this common set would depend on the resources available for the assessment. Collection of environmental data (e.g. soil and air temperature and moisture, mean annual precipitation) should be also considered.

Some final words:

Experimental designs in regenerative agriculture are strongly dependent on a variety of (contextual) factors that determine choices made. Important elements are:

- Purpose of the experiment? (from scientific with a global audience to locally relevant practical knowledge, or combinations).
- Financial means available? (this determines the number and types of indicators that can be included and the sampling design).
- Time period available? (this determines the characteristics of the temporal assessment, frequency, etc.).
- Field or produce focused? (some questions can be better answered in the field, other questions require laboratory assessments)
- Farmer participation? (experiments established and run by scientists or by farmers may require different designs and statistical methods for their analysis)

Based on our limited knowledge about the 100 hectare-experiments, a few options have been explored within the expert group. However, these can only be preliminary suggestions to start a dialogue. Based on the present knowledge of the expert group, a few options appear (among a long list of others).

Related to the present protocols "teeltaanpak", the idea behind these documents seems promising considering that it is closely related to pressing questions of farmers themselves. The farmers' choice for different scenarios opens the possibility to facilitate their learning path. Additionally, local testing on indicators can be adapted to the wishes of the farmers as long as there is a certain classification structure underlying the experiments. This would also create a basis for an issue that is less clear to us at this point. How will the chosen farm specific scenarios and developed experiences shared with other farmers that might be confronted with similar questions? Since there are also measurements done at the start of the experiment and follow ups have been planned, a system to disclose the results might be very useful for many reasons. Related to the underlying structure just mentioned, different indicators can be chosen for different scenarios and used to a kind of 'open-source' feedback system. Thirdly, the source of the present protocols is not directly clear to our experts. If not done yet, it might make sense to have a careful look if and how the different scenario suggestions are rooted in scientific literature summarized in this report. Embedding the choices in a scientific context, combined with the (field/produce) measurements done for different scenarios could lead to a very rich database that could be used to develop firmly rooted local knowledge about successful scenarios which could in turn lead to better established pathways. This way, the knowledge developed could not only spread among the farmers involved, but also communicate the value of the 100-hectare internationally to a broad audience.

Finally, this report points at a potential for the 100 hectare experiments for future research in general. The first results of the effects of RA seem positive. However, there are plenty of open questions to be answered. In that respect, research should focus on approaches that further establish the link between taste and plant health, between soil quality and taste, and ultimately the link between soil health, plant health, plant animal produce taste and human health. Combining these fields is also the core of the One health approach, the newest buzz word in aiming to merge environmental, agricultural and medical fields, and an important component of sustainable food systems. Several countries are trying to develop the concepts of One health, but are struggling with the multidisciplinarity of the topic. With the 100-ha project, Fascinating has the potential to set the tone from a practical and academic perspective.

Appendix A

Step 1 – Defining the strategy of the systematic review

Methodology

Weekly meetings were organized with the experts of the scientific research group to discuss three relevant points related to the overall strategy of the creation of this systematic review.

i) **Heterogeneity of RA definitions.** The concept of RA has developed over time and is interpreted in a variety of ways. A search strategy based on "regenerative agriculture" or "regenerative farming" would be useful to identify common principles, outcomes and practices of RA systems. However, the diverse ways in which RA is interpreted have consequences for the assessment of RA benefits on the soil quality, plant quality and food-produce quality in terms of nutrients, taste and secondary metabolites. Subsequently, it could affect the focus of our literature search strategies and alternative strategies should also be considered.

ii) **Practices are not exclusive of RA**. An alternative search strategy is to focus on farming management practices (for instance the principle of soil coverage can consist of mulching, cover crops or relay crops). By taking these farming management practices as a starting point of the literature search, the focus is much more on the activities of farmers, than on the rather abstract terminology of RA. Even more as practices of RA are also considered in other production systems (e.g. conservation and organic agriculture, agroecology), this alternative strategy can facilitate the collection of articles assessing practices in which the original RA terminology has not been used. In other words, articles that do not use RA as a reference point but do use similar practices would be included in this second strategy leading to a different set of articles in comparison to those recovered from the other search.

iii) **The possible list of keywords.** The alternative search strategy required the definition of a set of keywords. These keywords were used to execute preliminary searches on Scopus and Web of Science to determine the potential number of documents recovered and to refine the strategy that should be finally used for article collection (Appendix B).

Step 2 – Collection and selection of relevant articles

Methodology

The discussion about definitions of RA during **Step 1** highlighted the need to provide a method to disaggregate RA systems. This disaggregation requires to identify groups of RA systems using different combinations of practices (i.e. RA typologies) to improve the robustness analysis of the management practice effects on soil quality, plant quality and food-produce quality. In summary, considering advantages and disadvantages of the alternatives discussed, and taking into account preliminary searches on scientific databases, the expert's group decided to perform the literature review on Scopus and WoS databases, splitting the collection of articles in two parts (Figure A.1). The first part of the review was focus on "regenerative agriculture" (i.e. SR-A), while the second part was focus on "management practice" (i.e. SR-B). We expected than SR-A allows the identification of principles, outcomes and practices reported in regenerative agriculture literature, while the SR-B allows the identification of the most frequent combinations of practices assessed on the literature and the most relevant findings about their effects on soil, plant, and plant/animal produce quality. Both strategies would contribute to the identification of RA typologies.

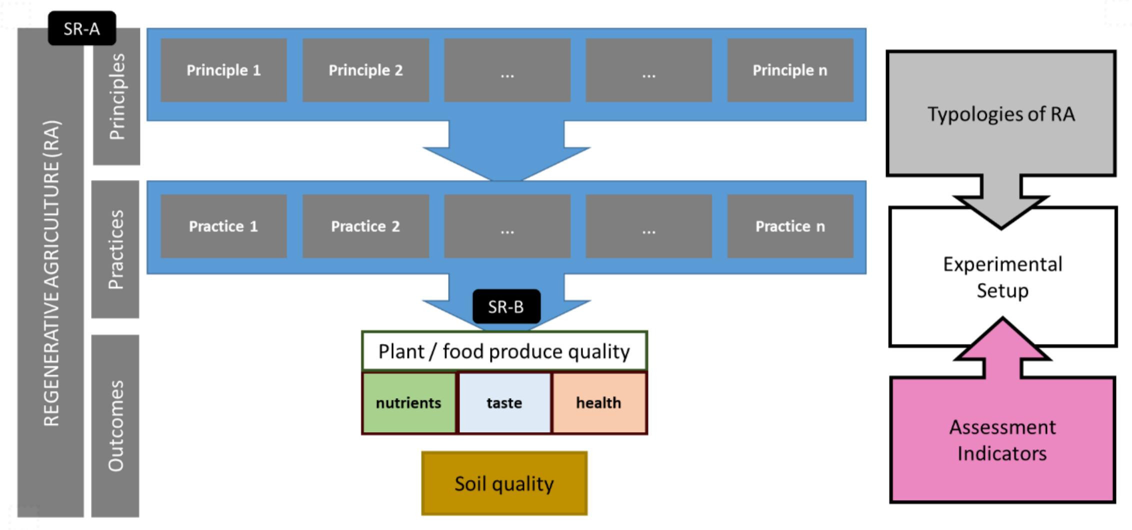


Figure A1. Schematic overview of the conceptual network behind the literature review.

The SR-A was performed on Scopus and WoS using the keywords "*regenerat* agricult**" OR "*regenerat* farm**", considering the *Title*, *Abstract* and *Keywords* fields. As the number of articles was higher than 800, the search was filtered focusing only on the *Title* field to be affordable in the period of this research. Articles recovered from this search were deduplicated and then articles that define regenerative agriculture using principles, outcomes and/or practices were identified and retained.

For the SR-B (alternative search), three blocks of keywords were defined, namely (a) the farming management practice, (b) the quality of the plant/food-produce, and (c) the target plant or food-produce of the search. For each of the three different blocks an associated list of keywords was defined. These keywords were cross-checked and improved by the experts. Preliminary searches on Scopus for the alternative strategy recovered a considerable number of articles (e.g. 2786). However, a general overview of the article titles of this preliminary search revealed a tendency of higher number of articles related to plant than to animal food-produce. After discussions and update of the list of keywords, the expert group decided to prioritize searches focus on management practices separately for plant and animal produce (Table A1). In both cases the search was performed on Scopus and WoS considering the three blocks of keywords covering management practices, quality and food produce (Table A1). The search was performed considering the *Title* field. Articles recovered from this search were deduplicated and used as input for selection of relevant articles assisted by ASreview. After selection articles with available full text pdf were retained, including two articles in Portuguese language. Full text articles available only in Chinese language were excluded.

Step 3 – Extraction data and analyses of relevant articles

Methodology

For the relevant articles recovered from SR-A, data extraction will be focused on the principles, practices and outcomes reported on each article, and then ranked according to their frequency.

For the relevant articles recovered from SR-B, data extraction was performed and organized based on a matrix of binary data covering three main topics:

i) *target of management practice effects*: Four main targets were considered, namely plant quality (or milk quality), yield & growth, soil quality & environment, and interrelationships. For the first three targets results reported in each article were categorized as positive, negative, neutral or ambiguous. Positive, negative and neutral categories were assigned when all or the majority of the evaluated variables were positively, negatively or not affected by the management practices assessed. Ambiguous category was assigned when results were positively, negatively or not affected depending on the variable considered or when the effects varied among years, species, cultivars, etc. A combination of categories was used when a low number of variables were assessed and they showed clear but differentiated trends depending on the variables.

ii) *assessed species/crop type*: All the species in which the effects of management practices were assessed where listed and then classified according to crop type, the main crop types assessed in each article were also registered.

iii) *assessed practices*: All management practices were listed and then regrouped. These groups were defined considering the practices recovered, the description of the practices reported in the articles and frequency of each practice in the database. For each article, the number of practices assessed, the condition in which practices were assessed (i.e. alone or in combination). For articles considering study designs combining management practices with warming, salinity or drought treatments, only results for management practices were recovered, but the use of the other treatment was also registered.

Additionally, the experimental approach (e.g. field, pot, laboratory assay), the soil texture and the percentage of sand/silt/clay of the soil were also extracted.

Frequency analysis was performed for these main topics. Additionally, for the third topic a hierarchical classification method was used to identify arrangement of practices based on the binary matrix of practices assessed.

Finally, to generate a summary table of the main findings, review articles available among relevant articles recovered from SR-A and SR-B were considered and combined with reference articles based on participant expert's knowledge. Then, for each article the main message, the principal outcomes, and the relevance for this project in terms of experimental setup and selection of indicator assessment were also presented.

Appendix B

Table A1. Complete list of keywords defined and tested during Step 1 of the stepwise methodological approach, considering prioritization of different blocks guiding the definition of the final blocks/keywords used for the SR-B in Step 2.

Block	Keywords	Priority
management practices	"reduced tillage" OR "non tillage" OR non-tillage OR zero-tillage OR "zero tillage" OR "direct seeding" OR "direct sowing" OR "minimum tillage" OR minimun-tillage OR "conservation tillage" OR "crop rotation" OR "crop diversity" OR "relay crop*" OR "perennial crop*" OR agroforestry OR "diversified rotation" OR intercrop* OR "crop residues" OR "cover crop*" OR "green cover" OR "green manure" OR "service plant*" OR "service crop*" OR "crop service*" OR mulch* OR "stubble retention" OR "overwinter stubble" OR leys OR "pasture crop*" OR "organic amendment*" OR compost* OR slurry OR digestate OR manure OR biostimulant OR bio-stimulant OR bioeffector OR "chicken litter" OR biochar OR biodigestate OR biofertilizer OR bio-fertilizer OR "mixed farming" OR "managed grazing" OR "intensive grazing" OR "silvopasture" OR "holistic grazing" OR "rotational grazing" OR "integrated grazing" OR "crop-livestock integration" OR "crop-livestock" OR "integrated livestock" OR "diverse pasture" OR "herb-rich pasture"	prioritized
productive systems	"cultivation system" OR "production system" OR "management system" OR "farming system" OR "agricultural system" OR "intensive system" OR "extensive system" OR agroecosystem OR "livestock system" OR "cropping system" OR "agronomic practice" OR "agricultural practice" OR "farming practice" OR "production practice" OR "management practice" OR "agroecological practice" OR "ecological practice" OR "integrated farming" OR "organic farming" OR "ecological farming" OR "conventional farming" OR "low-input farming" OR "intensive farming" OR "extensive farming" OR "inorganic farming" OR "agroecological farming" OR "regenerative farming" OR "integrated cultivation" OR "organic cultivation" OR "ecological cultivation" OR "conventional cultivation" OR "low-input cultivation" OR "intensive cultivation" OR "agroecological cultivation" OR "regenerative cultivation" OR "conservative cultivation" OR "integrated crop" OR "organic crop" OR "ecological crop" OR "conventional crop" OR "low-input crop" OR "agroecological crop" OR "integrated field" OR "organic field" OR "ecological field" OR "conventional field" OR "low-input field" OR "conservative field" OR "integrated agriculture" OR "organic agriculture" OR "ecological agriculture" OR "conventional agriculture" OR "low-input agriculture" OR "conservative agriculture" OR "regenerative agriculture" OR "agroecology" OR "agroecological agriculture" OR "agronomic management" OR "agroecological management" OR "ecological management" OR "conservative management" OR "organic management" OR "low-input management" OR "conventional management" OR "intensive management" OR "extensive management" OR "crop management" OR "horticulture management" OR "vegetable management" OR "livestock management" OR "soil management" OR "sustainable management" OR "fertility management" OR "diversified management" OR "agricultural diversification" OR "fertilization management" OR "fertilisation management"	non-prioritized
plant produce quality	nutritional* OR phytochemical* OR nutraceutic* OR biochemic* OR antioxidant* OR "proximate composition" OR phenolic* OR bioactive* OR "secondary compound*" OR "secondary metabolite*" OR anthocyan* OR caroten* OR polyphenol* OR (phenol* AND NOT phenology) OR flavon* OR "essential oil*" OR "fatty acid*" OR tannin* OR caffein* OR lycopen* OR "ascorbic acid*" OR vitamin* OR "trace element*" OR macroelement* OR microelement* OR "sensorial attribute*" OR "sensorial propert*" OR metabolom* OR volatilom* OR microbiom* OR microorganism* OR bacter* OR fung* OR carbohydrat* OR chemometric* OR "specialized metabolite*" OR terpene* OR digestibility OR protein* OR amino-acid* OR "amino acid*" OR taste OR endophyte* OR "plant health" OR "plant fitness" OR "shelf life" OR toxin* OR nitrate OR pathogen* OR "antimicrobial resistance" OR "antibacterial resistance" OR lignin	prioritized

animal produce quality	nutritional* OR phytochemical* OR nutraceutic* OR biochemic* OR antioxidant* OR "proximate composition" OR phenolic* OR bioactive* OR "secondary compound*" OR "secondary metabolite*" OR anthocyan* OR caroten* OR polyphenol* OR (phenol* AND NOT phenology) OR flavon* OR "essential oil*" OR "fatty acid*" OR tannin* OR caffein* OR lycopen* OR "ascorbic acid*" OR vitamin* OR "trace element*" OR macroelement* OR microelement* OR "sensorial attribute*" OR "sensorial propert*" OR metabolom* OR volatilom* OR microbiom* OR microorganism* OR bacter* OR fung* OR carbohydrat* OR chemometric* OR "specialized metabolite*" OR terpene* OR digestibility OR protein* OR amino-acid* OR "amino acid*" OR taste OR endophyte* OR "plant health" OR "plant fitness" OR "shelf life" OR toxin* OR nitrate OR pathogen* OR "antimicrobial resistance" OR "antibacterial resistance" OR lignin OR omega-3 OR omega-6 OR "conjugated linoleic" OR "alpha linolenic" OR "Eicosapentaenoic" OR "Docosapentaenoic" OR "Docosahexaenoic" OR polyunsaturated OR monounsaturated OR PUFA or MUFA	prioritized
plant produce	food* OR fruit* OR vegetable* OR grain*	prioritized
animal produce	milk	prioritized
soil quality	nutrient* OR biolog* OR functional* OR chemic* OR mineral* OR physicochemic* OR biochemic* OR enzyme OR respiration OR "trace element*" OR macroelement* OR macro-element OR microelement* OR micro-element* OR "soil organic carbon" OR SOC OR "particulate organic carbon" OR POC OR "mineral-associated organic carbon" OR "mineral associated organic carbon" OR MAOC OR "soil organic matter" OR SOM OR "total carbon" OR "total nitrogen" OR nitrate OR "mineral nitrogen" OR phosphorus OR pH OR "electric conductivity" OR density OR diversity OR biodiversity OR bio-diversity OR metabolom* OR volatilom* OR microbiom* OR microorganism* OR micro-organism* OR bacter* OR fung* OR fauna OR biota OR microb* OR "food web*" OR food-web* OR nematode OR protoz* OR mite* OR springtail* OR *worm OR protist* OR *arthropod OR rhizob* OR mycorrhiz* OR fatty-acid* OR "fatty acid*" OR "mineral associated organic matter" OR MAOM OR "particulate organic matter" OR POM OR "aggregate stability" OR porosity OR pore* OR "penetration resistance" OR "bulk density" OR "soil temperature" OR "soil structure" OR "water conductivity" OR "water infiltration" OR "water holding capacity" OR "field capacity" OR "permanent wilting point" OR "water retention" OR "water storage" OR drainage OR "soil redox" OR "redox potential" OR "soil-borne diseases" OR "nutrient bioavailability" OR "nutrient bio-availability" OR "root exudates"	non-prioritized
soil	soil OR sub-soil OR top-soil OR rhizosphere	non-prioritized

Appendix C

Fourteen groups of agronomical practices identified and ordered according to frequency:

1. No/Minimum Tillage (11/18): No-till; Minimized till; Minimum soil disturbance
2. Intercropping, Interseeding, Integration of Perennials, Hedgerows (10/18): Integration of perennials; Increase and maintain land with native vegetation; Intercropping, inteseeding, hedgerows; Deep-rooted perennials to draw and store water deep within the land; Intercropping; Intercropping, perennials, restore natural habitats; Promote natural complexity (considering contextual capacity); Maximized diversity (not only crop).
3. Cover Crops/Residue Mulch (10/18): Cover crops; Cover crops or Resident vegetation; Multispecies cover crops and residue retention; Multispecies cover crops; Cover crops and mulching; Cover crops, green manures, mulching; Vegetative soil cover; Soil cover.
4. Mixed Farming (Animals, Trees, Pasture, Crops) (9/18): Silvoarable systems, Silvopasture; Cropland grazing; Appropriate cropping integrated with animal enterprises; Integration of crops with trees and livestock; Integrate livestock; Pasture cropping; Mixed farming.
5. Reduction/Elimination of Pesticides and Herbicides (8/18): Reduce/eliminate pesticide and herbicide use; Reduce/eliminate synthetic agrichemicals; Reduce synthetic inputs; Eliminate pesticides use; Avoid artificial inputs; Minimize synthetic agrochemicals; Minimize external inputs.
6. Reduction/Elimination of Fertilizer Use (8/18): Reduce/eliminate fertilizer use; Reduce/eliminate synthetic agrichemicals; Reduce synthetic inputs; Integrated nutrient management; Eliminate synthetic fertilizers; Avoid artificial inputs; Minimize synthetic agrochemicals; Minimize external inputs
7. Diverse Crop and Animal Rotations (8/18): Diverse crop rotations; Progressive biological sequencing (rotation crop, pasture, grazing); Complex rotation (crop, pasture, grazing)
8. Compost/Manure/Integrated Nutrient Management (8/18): Organic ammendments (compost, manure, mulch, compost teas); Enhance nitrogen fixation; Compost and biostimulants; Organic fertilizers, compost, mulch, green manure, or crop residues; Compost, green manure; Manure and compost; Nutrient ammendments (they include inorganic); Organic amendment
9. Managed Grazing (5/18): high-intensity, short-duration time-controlled with frequent rotation of livestock between small paddocks with perennial native grasses (i.e., cell grazing) and long rest periods; Multi-paddock grazing systems, field rest following the punctuated disturbance of grazing; Progressive biological sequencing (rotation); rotational grazing; Grazed corn field.
10. Agroforestry (4/18): alley cropping, contour hedgerow, forest farming, living fence, multistory cropping, riparian forest buffer, silvoarable systems, silvopasture, and windbreak; contour hedgerows; Integration of crops with trees and livestock; Integration of trees
11. Biological Pest and Disease Control/Integrated Pest Management (4/18): Integrated pest management (resistant or tolerant species, rotations, management of crop residues, encourage natural predators); Pest-resilient food systems; Use natural pest control
12. Recuperation Periods (Fallow) (3/18): Long rest periods after grazing; Adaptive use and rest
13. Reduction/Elimination of Supplementary Feeding (2/18): Reduce/eliminate supplementary feeding; Minimize external inputs
14. Water Regulation and Purification (2/18): Grass water ways; Control of daily water cycle and hydrology