On the way to Regenerative Agriculture: indicators for measuring the state of regeneration of Dutch soils

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Abstract

Regenerative Agriculture (RA) is a relatively new transformative pathway for farmers to adapt to more sustainable principles while interlinking the practices they adhere to into the broader concept of sustainability in social, economic as well as environmental dimensions. How to make the effects of RA practices insightful remains to be defined. Therefore the aim of this research was to collect insight or evidence to indicators suitable for gaining a perspective on the state of the soil in scientific contexts linked to soil health and quality as well as a RA context. A method of systematic review of scientific databases was adapted to identify scientific, peerreviewed records on soil health or quality assessments as well as soil assessments in relatively new pool of RA science. The used indicators in these records were included in the results, showing a total of 40 indicators used in both soil health or quality assessments and assessments of the soil in RA-related research. These indicators were linked to themes in soil health as set up by the broader RA transition project ReGeNL. Discussion shows that RA literature follows usage of indicators as has been done in soil health and quality assessments. Furthermore, indicators were lacking to describe the theme of pest and disease suppression. Soil biodiversity and habitat provision indicators are underrepresented in literature. For continuation of the project and helping farmers adapt to RA it is advised to determine indicators and practices to use in future assessments in a bottom-up approach.

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Introduction

Need for sustainable farming

Europewide there has been a strong call for more future-proof agricultural frameworks to ensure food quality and cope with environmental problems. Initiatives such as the European Green Deal, which includes the Farm to Fork Strategy and the European Biodiversity Strategy, underline this call (European Commission, 2020a, 2020b). Closely linked to the Green Deal and interwoven with both strategies lies the EU Soil strategy, which emphasizes the need for healthy soils to battle degradation, greenhouse gas emissions, loss of nutrients and biodiversity loss (European Commission, 2021).

A central stakeholder group affected by, or responsible for achieving the European goals are farmers. 46.4% of the total land area of the EU is managed by this stakeholder group (Eurostat, 2022). In general, farmers or agricultural businesses are categorized into different farming systems. Conventional agriculture, circular agriculture, regenerative agriculture, organic agriculture, sustainable intensification and conservation agriculture are all examples of farming systems adhering different sets of principles that can sometimes be overlapping (Giller et al., 2015; Schreefel et al., 2020; Sumberg & Giller, 2022; Walthall et al., 2024). Whether or not a certain farming system, with corresponding practices, is adhered to by farmers has a major effect on the eventual goals that are outlined by the EU in different strategies and the Green Deal. Therefore, mapping out the effects of farming practices on different goals is crucial for safeguarding the right legislation and moving towards a just sustainable farming framework.

The Regenerative Narrative

Regenerative Agriculture (RA) has received more attention in recent years as a system or framework that is promising to be environmentally sustainable, socially just and economically viable (Giller et al., 2021; Jayasinghe et al., 2023; Schreefel et al., 2020; Walthall et al., 2024). The three dimensions (environmental, social and economic) are also referred to as dimensions of sustainability (Purvis et al., 2019). It is theorized that balancing out these three dimensions will lead to a state of sustainable management.

A comprehensive definition of RA has been described by Schreefel et al. (2020), wherein soil conservation is the entry point for regenerating ecosystem services that eventually will enhance environmental, social and economic dimensions. Even though there is a discourse on what RA exactly entails, there are basic sets of rules that can be pointed out as regenerative practices which can be used to join the regenerative movement (Gordon et al., 2023; Schreefel et al., 2020). Farmers that want to join the RA movement generally adhere to Conservation Agriculture principles such as no- or minimum-tillage and permanent soil cover, however also grazing regimes and agroforestry can be added as measures to improve the farming system mainly improving the state and health of the soil (Jayasinghe et al., 2023).

RA as a farming system is the entry point for a transformative pathway, wherein the goal is to shift farmers from conventional systems to a sustainable system. This shift comes forward in the ReGeNL project, a Public-Private Partnership to which context this research is executed. The aim of the project is to present a regenerative farming system for the Dutch agricultural sector to improve the state of the soil, maintain economic perspectives and be socially supported. For supporting this shift in farming system, a matrix is developed and evolving in which the

practices, results and outcomes are leading. Indicators can be used to track the different aspects in this matrix.

Soil health & Indicators

Soil health plays a key role in assessing the sustainability of farming systems because a healthy soil is able to function as a vital living system to sustain both plant and animal productivity and health while also maintaining water and air quality (*Soil Health Benchmarks*, 2024). As soil health is a complex system including biological, physical and chemical properties that altogether make up the soil environment. A collection of measurements or metrics on the three different properties can together make up a proximate for the overall state of the soil (Biswas et al., 2014; Sprunger & Martin, 2023). Since the soil environment is not characterizable by a single metric, the challenge lies in interpreting different indicators that explain properties altogether making up the soil environment. In this sense physical and chemical properties are more straightforward to measure and interpret while soil biological properties struggle with an underrepresentation in soil assessments (Bunemann et al., 2018; Van Leeuwen et al., 2017; Zwetsloot et al., 2022).

Likewise soil health, sustainability is a complex concept and therefore too made insightful by the use of indices or assessments. Frameworks set up for measuring sustainability are general and do not per definition fit to specific needs or situations of the end-user (De Olde et al., 2016; Soulé et al., 2021). Aggregation of different indicators subsequently delivers an index. Indicators themselves are quantitative with for example soil pH, soil texture, soil organic carbon content or soil microbial biomass; however also presence or absence of ground cover or presence or absence of a tillage plan can be used as more general indicators.

As pointed out by the study of Soulé et al. (2021) scholars have been talking about *'indicator explosions'* or *'zoos of indicators'*, showing that the science behind indicators is vast and selection of the right ones is a task in itself. A much used categorization of indicators in different contexts are Key Performance Indicators (KPI's) which are quantifiable and time-bound indicators to monitor the performance of a change in settings, requiring a before set target to see the indicator move towards (Domínguez et al., 2019). Another way of categorizing indicators is by basing them on target, practice, result or outcome and in this way linking interventions to a quantifiable unit (Schreefel et al., 2023).

Problem statement

A more future-proof farming system is rewuired in order to regenerate European soils, improve biodiversity, deliver healthy food and enable farmers to still make a livelihood. The RA movement is a promising way to follow when we want to move towards this state of being. It is up to farmers to implement the principles. The ReGeNL project is an entry point for understanding RA, and subsequently can help farmers adhere to the regenerative principles.

As being said that RA as a defined system is under discourse, research on this farming system is necessary to contextualize what it implies in specific situations. Besides the implementation of practices linked to RA, the following task is to make insightful how implementation actually regenerates the system. To this end the right indicators should be collected and contextualized in order to not only understand and help stakeholders make informed decisions, but also to prevent co-optation and greenwashing of the term RA.

Research aim

In this thesis the provided definition of RA by Schreefel et al. (2020) was used as a starting point. The main area of interest lies in the environmental aspects of the soil under RA, rather than social and economic dimensions. Therefore, the main question within this research is stated as: *"What indicators are most suitable to acquire a perspective on the state of regeneration of the soil in a Dutch farm-level context?"*

Two research sub-questions aiming to understand underlying themes in this question were formulated.

SQ1: 'What indicators are usable in a Dutch farm-level context for measuring soil health?'

SQ2: 'What soil indicators can be linked to a Regenerative Agriculture context?'

Altogether, the aim of this research was to provide insight on indicators used in soil health assessments and indicators used to assess the effects of RA on soil health to be able to find synergies and advice for further research.

Material and Methods

Search strategy

Previous literature has reported on large numbers of indicators used for both soil health and RA indicators. Therefore, to guide ourselves through this 'zoo of indicators' a systematic literature review using the Preferred Reporting Items System for Systematic Review (PRISMA-P) tailored to the agricultural sciences was identified as a suitable approach. (Koutsos et al., 2019; Page et al., 2021). This method follows a structural framework to guide through the phases of scoping, planning, identification, screening, assessment and presentation. Databases found eligible for the literature search were Scopus and Web of Science (WoS) because of the peer-reviewed content of these databases and the provided access during the research period.

The general description of the systematic review started with the (1) scoping phase in which the questions were phrased, and a preliminary literature search was executed. Here the aim was to find meta-analyses or systematic reviews to see what themes have already been researched and find keywords for this research. Followed in the (2) planning phase, the search queries were set up using Boolean operators for in- or exclusion of specific words that were found in the literature. In the (3) identification phase (the start of flowcharts in Figure 1, Figure 2 and Figure 3) the search query was given into the respective database, what resulted in a pool of literature to which automation screening was done. With this automation screening, the pool of literature was limited for the first time. Subsequently the (4) screening phase followed during which duplicates from both databases were removed. Within the screening followed (4a) assessment which consisted of screening titles and abstracts of the pool of literature and testing them to eligibility criteria as depicted in the flow-out during screening in Figure 1, Figure 2 and Figure 3. If a source was found ineligible, it was excluded from further consideration. With the remaining included literature, a strength of evidence assessment was conducted following the strength of evidence classification in Table 1. Note that in this research, as opposed to the framework, The Low and Very Low categories were merged due to low numbers of records in these categories. The literature found eligible and included in the eventual (5) presentation and interpretation phase were all in the High strength of evidence category. The last phase consisted of reading all literature and filtering out the indicators used in the research. The corresponding reference

databases to each executed search were saved in the form of .BibTex files in the supporting folder 'Review process' > 'SQ1'/'SQ2' > 'References' > 'search_1'/'search_2'.

Strength of evidence	Mode of research to include
High	Systematic reviews, Meta-Analyses, Experiments, Field trials
Moderate	Narrative reviews, Case studies, Simulations
Low	Qualitative research, Opinion papers, Reviews with inconclusive results

The manual filtering of indicators focussed on quantifiable indicators and author implied indicators. Indicators encountered were scored for how often they were used in the total set of included records. To each search query the review method and filtering of indicators was gone through once. This resulted in two indicator databases which were compared for duplicates in order to find the relevant connection between soil health and RA research. The list of duplicates was the final list of indicators used for further investigation and discussion of the insight they provide, based on the records they were retrieved from.

The eventual indicator list investigated was linked to the ReGeNL matrix outcomes set up for soil health. The definitions of outcomes as used in ReGeNL are listed in *Table 2*. Investigation of the use of each indicator in the reviewed literature lead to discussion over the depth of insight the indicator has on the matrix outcomes. In the broader project, the work of Brouwers (2025) aimed to link management practices of RA to these outcomes.

Table 2 Section of the ReGeNL Regenerative Agriculture matrix considering soil health and sub-outcomes with definitions to each

1	Soil Health	Soil health is the ability of a soil, at a specified point in time, to function as a vital living system, within natural or managed ecosystem boundaries and land-use boundaries, to sustain plant and animal productivity and health, maintain or enhance water and air quality and to further provide ecosystem services in the long-term without (increased) trade-offs between ecosystem services.
1.1	Nutrient Cycling	Nutrient Cycling is defined as the capacity of a soil to take up and recycle nutrients from different inputs (e.g., plant residues, manure) and to support the uptake of nutrients from soil minerals and organic matter, water and air by plants and the soil community.
1.2	Carbon and Climate Regulation	Carbon and climate regulation includes soil processes that contribute to the retention of carbon in the soil and regulate the release of major greenhouse gases. The function can be defined by three main groups of processes which take place in the soil: (1) Decomposition; (2) Biochemical transformation and (3) Resource reallocation
1.3	Structure and water regulation	The capacity of the soil to receive, store and conduct water for subsequent use and to prevent droughts, flooding and erosion.
1.4	Pest and Disease Management	The disease and pest management function represents the capacity of soils to prevent the establishment and development of soil-borne plant pathogens and pests despite their presence in the field, the availability of a susceptible plant host, and a suitable environment.

1.5	Soil Biodiversity	Soil biodiversity refers to the variability of living organisms in
	and Habitat	soil and the ecological complexes of which they are part; this
	Provision	includes taxonomic and functional diversity within species,
		between species and of ecosystems. Habitat provision refers to
		the capacity of soil to create and sustain suitable habitats for a
		wide range of organisms, including microorganisms, plants, and
		animals. It encompasses the physical, chemical, and biological
		characteristics of the soil environment that enable the
		establishment and maintenance of diverse communities.

Soil health indicators

The first research question was focussed on finding soil health indicators suitable for use in a Dutch farming context. The search for keywords resulted in the finding that 'soil health' and 'soil quality' are, or have been used interchangeably in research. Secondly, the word 'indicator' has a set of synonyms such as *metric, measurement, index, benchmark*. Subsequently, the research question was broken down into object (indicator), theme (soil health), geography (The Netherlands) and context (agriculture). For agriculture, the word itself and farming types (livestock or arable) were considered. In a first search attempt, the search term 'The Netherlands' was included in the query. The result of this search was zero. Thus, alternative search terms were set up for the geographical context. This resulted in setting up a first search query considering soil and climate keywords. Subsequently a second search query considering Europe as geographical limitation was used. This resulted in the following search queries:

For search 1:

(indicator OR metric OR measurement OR index OR benchmark) AND ("soil health" OR "soil quality") AND (sand OR clay OR peat OR loam OR temperate) AND (arable OR livestock OR agriculture)

For search 2:

(indicator **OR** metric **OR** measurement **OR** index **OR** benchmark) **AND** ("soil health" **OR** "soil quality") **AND** europe **AND** (arable **OR** livestock **OR** agriculture)

Using these search queries the identification phase started after which exclusion of records was performed. The excluded records per phase are depicted for search 1 and search 2 in *Figure 1* and *Figure 2* respectively. A first refining using automation screening was executed by screening the literature for the keyword field using the search term 'indicator'. After screening titles of thus far included literature, the exclusion criteria geographical context and agricultural context were set up. For the geographical context, the Köppen climate classification as a reference, excluding records set in non-temperate climates. Records that do not have an agricultural context were excluded from the review. What followed was the strength of evidence assessment of the mode of research in the records, Moderate and Low assessed records were excluded from the analysis for indicator review. However, they remain identified to be included in future research. In the presentation and interpretation phase, the records were read; summarized for mode of research aim; and the used indicators were listed. Comparing the outcomes of both searches, 4 duplicates were found resulting in an overall set of 47 records.

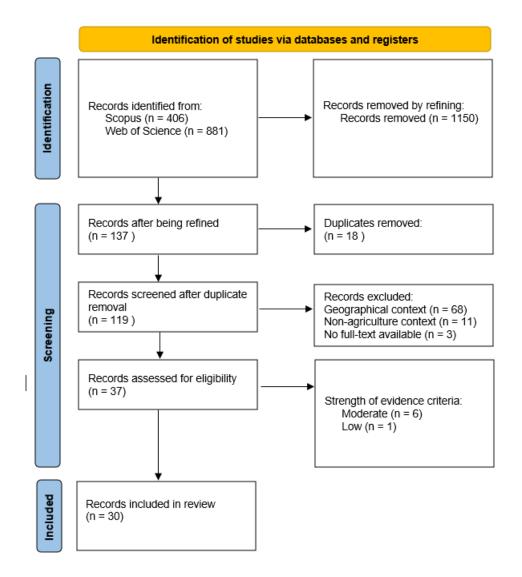


Figure 1 Systematic review process flowing through identification phase using the search term(indicator OR metric OR measurement OR index OR benchmark) AND ("soil health" OR "soil quality") AND (sand OR clay OR peat OR loam OR temperate) AND (arable OR livestock OR agriculture) in web databases Scopus and Web of Science. Screening phase consisting of exclusion by criteria and strength of evidence.

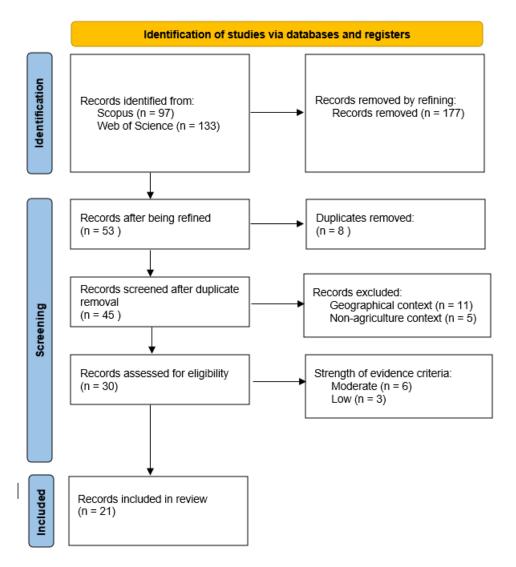


Figure 2 Systematic review process flowing through identification phase using the search term (indicator OR metric OR measurement OR index OR benchmark) AND ("soil health" OR "soil quality") AND europe AND (arable OR livestock OR agriculture) in web databases Scopus and Web of Science. Screening phase consisting of exclusion by criteria and strength of evidence.

RA indicators

After investigating the first research question, the second followed with RA as the central theme. The scoping brought forward a systematic literature review that asked a comparable question as this thesis: "What are the commonly used indicators, frameworks, tools, and models that can be employed to evaluate the biophysical and economic outcomes of RA?" Jayasinghe et al. (2023). This review had a broader focus, including frameworks, tools, models and economic dimension.

The research question was broken down to the object (indicators), theme (RA), and context (soil or environment). The search for keywords in the scoping resulted in the inclusion of wildcards to the terms 'Regenerative Agriculture' and 'Regenerative farming' which enabled the database to include records containing differently spelled terms. This resulted in the following search query:

(indicator **OR** metric, **OR** measurement **OR** index **OR** benchmark) **AND** ("regen* ag*" **OR** "regen* farm*") **AND** (soil **OR** environment)

Using this search query the identification phase started after which exclusion of records was possible. The excluded records per phase are depicted in *Figure 3*. An attempt was made for refining literature using automation screening. The attempts limited the records to sets of records below 10. Therefore, no refining using automation screening was done. Exclusion criteria were set-up after screening titles for themes coming forward. To this end records without agricultural context, RA-context, environmental-RA-context or full-text availability are excluded from review. During the reviewing two other records were found to be unrelated to the question posed and separately excluded from reviewing. Last, the strength of evidence assessment was conducted resulting in an exclusion of moderate and low evidence records, which remain identified for discussion in future research. In the interpretation phase, the records were listed and investigated for their use in records.

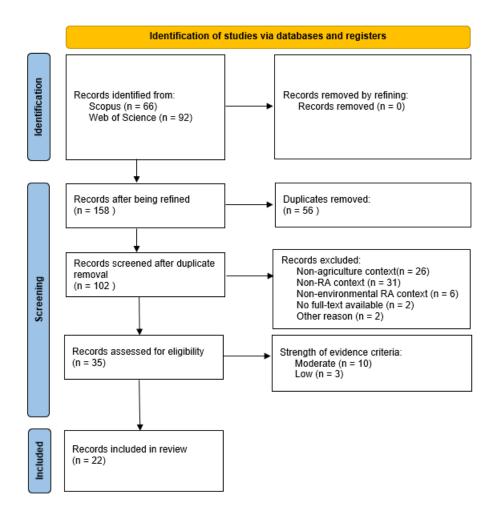


Figure 3 Systematic review process flowing through identification phase using the search term (indicator OR metric, OR measurement OR index OR benchmark) AND ("regen* ag*" OR "regen* farm*") AND (soil OR environment) in web databases Screening phase consisting of exclusion by criteria and strength of evidence.

Results & Discussion

Indicators in literature

From the included 47 records for the search for soil health indicators, 12 did not make use of indicators or were descriptions of methodologies. Therefore, these records were set aside to be used for later discussion. Themes of these records are Visual Soil Examination and Evaluation (VSEE) techniques, individual multi-criteria indices, Visual Near Infrared (VIS-NIR) spectroscopy, Soil Quality Index, the MicroResp[™] method, species specific indicators and a report on soil compaction. The rest of the 35 records were read and indicators used in the research are added to the list, resulting in 101 indicators.

For the included 22 reports for RA indicators, two records did not make use of indicators and one report consisted of a literature review on biophysical and economic indicators for RA. This record was excluded from first analysis to be used later for comparison. From the remaining 19 records, the eventual RA indicator list was composed, resulting in a total of 61 indicators.

The two lists of indicators retrieved from the 54 records were screened for duplicates. Resulting in a list of 40 indicators shown in *Figure 4*. Indicators are here categorized to their corresponding dimensions of soil health. A separate category is made for soil carbon indicators, as this indicator relates to all three dimensions (biological, physical and chemical) depending on the interpretation of the indicator. The most prevalent indicators were linked to the themes in the ReGeNL matrix and visualized in *Table 3*.

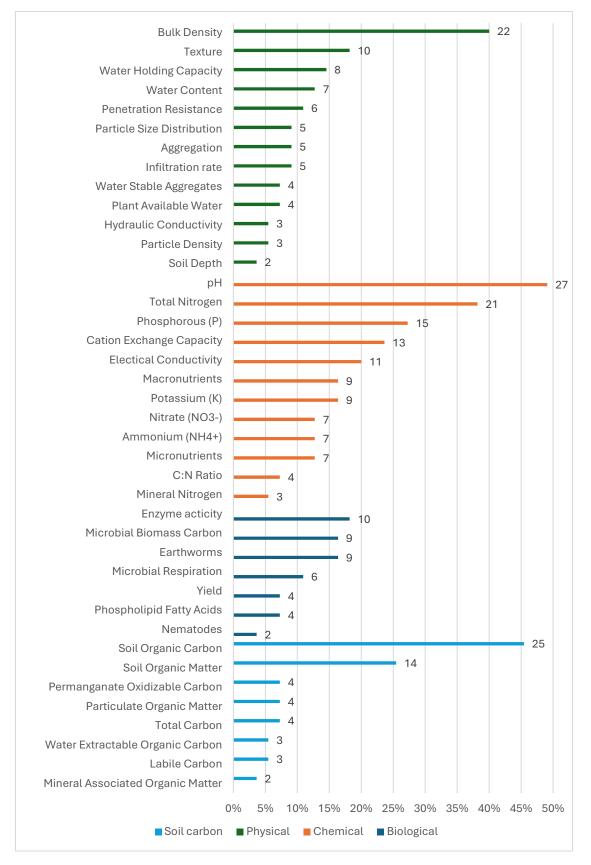


Figure 4 Soil indicators collected in literature review depicted in relative presence in records with corresponding absolute values per indicator with a total amount of analyzed records of n=54. Categorized to the soil health dimensions (physical, chemical, biological) with addition of soil carbon as a category.

Physical indicators

In total 10 indicators from the analyzed records have been categorized as physical. The most notable indicator, based on their occurrence in this review, is Bulk Density (BD) (n=22), followed by texture (n=10). Other physical indicators have an occurrence in less than 10 records.

Bulk Density, Infiltration, Aggregation & Penetration resistance

BD is generally considered as an indicator for water regulation, aeration and compaction (Adeli et al., 2018; Bolat, 2019; Do Nascimento et al., 2019). In this case, the higher the value of BD, the less pore space available, the less air and water the soil can hold and the more compacted the soil is. BD is found to be correlated negatively to soil organic matter and soil organic carbon (Koorneef et al., 2024; Xu et al., 2022). Soils with low BD are therefore expected to show higher values of soil organic matter and soil organic carbon. This can be partly referred back to the physical indicator Aggregation, since stable aggregates retain particulate organic matter in the soil. This follows too for infiltration rate, as infiltration rate is seen to increase with higher soil carbon levels and positive relation is seen between infiltration rate and aggregate stability (Adeli et al., 2018; Daverkosen et al., 2022). Similarly to BD, penetration resistance is seen to be negatively correlated to soil carbon (Adeli et al., 2018).

Linking the back to the ReGeNL matrix, BD can be linked to theme 1.2 Carbon and climate regulation and 1.3 Structure and water regulation. Infiltration is more linked to theme 1.3 Structure and water regulation, however could also explain the aggregation and carbon content in the soil. Aggregation as an indicator relates more to theme 1.2 Carbon and climate regulation, however also has its explanatory value within structure, thus theme 1.3 Structure and water regulation. BD in this case could be a general indicator to the themes.

In a RA context, the participatory approach by Lujan Soto et al. (2020) suggests BD to be measured in combination with visual field observations such as puddles or anomalies in soil vegetation cover to find whether the local problem is explained by the indicator. Since BD is related to soil organic carbon and aggregation, but provides insight in water capacity too, the indicator could be used as an early indicator following from observational investigation with either aggregation or infiltration measurements following to relate the problem to either soil carbon or issues with compaction.

Practices or management schemes that are brought in relation to BD, aggregation and infiltration coming forward in the literature included this review are tillage, cover crops & crop diversity, organic amendments and livestock integration. Wherein the stated effects of minimum tillage were generally showing more improvement in BD than no-tillage.

Texture, Particle Size Distribution

The use of soil texture is often stated to be within the range of indicators investigated for research. In their review Bunemann et al. (2018) found that texture is used in over 40% of soil health or quality assessments (n=65). Its use is mainly to distinguish the soil type classification using the sand, silt and clay fractions of the soil. In case of subsequent analysis, most records use the clay content of the soil as part of soil texture. A general statement for clay fraction was found in *"more is better"* (Juhos et al., 2019). Clay fraction is related to properties like soil structure, nutrient cycling, water dynamics (Do Nascimento et al., 2019; Juhos et al., 2019; Matos et al., 2023). As a response indicator Bartley et al. (2022) state that the effects of managerial changes on texture could be seen in 7 to 12 years after implementation. Under long

term soil cover, differences in texture are observed, probably affected by runoff and soil erosion (Andrés et al., 2022).

Records using Particle Size Distribution (PSD) in this review generally were inconclusive on PSD as an indicator. The methods used to retrieve PSD were mainly used to subsequently classify soil texture. In a farm-level context therefore, the use of VSEE techniques for determining soil texture is sufficient, since PSD values need more sophisticated methods of retrieving the indicator.

Chemical indicators

The overall most used indicators are in the chemical categorization, with pH (n=27), (Total) Nitrogen (n=21), Phosphorous (n=15), Cation Exchange Capacity (CEC) (n=13) and Electrical conductivity (EC) (n=11). Notable is that nitrogen is more referred to in soil health assessments (n=14) than in RA related research (n=7). While the amount of records using the other indicators is more balanced between soil health and RA research.

The indicator 'macronutrients' is often referred to as 'other macronutrients' or 'other cations'. In some cases macronutrients has been broken down to each separate cation as an indicator. Essentially nitrogen and phosphorous are part of this group of nutrients, however are often stated to be used as separate indicators. Logically, macro- and micronutrients are linkable to theme 1.1Nutrient cycling in the ReGeNL matrix.

pН

As the most discussed indicator in the category chemical indicators, pH came forward in more soil health related research records (n=16) as compared to RA-related research (n=11). Several records stated pH to be an important indicator showing fast responses to organic amendments (Lekberg et al., 2024; Ponnusamy et al., 2024; Valarini et al., 2003). Site specific conditions such as parent material, climate or management type determine optimum values of pH (Bai et al., 2018). Overall, pH or soil acidity plays an important role in nutrient availability, as well as indicated to have a prediction power to microbial community (Andrés et al., 2022; Harvey et al., 2019; Juhos et al., 2019; Lujan Soto et al., 2020). This linked pH to ReGeNL matrix theme 1.1 Nutrient cycling and theme 1.5 Soil biodiversity and habitat provision. The review by Bunemann et al. (2018) stated that pH is often mentioned to underlie suppressiveness, though without validation. This could link pH to theme 1.4 Pest and disease management, however further research will be needed.

Nitrogen

Nitrogen (Total nitrogen and mineral nitrogen) as an indicator was used more in soil health related records (n=15) than in RA- related records (n=7). The RA related records were diverse in conclusiveness based on nitrogen. Firstly, Andrés et al. (2022) mentioned nitrogen to play an important role together with other cations in soil biodiversity and stated that high nitrogen levels favor soil predators. Montgomery et al. (2022) aggregates nitrogen together with other indicators into the Haney soil health score that gauges microbial activity. Xu et al. (2022) presented strong negative correlations between bulk density and Total Kjeldahl Nitrogen; strong positive correlation of Total Kjeldahl Nitrogen with organic matter and mean water holding capacity; and soil protein and nitrogen showed strong correlation since nitrogen is bound in soil protein. Mahmud et al. (2024) presented in-field infrared methods to estimate nitrogen levels, for supporting change to RA especially considering nitrogen to be important for plant growth and

microbial activity. For soil health related research the same patterns of broad interpretation of nitrogen were be observed. Adding on to (total) nitrogen, the soil nitrogen pool also contains ammonium, nitrate and mineral nitrogen which provide their insights in acidity, nutrient availability and biological activity in the form of nitrification and denitrification.

This makes nitrogen perhaps the most insightful chemical indicator as it could link back to ReGeNL themes 1.1 Nutrient cycling, 1.3 Structure and water regulation and 1.5 Soil biodiversity and habitat provision.

Cation Exchange Capacity

Cation Exchange Capacity (CEC) is a measure for the capacity of soils to retain and exchange cations (Koorneef et al., 2024). Soil health related research (n=7) linked CEC to soil fertility, increased enzymatic activity, tillage practices or cover cropping (Mijangos et al., 2010; Valarini et al., 2003; Wulanningtyas et al., 2021). The RA-related research (n=6) links CEC to soil organic carbon or soil organic matter levels, showing strong correlations (Baker et al., 2022; Lekberg et al., 2024; Xu et al., 2022). The participatory approach of Lujan Soto et al. (2020) indicate CEC to enable fast quantitative insight in the qualitative observations farmers have over their crops performance. The main theme in the ReGeNL matrix to which CEC relates was theme 1.1 Nutrient cycling and the strong correlations with soil organic matter linked it to theme 1.2 Climate and carbon regulation as well.

Electrical Conductivity

Electrical Conductivity (EC) as an indicator in RA-related research (n=6) was used inconclusively by four records. The remaining records concluded contradictory to the effect of alternative versus conventional management on EC (Andrés et al., 2022; Ponnusamy et al., 2024). Soil health related records were more conclusive on EC as an indicator. Bunemann et al. (2018) stated that among the chemical indicators, EC was one of the more frequently used and that it could support the strength of Visual Near Infrared (VIS-NIR) measurements of soil properties. Using organic amendments as a treatment, Valarini et al. (2003) saw a short term (3 month) increase of EC after addition of organic matter and microorganisms, attributed to organic matter mineralization. In soils that have been affected by flooding conditions, EC was also seen to be higher, expected to be caused by higher salinity and less plant nutrient uptake (Harvey et al., 2019). Another record integrated EC in a nutrient index together with pH, nitrogen & phosphorous content, exchangeable calcium & magnesium and bioavailable copper, zinc, iron and manganese (Vanino et al., 2022). This seems to be in accordance with Juhos et al. (2019) who state that EC is most used for estimating nutrient cycles. They indicate that for EC the rule of thumb "less is better" applies. Because of the relevance to nutrients, EC as an indicator was linked to ReGeNL matrix theme 1.1 Nutrient cycling.

Biological indicators

Of the indicator categories, biological indicators showed the least prevalence in both soil health research and RA-related research.

Enzyme activity, Microbial biomass carbon & Microbial respiration

Enzyme activity was mentioned as an indicator, however in the assessed records it actually was a group of enzymes varying in size between records from three to seven differently assessed enzymes. No single common enzyme was found throughout the analysis of enzyme activity as an indicator in neither soil health related records (n=6) nor the RA-related records (n=3). The

interpretation of the enzymatic activity in soil health related records was mostly paired with microbial respiration, microbial biomass carbon (MBC) or mineralizable nitrogen (Bending et al., 2004; Giacometti et al., 2013; Marinari et al., 2006; Mijangos et al., 2010; Muscolo et al., 2015; Valarini et al., 2003). The RA-related literature was more limited on enzymatic activity with Lujan Soto et al. (2020) mentioning it as a technical indicator for soil quality, with an expected positive response to RA practices providing farmers with information on nutrient cycling, Diaz de Otalora et al. (2021) investigated the effects of rotational grazing management on enzymatic activity and did not find significant differences between treatments whereas Ponnusamy et al. (2024) aggregated enzymatic activity in an index.

Microbial Biomass Carbon (MBC) was considered part of the biological category rather than soil carbon category because of its direct link to biological activity. Throughout soil health related research (n=7) different units are used to report on this indicator. It is brought in relation to other indicators such as Total Organic Carbon (TOC), microbial respiration or enzymatic activity. No conclusions were drawn based on the single indicator, it is however discussed in combination with other indicators to show increases in microbial activity under zero- or minimum-tillage, slurry application or organic amendments and incorporation of trees (Antisari et al., 2021; Bending et al., 2004; Beuschel et al., 2019; Marinari et al., 2006; Mijangos et al., 2010). The RA-related research using MBC was more limited (n=2). Daverkosen et al. (2022) related MBC to TOC in a MBC:TOC ratio that negatively responded to organic amendments, probably due to higher TOC levels.

Together, respiration, enzyme activity and MBC are aggregable with each other as an index for microbial activity which has not been done in a unique approach in the investigated research. This partly resulted from the difference in enzymes assessed. The level of insight each individual indicator gives on the composition of the microbial community in the reviewed literature was unclear, as well as the response of microbial community to management practice.

Overall these microbial community indicators related best to themes 1.1 Nutrient cycling, 1.2 Carbon and climate regulation and 1.5 Soil biodiversity and habitat provision. If further analysis of the indicators is able to distinguish more between the type of organisms, it could be useful for theme 1.4 Pest and disease management.

Earthworms

Earthworm abundance or biomass were the only organism specific indicators that come forward in both types of research. These indicators however can still be made more specific by determining the functional groups of earthworms. In soil health related research (n=6) the main investigated impact on earthworm abundance and biomass were tillage practice or fertilization regime. Reduced- or no-till systems showed increasing number and biomass of earthworms (Anken et al., 2004; Drakopoulos et al., 2018; Mijangos et al., 2010). Organic amendments as a fertilization regime was shown to increase number and biomass of earthworms (Bai et al., 2018; Mijangos et al., 2010; Willoughby et al., 2023). Of the RA-related research (n=2) the integration of livestock and effect of grazing regime were considered on top of tillage and fertilization (Daverkosen et al., 2022; Trickett & Warner, 2022). Trickett & Warner (2022) specifically investigated earthworm populations and were the only ones specifying on earthworm functional groups. Their discussion showed that fertilization regime was mostly affecting the functional groups, because of the source of nutrition provided.

In relation to other indicators, bulk density was mentioned to correlate positively with earthworm number and biomass (Bunemann et al., 2018; Drakopoulos et al., 2018). This due to

the creation of macropores. Trickett & Warner (2022) however found a negative correlation between earthworms and bulk density which probably was the result of the overall lower earthworm abundance. With this relation to bulk density, on top of linking earthworms to the ReGeNL matrix theme 1.5 Soil biodiversity and habitat provision, earthworms can be used as indicators for theme 1.3 Structure and water regulation. It should be noted however, that absence of earthworms will cause problems for assessing the effects of RA practices in terms of theme 1.3 Structure and water regulation.

Indicator species

Records excluded before screening the indicators in literature used different specific species in their research. These investigated soil organisms consist of 1) collembola species *Folsomia candida*; and 2) Bacteria species *Gemmatimonas sp., Mycobacterium sp. and Sorangium sp.* (Gorska et al., 2022; Nelson et al., 2011). Collembola are pointed out to response to soil quality properties in their appearance, and therefore good indicators for soil quality. The bacteria species are determined to be indicator species for long term effects of management and determined using metagenomic analysis.

Multiple Substrate Induced Respiration

A description of the use of the MicroResp[™] method came forward in the soil health literature and was found to be used as an index in two soil health related records as well as in one RA related record. This method was used for determining microbial community level physiological profiles to eventually calculate the multiple substrate induced respiration and functional diversity of soil microbes. (Andrés et al., 2022; Beuschel et al., 2019; Bongiorno, 2020; Creamer et al., 2016). The occurrence of MicroResp[™] as a method for retrieving indicators is expected to be growing in future publications, as it wass pointed out by Bongiorno (2020) to be a novel soil indicator. (Andrés et al., 2022) indicate the use of the MicroResp[™] method affordable for farmers. The relatively small occurrence in this review was possibly due to the search for indicators and not methods of retrieving soil indicators.

Soil Carbon

Soil carbon was determined to be a separate category for investigation since it relates to both chemical and biological processes and affects the physical state of the soil as well. From the reviewed literature, most notable was that Soil Organic Carbon (SOC) compared to Soil Organic Matter (SOM) was more prevalent in soil health related research with 17 and 8 records to the respective indicators. RA-related research had an equal occurrence in SOM and SOC usage with 8 records to each indicator. Since SOC is a fraction of SOM they were interchangeably used as an indicators for soil quality in several records, or converted from SOM to SOC (Bunemann et al., 2018; Juhos et al., 2019; Trickett & Warner, 2022).

Since SOC is generally slow in response to changing management, changes in this SOC were only reported on changing in long term experiments. Other research still measured SOC to relate it to soil structural properties. Analyzed long term experiments using SOC in this review (n=3) indicated an increase in SOC over long term periods depending on the management strategy. These analyzed long term experiments came forward in soil health related literature as long term soil quality assessments. This included tillage experiments in Switzerland that showed no significant differences in SOC levels between tillage practices attributed to the Swiss conditions, however stating that SOC in natural grasslands was significantly higher (Anken et al., 2004). The same management practice (tillage) combined with crop rotation and low-input

strategy was tested in Mediterranean context (Italy and Spain), where SOC is one of the positively affected soil quality indicators (Vanino et al., 2022). A German alley cropping experiment on former arable land showed increasing SOC after five to eight years in the 0-5 cm topsoil layer (Beuschel et al., 2019). Other research included long term managed plots, without experimental basis. An Italian experiment investigating adjacent organic and conventionally managed fields, showed no increase in SOC over seven years (Marinari et al., 2006). The RArelated literature did not bring forward long term 'RA-management' experiments. Nevertheless, one long term experiment and three long term plot changes were assessed to their SOC levels indicating a five-year period in the United States that can not draw conclusions on the effect of living mulch on SOC levels, a 25-years stewardship program in Canada showing increasing levels of SOC, an experiment assessing agroforestry in temporary differently established plots (minimum eight years) in Brazil showing an increase in SOC and a twenty-year multispecies pasture grazing regime indicating increasing SOC levels (Baker et al., 2022; Kersey et al., 2024; Matos et al., 2023; Rowntree et al., 2020). Other included records that used SOC as an indicator investigated it for providing insight in soil health in general, or to relate it to other soil properties. Altogether, SOC levels are prone to change under long-term management regimes. With a preliminary indication that RA-practices could increase SOC levels after at least five years, depending on the original state of the soil and management practices adapted.

Since SOC responds to management practices, however only slowly, short term effects to soil carbon are better distinguished by fractions of SOC. Increasing the use of harder to determine soil carbon fractions Particulate Organic Matter (POM), Mineral Associated Organic Matter (MAOM), Potassium Permanganate Oxidizable Carbon (POXC) or Water Extractable Organic Carbon (WEOC) can provide more insight in the effects of management on the specific fractions in short term, and relate to microbial processes, nutrient cycling and seasonality though further and deeper research is recommended (Bongiorno, 2020; Bunemann et al., 2018; Giacometti et al., 2013; Koorneef et al., 2024).

In terms of The ReGeNL matrix, the indicators in the soil carbon category are logically related to theme 1.2 Carbon and climate regulation. Considering the relations made between physical indicators and SOC, theme 1.3 Structure and water regulation could also be explained through SOC values. The fractions within SOC need to be further examined to relate these to themes 1.5 Soil biodiversity and habitat provision.

ReGeNL matrix themes

From the before discussed insights of included records in this review the links to the ReGeNL themes as described in *Table 2* are depicted in *Table 3*. The links specifically refer to improvement of each theme and the indicators best describing this improvement. Indicators are not deemed to be solely affecting the corresponding themes, as they can hamper improvement of other indicators or themes as well. This can be exemplified with bulk density and infiltration as soils compacted soils that do not drain any water will logically not provide a good habitat or provide a good basis for nutrient cycling as well. Most notable is that theme 1.4 Pest and disease management is underrepresented in the reviewed records and indicators. Links that are made in included records are advised for further investigation. A lead to further investigation into this theme can be found in the records by Bongiorno (2020) and Koorneef et al. (2024) as they investigate disease suppressiveness using different methods. Additionally, methodologies were not part of the search in this review. As came forward in the biological indicator selection, methods such as metagenomic analysis or the MicroResp[™] can provide insight in novel indicators. Another methodology that came forward in the reviewed records is the Visual Near

Infrared (VIS-NIR) spectroscopy that as a method can measure soil chemical properties using infield devices.

From the systematic review by Jayasinghe et al. (2023) the only corresponding indicator defined by this review is soil organic matter. Other indicators used in their review correspond to whole themes within the ReGeNL matrix such as nutrient balance.

Table 3 Link of indicators from literature revie (n=54) to ReGeNL themes 1.1: Nutrient Cycling, 1.2: Carbon and Climate regulation, 1.3: Structure and Water regulation, 1.4 Pest and Disease Management, 1.5 Soil Biodiversity and Habitat Provision. ¹Links stated to be prone to further research

Indicator	Matrix theme				
	1.1	1.2	1.3	1.4	1.5
Bulk density		Х	Х		
Infiltration			Х		
Aggregation		Х			
Penetration resistance			Х		
Macronutrients	Х				
Micronutrients	Х				
рН	Х			X ¹	Х
Nitrogen pool	Х		Х		X
Cation exchange capacity	Х	Х			
Electrical conductivity	Х				X
Enzyme Activity	Х			X ¹	X
Microbial biomass carbon	Х			X ¹	X
Microbial respiration	Х			X ¹	Х
Earthworms			X		Х
Soil organic carbon/Soil organic matter		Х	Х		
Fractions of soil organic carbon	X ¹	Х	X		X ¹

Literature pools

The two pools of literature, one to each research question, consist of literature from different time periods. Search query 1 and 2 resulted in literature dating from the years 2000-2024, whereas query 3 resulted in literature dating from 2020-2024. This clearly shows that RA research is upcoming more recently, especially considering indicators. This is in accordance with the findings of Giller et al. (2021) who see a drastic increase in scientific publications referring to RA with the year 2016 as turning point and by 2020 a finding of 52 academic references having terms Regenerative Agriculture or Regenerative Farming in their abstract. A fraction of this literature considers agronomic or soil health perspectives, as this is only a part of the RA movement. The limitation to peer-reviewed scientific sources enables for deeper insights into the correlation and explanatory values of certain indicators, however also excludes standard testing reports or public knowledge. An example of an already marketed RA indicator framework is the <u>SoilMentor</u> app developed by VidaCycle. This framework does show common indicators with scientific records, however includes arbitrary values as well. It remains unclear how a system like this analyses the state of the soil and provides farmers with the right insight except for scoring 'good' or 'bad'.

Not included in the eventual review for indicators are the records assessed moderate or low according to the strength of evidence grading. These records however have the potential to be

explanatory to the relationship of indicators, especially considering modelling approaches. Inclusion of the studies for further investigation of relationships between indicators is advisable.

Prospectives & conclusions

Regenerative indicators

For acquiring a perspective on the state of regeneration, the list of 40 indicators are all usable for acquiring perspectives within their corresponding dimensions as brought forward by this review. The value for each indicator however is highly context dependent. The use of indictors in almost all literature was on a field level and therefore deemed suitable in a farm-level context.

From the identified physical indicators, Bulk Density is the most important enabling insight in structure and water regulation of the soil, as well as climate and carbon regulation due to its relation with soil carbon. Other physical indicators relate more structure and water regulation. Texture is often stated in literature as being an indicator, however should better be described as a given soil property.

From the chemical indicators, most are best linkable to nutrient cycling. Nitrogen, electrical conductivity and cation exchange capacity are exceptions. Cation exchange capacity is relatable to carbon and climate regulation. Nitrogen and electrical conductivity both are also linkable to biodiversity and habitat provisioning. Additionally nitrogen is linkable to structure and water regulation as well as climate and carbon regulation considering the other parts of the soil nitrogen pool as being part of this indicator.

Soil pH could provide insight in soil biodiversity and habitat provision mainly in terms of comparing it to microbial activity and could as well provide insight into disease suppressiveness after further research.

An underrepresentation of biological indicators is seen in the scientific literature. Microbial community profiling is possible using the set of indicators enzyme activity, microbial biomass carbon and microbial respiration. The MicroResp[™] method came forward as another pathway for analysing soil microbial communities, as well as metagenomic analysis. The search for indicators resulted in exclusion of finding specific methods for retrieving soil indicators. Therefore, future research should consider research methods as well.

Soil organic carbon and soil organic matter have the potential to provide insight in the effects of management practices for improving soil health over long time periods. The fractions of soil organic matter have the potential to provide these insights on shorter term, though future research is necessary since the literature included in this review do not provide this insight.

Assessing and adapting to RA

The 'Indicator Zoo' to collect indicators for assessing the state of regeneration provides indicators suitable to each and every end-user. The literature included in this review did not use or approach every indicator or set of indicators in the same way. Aggregation in indices or analysis without discussion or conclusion based on several indicators hampers the understanding of their importance. Especially when such an index is referred to as being an indicator. This makes the 'Indicator Zoo' an unexplorable world to whoever tries to navigate through the science behind it. In general, RA-related research tends to use the 'classical' soil health assessments as an approach for providing insight in the effects of regenerative practices, sometimes too without further discussion on the insight an indicator gives.

For further research conducted within the ReGeNL project it is therefore advised to include a motivation to each indicator used. With this review and the work of Brouwers (2025) a starting point can be made to further explore the matrix themes concerning other dimensions besides soil health. A participatory approach as used by Lujan Soto et al. (2020) could be set up in order to define regenerative practices and indicators in a bottom-up approach. This can ensure that farmers and scientists are able to work with the defined indicators in order to move towards regenerating systems each concerning their own site-specific challenges and with their own comprehension of what insight indicators give into regeneration. This too could provide a tool for policymakers to include the defined indicators and practices in monitoring frameworks that fit their goals, so that each and every end user is able to follow the impact RA practices have on our environment.

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