Relating the impacts of regenerative farming practices to soil health and carbon sequestration on Gotland, Sweden

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Abstract

Land degradation, greenhouse gas emissions and biodiversity loss through agriculture are some of the greatest challenges we are facing today. Fertile and productive soils are the basis of life on this planet and need to be protected and restored to support a growing population and lower negative impacts of climate change.

Regenerative agriculture (RA) claims to improve environmental, social, and economic facets of food production. Its emphasis lies on carbon sequestration for climate change mitigation, biodiversity, and food security through the regeneration of degraded land. The concept of regenerative agriculture has gained attention both in mainstream media and in academic literature in recent years. However, there is no uniform definition of the term so far, and further there is a lack of comprehensive scientific studies on "real-life" farms that are changing their management from conventional to regenerative practices.

This thesis investigates the contemporary and historical context of the emerging term *regenerative agriculture* and identifies the main themes, movements, and debates associated with it by a broad literature research. Further, we compare regenerative farms with conventional farms on Gotland, Sweden in order to draw first conclusions about the impact of certain farming practices on soil physical, chemical, and biological parameters. The soil health on 24 different plots is assessed by a variety of indicators, i.a. total, organic, active, and microbial biomass carbon, C:N ratio, wet aggregate stability, root depth and abundance, earthworm number, nutrient leaching, and soil texture. These parameters are related to four main management practices: application of organic matter, soil disturbance through tillage, crop diversity, and share of legumes through a principal component analysis and multiple linear regressions. We found that the amount of carbon added to the soil had a significant impact on several soil health indicators, mainly organic and active carbon, bulk density, number of earthworms, root abundance, water infiltration, and vegetation density. Reduced tillage was connected to higher wet aggregate stability, and vegetation density. These findings need to be confirmed in the coming years; however, they show that higher organic inputs and less soil disturbance generally had a positive impact on soil health on the investigated farms.

Soil sampling will be continued on the same plots in the future to thoroughly investigate the impacts over a longer time period, as the thesis is part of the project *Time Zero! Land surveys during farm conversion from abandoned land to regenerative agriculture* performed at the Department of Soil and Environment at the Swedish University of Agriculture, Uppsala.

Popular scientific summary

Land degradation, greenhouse gas emissions and biodiversity loss are some of the largest challenges we face as humanity today. While agriculture is a major contributor to these issues, the suggested potential to contribute as the solution to the same problems needs to be thoroughly investigated. Carbon can potentially be stored in soils through improved farming techniques and thereby contribute to decreasing the impacts of the climate crisis. Higher soil carbon also helps to maintain and enhance soil fertility which is needed to continuously feed the world population.

Regenerative agriculture is gaining more and more attention both in mainstream media and academic literature. It claims to provide tools that can be part of a solution to combat the climate and biodiversity crisis and ensure long-term food security. Highlighted management practices within regenerative agriculture are the addition of organic matter, no/reduced tillage, cover crops or permanent soil cover, and integration of livestock and crops. Scientific studies on "real-life" farms that change their management from conventional to regenerative practices are however rare.

Moreover, there is no commonly accepted definition of the term so far. In order to enable scientific studies, public incentives and other support for farmers to increase soil health, biodiversity, and carbon storage in soils, we conclude that a context-specific definition should always follow along with claims about regenerative agriculture. In this thesis, we provide a holistic definition to include the broader ideological potential behind regenerative agriculture, along with a tangible and verifiable working definition that enables the design of a soil health study on regenerative farms on Gotland, Sweden.

The current and historical understandings of regenerative agriculture and the main debates connected to it are investigated in this thesis. This is followed by a comparison study of farms applying regenerative practices with conventional farms to see if there are differences in soil health. A variety of soil health indicators were assessed on 24 different field plots and related to four main management practices. These are the application of organic matter, the reduction of soil disturbance through tillage, the number of plant species present on the field, and the percentage of legumes within the field. We found that the amount of organic matter added to the soil and reduced tillage generally had a positive impact on several soil health indicators on the investigated farms. The examined regenerative practices seem promising; however, the present findings need to be confirmed in the coming years.

The thesis is part of the project *Time Zero! Land surveys during farm conversion from abandoned land to regenerative agriculture* performed at the Department of Soil and Environment at the Swedish University of Agriculture, Uppsala.

Table of contents

List of tables

List of figures

List of appendices

Abbreviations

AC	Active carbon
BD	Bulk density
C	Carbon
CC	Climate change
CFE	Chloroform fumigation extraction
COP21	Conference of the parties 2021
CO_2	Carbon dioxide
GHG	Greenhouse gas
IC	Inorganic carbon
IPCC	Intergovernmental panel on climate change
MBC	Microbial biomass carbon
MLR	Multiple linear regression
MRT	Mean residence time
N	Nitrogen
OM	Organic matter
P	Phosphorus
PAW	Plant-available water
PCA	Principal component analysis
RA	Regenerative agriculture
ROA	Regenerative organic alliance
ROC	Regenerative organic certification
SOC	Soil organic carbon
TOC	Total organic carbon
TC	Total carbon
WAS	Wet aggregate stability

1 Introduction

An increasing popularity of regenerative agriculture (RA) is becoming apparent in the last five years, following a surge in climate change (CC) awareness. Regenerative agriculture has a core focus on carbon (C) sequestration for climate mitigation, and the associated benefit of increased soil health. This thesis presents a literature study on the emerging concept of RA in combination with a soil health study on 'real-world' regenerative farms on Gotland (Sweden).

Regenerative agriculture is a complex, context-dependent, and ever-developing term. In the following chapter the present urgency of CC and soil degradation (1.1) and agriculture's role within it (1.2) will be mapped out. Furthermore, the diversity of the historical (1.3) and contemporary understanding of RA (3.2.1), and the position in relation to other alternative agricultural approaches (3.2.2) are unfolded with the aim of creating our own definitions (1.4) – a broader holistic interpretation of the concept and a practical working definition making the concept tangible for this thesis.

The working definition sets a framework for the soil health study on 24 regenerative fields on Gotland, Sweden. Based on previous soil health studies, a set of soil health indicators were selected (1.5) and analysed through field and laboratory work (2.3). Management and soil health indicators are first analysed and presented individually (3.3 and 3.4) and afterwards related to each other through a principal component analysis (3.5) and multiple linear regression (3.6). Additionally, a nutrient analysis was performed, relating nutrient content and loss to management indicators through multiple linear regressions (3.7). At last, the soil health results will be discussed (4.2), together with a discussion of the study design (4.1), key parameters for future recommendations (4.2.17), and limitations within the thesis (4.2.18).

The concept, ideas and approaches of this work were composed in close collaboration between Alena Holzknecht and Lærke Daverkosen. Individual parts are indicated by the author's first name in chapter headings. If no name is indicated, the work was done jointly.

1.1 The issues: climate change and soil degradation

In the most recent report from IPCC (2021), it is stated that global warming is unequivocally caused by human influences (*Figure 1*, b) and that the present state of the climate systems together with the scale of the changes in the period 1850 – 2020 are unprecedented in more than hundred thousand years (*Figure 1*, a). Global warming was observed to be slightly above 1 °C today relative to 1850 – 1900, and a warming of 1.5 °C and 2 °C relative to 1850 – 1900 is expected to be exceeded during the 21st century (ibid.). A raise in global temperatures of 1.5 °C is expected to increase the frequency and intensity of heavy precipitation and flooding in most regions of the world, including Northern Europe. On the other hand, an increased frequency of severe droughts with adverse impacts on food security and terrestrial ecosystems is to be expected. Furthermore, it contributes to desertification and land degradation in worldwide creating additional stresses on land, exacerbating existing risks to livelihoods, biodiversity, human and ecosystem health, and food systems (IPCC 2019).

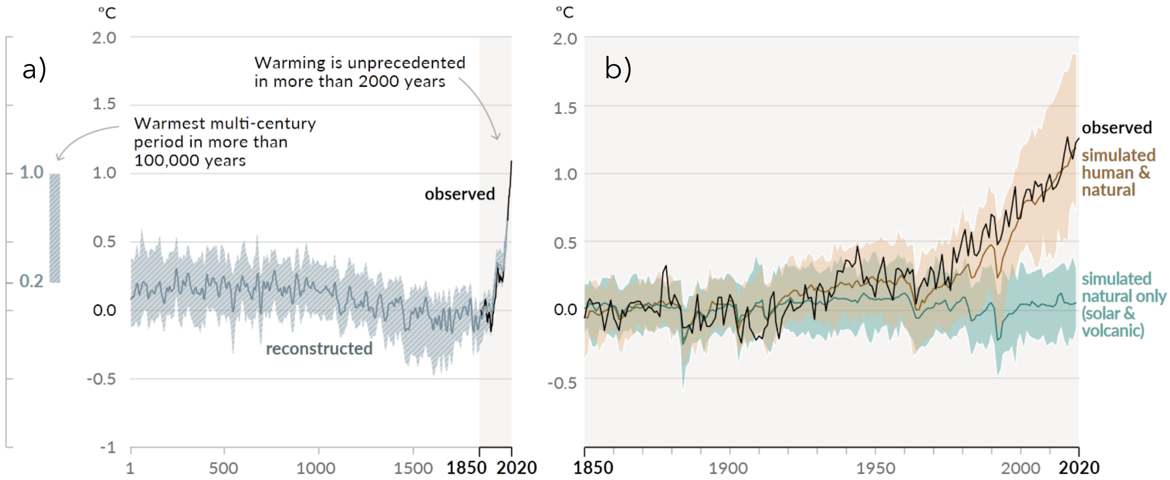

Figure 1: a) Changes in global surface temperature on a decadal average relative to 1850 – 1900, and b) annual average as observed and simulated using 'human & natural' (brown) and 'only natural' (green) factors for 1850 – 2020. Source: IPCC (2021)

Land is simultaneously a source and a sink of carbon dioxide (CO_2) and plays a key role in the climate systems and greenhouse gas (GHG) exchange between the land surface and the atmosphere (IPCC 2019). The conversion of natural ecosystems to managed ecosystems changes the land to a GHG source and depletes the terrestrial C stock (Poeplau & Don 2015). Thus, ecosystems have been turned into GHG sources since the onset of agriculture approximately 10,000 years ago (Lal et al. 2018). A meta study found that the conversion of forest and grassland to cropland causes a soil organic carbon (SOC) decline of 30 – 80% in the upper soil layers (Singh *et al.* 2018). Emissions from agriculture and expansion of the agricultural land represent 16 – 27% of total anthropogenic emissions. When emissions associated with pre- and post-production activities in the global food system are included, the emissions are estimated to be 21 – 37% of total net anthropogenic GHG emissions. Emissions from the agricultural sector are expected to increase, due to population and income growth, together with CC-induced land degradation. Expansion of areas under agriculture and forestry have supported consumption and food availability for a growing population, but simultaneously contributed to increasing net GHG emissions, loss of natural ecosystems and declining biodiversity. On the brighter side, the natural response of terrestrial land to human-induced change caused a net sink of around 11.2 Gt CO_2 yr^{-1} during 2007 – 2016, which is equivalent to 29% of total CO_2 emissions. However, the persistence of this sink is uncertain (IPCC 2019). According to IPCC (2019), about a quarter of terrestrial land is subject to human-induced degradation. Poor management practices have led to low productivity and increased risks of food insecurity (Gupta 2019).

Political initiatives for climate mitigation and land restoration are numerous and ambitious. In 2015, the European Union set the goal at the conference of the parties (COP21) of reducing GHG emissions by 80 – 95 % (relative to emission level in 1990) before 2050, together with the voluntary plan "4 per 1000" to increase C stocks with a rate of 0.4 % per year in topsoils of the world (Lal *et al.* 2018; Al-Kaisi & Lal 2020). The urgency of climate mitigation and need for drastically faster emission reductions and C sequestration strategies are emphasised by the new IPCC report (IPCC 2021). Every tonne of CO_2 emissions adds to global warming and it will require at least net zero CO_2 emissions, along with strong reductions of other GHG emissions to limit human-induced global warming (IPCC 2021). Agricultural practices based on indigenous and local knowledge can contribute to overcome the combined challenges of CC, food insecurity, biodiversity conversion, and land desertification and degradation (IPCC 2019).

Indiscriminate use of adverse agricultural practices like continuous monoculture and intensive tillage have contributed to widespread land degradation. This leads to the risk of exceeding the soil's capacity to overcome climate disturbances, such as drought and severe and frequent weather events (Lal 2015). Apart from contributing to CC, agriculture itself is vulnerable to global warming and the increase in extreme weather events (IPCC 2019). Additionally, agriculture faces the challenge of increased food demand caused by population and income increase (Olson *et al.* 2016; IPCC 2019). According to Giller *et al.* (2021), the solutions to this challenge include either increasing food production within or beyond the current land under cultivation. Expansion of cultivated land would involve inclusion of less productive land currently functioning as C sinks and would lead to habitat loss and altering of biogeochemical and hydrological cycles. A solution that does not require vast land use changes relies on improved land management. In general, RA aims at broadening the agricultural priority from producing food to land restoration and C sequestration. Hereby, reframing agriculture from being part of the problem to being part of the solution.

1.2 Regenerative agriculture – part of the solution?

Regenerative agriculture emerged as an agricultural system with the aim of regenerating and restoring land – and thus soil. The same principle progressed to more recent understandings of RA, but with a more extensive emphasis on restoration of agricultural land through an increase of the SOC pool. The historical understanding presents RA as a solution to land degradation and biodiversity loss, while the latter further includes an answer to CC through C sequestration. The importance of SOC lies in its potential as a land-based solution to climate mitigation through a combination of preventing C emissions, removing atmospheric CO_2 and delivering ecosystem services. This can be achieved through a combination of improving crop lands so land conversion for food production and thus C loss from soils become unnecessary, as well as active C storage in agricultural land (Bossio *et al.* 2020).

Soil organic C comprises about 58% of soil organic matter (SOM), which consists of a wide range of heterogeneous dead and living organic compounds of varying size with different stability and decomposition levels. Soil organic C can be partitioned into three pools: active or labile, slow, and passive C pools with residence times of 1 – 5 (< 1 – 10), 20 – 40 (10 – 100) and 200 – 1500 (> 100) years respectively, depending on the conceptual model. Naturally, the pools increase through C additions via photosynthesis of growing plants, decaying plant, animal and microbial matter and decrease through losses from decay, mineralization and erosion (Singh *et al.* 2018; Ramesh *et al.* 2019; De Moraes Sá *et al.* 2020). The labile pool consists of microbial biomass carbon (MBC), plant residues, roots, and fungal hyphae and is easily disturbed by soil use. The intermediate, or slow pool contains decomposed residues that are stabilized through organic matter (OM) occlusion and interactions between OM and minerals that make this pool less sensitive to land use and management. The passive pool is comprised of recalcitrant, stable, or mineral-associated C. It is further stabilized by micro-aggregation and thus least influenced by land management (De Moraes Sá *et al.* 2020).

As SOC stocks in agricultural land have been reduced considerably through land-use changes, there is a potential to restore SOC by improved management practices (Singh *et al*. 2018). However, the maintenance of higher SOC requires improved management in the long term, as SOC stocks can decrease again if such are ceased. Further, the storage capacity of SOC depends largely on climate, topography, and soil characteristics. A basic strategy for terrestrial C sequestration for climate mitigation in agriculture consists of 1) increasing C inputs and 2) maximizing the mean residence time (MRT) of C in the soil (Lal *et al*. 2018).

The amount of SOC is a net balance of organic C inputs and outputs. SOC input is largely a function of the amount and quality of OM added to the soil in combination with soil texture. To minimise C outputs, SOC needs to be stabilized in the long term and aggregation is the most important process, which will be discussed in chapter 4.2.7. Carbon decomposition is further regulated by climatic factors like temperature and water content, soil properties like texture, carbon to nitrogen ratio (C:N), specific surface area of soil particles, biological composition of SOC and soil microorganisms (Ussiri & Lal 2017).

Further, a new equilibrium at high SOC levels can be reached after some years with improved management practices. According to a meta study by Han *et al*. (2016), this can take about 30 – 70 years in warm temperate regions and 20 – 27 years in tropical regions. However, fertile soils in the same climate may be closer to the C saturation potential than largely degraded soils (Six *et al*. 2002).

Agricultural practices to increase SOC include perennial cropping systems, reduced or no tillage, mulch application, managed grazing, crop-livestock integration, and cover cropping. Another option to increase organic C contents is adding biochar to the soil which can persist from 100 to 1000 years. Most documented soil health co-benefits of RA are due to improvements in SOM content (Toensmeier 2016). SOM serves many functions within the soil and an increase will positively affect biological, physical, and chemical properties of the soil, such as nutrient supply, soil structure, water holding capacity, and microbial soil life (Watts & Dexter 1997; Johnston *et al*. 2009). Additional benefits from increased SOM include increased soil fertility and CC resilience, reduced soil erosion and habitat conversion. Further, increased SOC does not require additional land area, minimizes water footprints and related practices are readily implementable as they do not necessitate land use changes (Bossio *et al*. 2020). Bossio *et al*. (2020) call these SOC enhancing opportunities "no-regrets opportunities", as they have a variety of positive outcomes on different environmental and social levels.

A report by the IPCC (2019) underlines that the challenges of sustainable land and CC are based on a high level of complexity and a high diversity of actors involved. Sustainable land-use management, food security and low emission trajectories are facilitated by policies that involve changes across the food system. This could include the reduction of food loss and waste, change in dietary behaviour, as well as the empowerment of women and indigenous people, supporting community action, ensuring long-term access to markets and land, as well as advisory services, and reformations of trade systems. However, all of the mentioned activities need to be seen in context with previous land use, geographies, feasibility and social and environmental circumstances (Bossio *et al*. 2020).

Bossio *et al*. (2020) found that soil C represents 25 % (or 23.8 Gt CO_2-equivalent yr^{-1}) of the potential of natural climate solutions. Forty percent of this potential can be found by protecting existing soil C pools, whereas 60 % are represented by rebuilding depleted C stocks. Agriculture and grasslands account for 47 % of this mitigation potential, whereas the rest can be accounted to forests, and wetlands. Other land-based opportunities for C sequestration besides improved agricultural management are afforestation, reforestation, and C storage in harvested wood products (IPCC 2019), as well as trees in croplands (agroforestry), peatland and coastal wetland restoration, avoidance of forest and grassland conversion, and the use of biochar. A less established option for SOC increase in deeper horizons is the application of organic biosolids from urban areas (Bossio *et al.* 2020). Regenerative agriculture is one opportunity in a long row of actions needed to achieve CC mitigation and adaptation goals. Only a quick implementation and combination of the above-mentioned practices and other measures to rapidly decrease global GHG emissions will make it possible to keep global warming below 1.5°C.

1.3 Origins of regenerative agriculture

The word *regeneration* stems from Latin *genero* [to produce or procreate] and *re-* [back or again]. Used in biology, the term is applied for the process of restoration and growth (Hermani 2020). In an agricultural perspective this can be translated into the restoration of the soil, which means that the application of RA practices depends on the current state of the cultivated land. The word *regeneration* itself is in conflict with the transformation of pristine ecosystems to agricultural land. The connotation emphasizes a reorientation from not only reducing harm and damage, but actually creating net-positive environmental and societal outcomes (Robinson & Cole 2015).

Originally, the term *regenerative agriculture* was coined by Robert Rodale, son of organic pioneer Jeremy Rodale, in his article *Breaking New Ground: The Search for a Sustainable Agriculture* (Rodale 1983). He envisioned an agriculture beyond the present system and "beyond sustainability, to renew and regenerate our agricultural resources (Rodale 1983)" (Mang & Reed 2012; Hermani 2020). This should be achieved through a core focus on restoration, as "one that, at increasing productivity, increases our land and soil biological production base […] it has minimal to no impact on the environment beyond the farm or field boundaries (Rodale 1983)". Even though Rodale was the first to coin the term *regenerative*, pioneers of permaculture had already introduced an ecological approach of emphasising the regenerative potential of ecological systems by changing the human relationship to nature in 1978 (Mang & Reed 2012).

Throughout the 1990s, the term *regenerative agriculture* became almost invisible in agricultural literature and research. This absence occurred parallel to the development of organic certification and the institutionalization of organic agriculture (Hermani 2020) (see chapter 3.2.2.1). Throughout this decade a regenerative approach was instead detectable in the context of design and development (Mang and Reed 2012; The Center For Regenerative Studies 2021; Regenesis Group 2021). John T. Lyle, founder of the Center for Regenerative Studies, together with the Regenesis Group was one of the prominent developers. These movements continued Rodale's discourse of regeneration as going beyond sustainability by calling for fundamental perception changes that would create self-evolving systems (Lyle 1994; Mang & Reed 2012).

The original term of *regenerative agriculture* from Rodale Institute did not include a specific viewpoint on synthetic inputs. As the term developed, included more stakeholders, and organic agriculture diverged into branches of ideological and institutionalised perceptions of practices, Rodale Institute moved towards a regenerative organic agriculture, which is also still the term used by them. RA can thus be interpreted as a revitalization of the more radical early ideas of the organic movement, or as an update of organic principles with a focus on C sequestration (Hermani 2020). The redefinition to regenerative organic agriculture emphasises the need to distance itself from use of synthetic inputs and underlines the view of an RA system as a semi-closed system, that "takes advantage of the natural tendencies of ecosystems to regenerate when disturbed (...) marked by tendencies towards closed nutrient loops, greater diversity in the biological community, fewer annuals and more perennials, and greater reliance on internal rather than external resources (Rodale Institute 2014)" a principal which is not uniformly adapted by advocates of RA today (Giller *et al.* 2021). However, fully closed nutrient loops are not possible in cropping systems, as nutrients are always exported in the form of harvested goods. Nutrients as well as C need to originate from external sources if not all products are consumed at farm level and reintroduced through waste cycling. Another question is whether regenerative systems as described here can produce the same amount of food on the same area as the current systems.

The foundation of "Regeneration International" in 2015, an international foundation based on the ambitious goal "to reverse global warming and end world hunger by facilitating and accelerating the global transition to regenerative agriculture and land management (Regeneration International 2019)" was an important milestone for the increased attention to RA which has been detected both in mainstream and academic literature within recent years (Hermani 2020, also see 3.1). Furthermore, RA has gained political attention and was listed as a "sustainable land management practice (IPCC 2019)" in IPCC's special report on *Climate Change and Land* in 2019.

Today, RA is trying to find its place in a complex landscape composed of many different actors with a wide range of goals, ideologies, and histories. Many authors tried to define the term in the last years, coming up with different outlines based on various criteria. Others are using the term but are not defining it or are using it interchangeably with other terms in agriculture like agroecology, alternative, biodynamic, organic, carbon farming, conservation, or sustainable agriculture. General themes are primarily based on an environmental dimension, but also economic and social dimensions are included on farm- and food-system levels in more encompassing definitions (Lal 2020; Schreefel *et al.* 2020).

1.4 Our definitions

Despite the gained popularity of RA, neither a uniform definition of RA exists, nor a legal or regulatory framework for it. Due to the wide range of definitions and descriptions behind the term *regenerative agriculture,* research on the topic highlights the need for a clear definition of the term for any given use and context (Newton *et al.* 2020; Giller *et al.* 2021)

Two main issues are caused by publications making claims about RA without defining the concept. Firstly, the authors might not have developed a clear understanding of the concept themselves, in which case it is not appropriate to make claims about it. Secondly, when authors have a sound conception but leave it unspoken, this hands over the task of defining to the reader which can just as well cause misinterpretations (Newton *et al.* 2020).

In order to avoid misunderstandings and guide the reader, we formulated two definitions for this study. The first is a holistic definition to encompass our broader understanding of RA including more theoretical ideologies and philosophies. The second is a working definition, with more explicit statements that can be tested within the hypotheses of this study. It consists of a set of agricultural practices that were extracted from the broader understanding of RA.

Holistic definition:

Regenerative agriculture is an ever-developing, complex, and context-dependent agricultural approach aiming to restore and regenerate degraded land and contribute to mitigate climate change. In regenerative agriculture, soil is the entry point to rethink food systems with the aim of enhancing biological, physical, chemical, as well as cultural ecosystem services in response to ecological conditions and the climate crisis, on a local as well as a global level.

Working definition:

Regenerative agriculture is an agricultural approach that aims to improve the current state of the soil and includes the combination of two or more of the following practices: reduced/ or no till, increased complexity of crop diversity, addition of carbon through organic amendments or grazing animals, and/or inclusion of legumes, with the specific aim of restoring soil health and /or sequester carbon.

1.5 Soil health & selection of indicators

According to Mitchell *et al.* (2019), the concept of soil health is based on the perception of soil as a living biological entity, impacting plant growth and being intertwined with the wellbeing of animals, humans and ecosystems. It is associated with SOC dynamics and the supply of nutrients in the soil-plant-atmosphere continuum and has a focus on long-term food security. Giller *et al.* (2021) mention that soil health has gained more attention in conjunction with RA, and while it can be something favourable to strive for, they call it a problematic term that is abstract and needs to be specified to be measurable. In contrast, soil quality is more associated with soil functions like plant growth, C sequestration, and nutrient cycling and might have an orientation towards the production of particular crops. Soil fertility has a primary focus on crop yields (Bünemann *et al.* 2018).

Other authors like van Es & Karlen (2019), Rinot *et al.* (2019), and Jian *et al.* (2020) use the terms soil health and quality interchangeably and define it as the capacity of a soil to function as a biodiverse organism and provide ecosystem services. It can be assessed through physical, chemical, and biological indicators for both agro-ecosystems and natural ecosystems (Bünemann *et al.* 2018). There is a myriad of indicators to choose from, as the awareness about effects of agriculture on the environment and soil health is rising. However, there is a lack of consensus about the selection and degree of simplicity of indicators (Hermani 2020).

For the present study, the aim was to capture the current state of soil health of the investigated soils from a diversity of perspectives. The goal was to detect management effects on a multitude of soil health indicators, coming from the understanding of soil being a complex and dynamic system that cannot be apprehended if the measured variables are reduced to a minimum. Bünemann *et al.* (2018) emphasize that management effects are limited on inherent soil characteristics like texture or mineralogy. Therefore, in order to detect short-term effects, dynamic indicators are needed, as well as a certain sensitivity to management. Seasonal variation also needs to be considered.

The starting point for the choice of parameters was the Comprehensive Soil Health Assessment (CASH) from the Cornell University Soil Health Lab (Moebius-Clune *et al.* 2016) that was evaluated and recommended by many authors (e.g. Bünemann *et al.* 2018; van Es & Karlen 2019) and is used as a baseline in the Regenerative Organic Certification (ROC) (Regenerative Organic Alliance 2018) and a recent soil health study in southern Sweden by Williams *et al.* (2020). Further, other soil health studies and reviews, e.g. Al-Kaisi & Lal (2020), Bünemann *et al.* (2018), and van Es & Karlen (2019) acted as decision support.

CASH manual indicators included in the study were texture, plant-available water (PAW), (sub-) surface hardness/ soil penetration resistance, wet aggregate stability (WAS), OM content, active carbon (AC), and standard nutrient analysis. Additionally, we measured bulk density (BD), dry matter, pH, electric conductivity, infiltration rate, vegetation density, rooting depth and abundance, earthworm number, microbial biomass carbon (MBC), total organic carbon (TOC), total nitrogen (N), and N and phosphorus (P) loss together with some background information that was recorded in the field. The precise relevance of these indicators is presented in the methods chapter (2.3).

We consciously decided against an aggregation of indicators into a single index value. Firstly, because it is difficult to combine variables that carry completely different levels of information into one value and its interpretation underlies a high level of subjectivity. Secondly, because the aggregation process reduces the informative value and would impede the quantification of the influence of management on individual indicators. An alternative to a single index value is statistical data reduction through principle component, redundancy or discriminant analysis (Bünemann *et al.* 2018) of which the first was carried out in this study, in combination with multiple linear regressions (MLRs). In future studies within the same project, some parameters may be omitted, depending on their informative performance in the present study.

1.6 Objectives

This thesis is part of a recently started SLU project about regenerative agriculture, *TidNoll! Från övergiven jordbruksmark till regenerativt jordbruk: markfysikaliska- och kemiska undersökningar vid gårdsomställning (TimeZero! From abandoned agricultural land to regenerative agriculture: soil physical and chemical studies during farm conversion)*. The aim of the project is to investigate the soil development on a newly started regenerative farm and to study long-term soil health changes from the baseline year, starting in August 2020. The aim of the present thesis was to integrate measurements for this project in combination with a broader study design, that could give answers on the outcome of RA within the scope of a thesis. This resulted in the inclusion of 10 new farms and a combined theoretical and practical study with the following objectives:

i) To explore how the concept of regenerative agriculture has emerged and evolved, and what is currently understood by the term.
ii) To translate the current understanding of regenerative agriculture into a practical definition, which can be analysed from a soil health perspective.
iii) To measure how soil health develops with regenerative agriculture.
iv) To evaluate the position of RA in the greater discussion about the future of agriculture.

2 Methods

2.1 Literature study

Prior to the practical soil health study, a theoretical part was conducted to analyse and understand the concept, philosophies and practices behind RA. Inspired by the extensive literature review of Hermani (2020), a literature review was conducted as a basis for designing the framework of the soil health study on Gotland. Newton *et al.* (2020) highlight that many of the innovative experiences and ideas about RA are not represented in scientific papers, as they originate from farmers and other stakeholders. Therefore, relevant non-academic literature like blog articles, company websites, Facebook groups, Instagram posts, etc. were included in the analysis to broaden the perspective beyond the academic perception of RA and include the broader public understanding. A quantitative literature review with an academic and non-academic focus was further conducted to explore the popularity increase of RA. Annual results for the academic search engines Google Scholar, ScienceDirect, and Web of Science were collected, using the search term *regenerative agriculture* within the years 2010 – 2021. An approximated number of monthly Google searches of the same search term was collected through Google Trends for the same period. Graphs for the quantitative literature review were created.

2.2 Field study

2.2.1 Site description (Lærke)

This study was carried out on 24 fields distributed over 11 farms and gardens on Gotland. The region Gotland consists of the main island Gotland, together with the smaller islands Fårö, Gotska Sandön, Furillen, Stora Karlsö, and Lilla Karlsö. This study is limited to the main island Gotland, which is the largest island of Sweden, with an area of 29,810 km^2. The region of Gotland has a population size of approximately 60,000 people (Region Gotland 2017), and is placed in the Baltic Sea at 57.4 °N ; 18.5 °E. Gotland has a mean annual temperature of 7 – 8 °C and a mean annual precipitation of 500 – 650 mm (SMHI 2021), with the lowest variation at the coast and higher fluctuations inland. In comparison to the rest of Sweden, the island gets milder winters and prolonged summers (Region Gotland 2017). As a result, the growing season is longer than for mainland Sweden. Lithologically, Gotland consists of limestone and shale (SGU 2021b), resulting in calcareous soils. The main soil types found inland on Gotland (*Figure 2*) are moraine clay soils with stripes of clay loams and peatlands, whereas postglacial coarse sand, and bedrock are represented along the coast (SGU 2021b). On Gotland, 38 % of the population lives in rural areas and 70 % of the land area is used for forest and agricultural use. Sheep are the most common production animals on Gotland, followed by cattle (Region Gotland 2017).

Field work and sampling were conducted in the period of 20th – 27th of April 2021. The local weather in this period varied between clear days with 20 °C, rainy days with 14 °C and snowy days with approximately 0 °C.

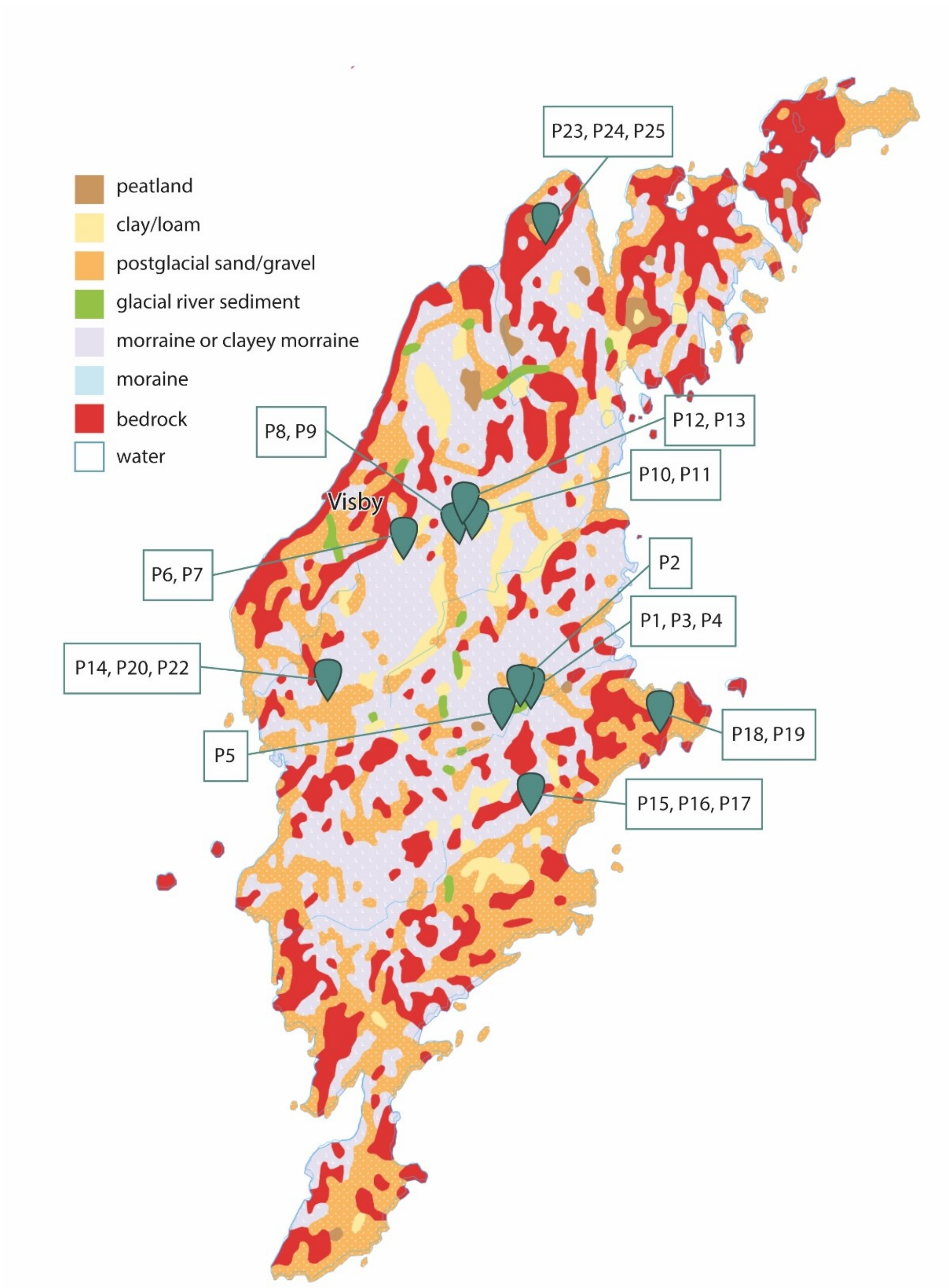

Figure 2: Soil map of Gotland with farm locations (modified after SGU 2021a)

Management information was collected through a survey and interviews with participating farmers. This was carried out by another thesis student working on the social aspects of the RA project at SLU. The included farms were chosen based on a self-definition as regenerative practitioner or through agreement with the practices we defined as regenerative farming. The latter included two or more of the practices *reduced/ or no till, increased complexity of crop diversity, addition of carbon through organic amendments or grazing animals, and/or inclusion of legumes*.

The 24 plots were categorised into three field categories: control, transition, and regenerative fields. The control fields (three plots) were chosen to include a combination of crop production with a limited number of species per year, none or low addition of organic amendment, no grazers, and regular tillage. Fields with regenerative practices such as minimum tillage, addition of organic amendment and/or grazers, more diverse crop systems, and incorporation of legumes or perennials, were divided into transition (eleven plots) and regenerative (ten plots). If these practices had been implemented for more than 3 years, they were classified as regenerative, otherwise as transition fields. This was based on the strictest EU conversion rule for conversion of non-foraging perennials in organic farming (Commission Regulation (EC) 889/2008) since no certification exists for RA to date. In Table 1 the different plots are presented with their representative P-number used for farmers anonymity, together with land use of the field, farm category, prevalence of grazers and application of organic amendments within the period 2015 – 2020. The study included both commercial farms and private gardens which is indicated in the table by the farm type. The unmanaged forest plot P21 was excluded from this thesis due to the absence of agricultural management.

Table 1: Management information for individual fields

Field name	Type	Land Use	Category	Grazers	Added Organic Amendment
P1	Farm	F	R	No	Yes
P2	Farm	F	C	No	Yes
P3	Farm	F	R	No	Yes
P4	Farm	F	R	No	Yes
P5	Farm	G and F	R	Yes	Yes
P6	Farm	F	C	No	Yes
P7	Farm	F	T	No	Yes
P8	Garden	V	R	No	Yes
P9	Farm	F	C	No	No
P10	Garden	V	T	No	Yes
P11	Garden	V	T	No	Yes
P12	Garden	V	T	No	Yes
P13	Garden	V	T	No	Yes
P14	Farm	G	T	Yes	No
P15	Farm	G	R	Yes	No
P16	Farm	F and G	R	Yes	No
P17	Farm	G and F	R	Yes	No
P18	Garden	G	T	Yes	No
P19	Garden	V	T	No	Yes
P20	Farm	G	T	Yes	Yes
P22	Farm	G	T	Yes	No
P23	Farm	G	R	Yes	Yes

P24	Farm	V	R	Yes	Yes
P25	Farm	G	R	Yes	Yes

F = fodder fields, V = vegetables, G = grazing, R = regenerative, T = transition. C = control.

2.2.2 Management information (Lærke)

Management information was quantified into four main categories, based on literature on regenerative practices: amount of C added, crop diversity index, years without tillage, and percentage of legumes.

The amount of C added was calculated from the application of OM, which included both external inputs and manure from grazing animals. External inputs were converted to t ha^{-1} $year^{-1}$ and summed up to total organic amendments added within the years 2015 – 2020.

$$total\ organic\ amendments\ [t\ ha^{-1}] = \sum_{i\,=\,1}^{6} organic\ amendment\ in\ year_i\ [t\ ha^{-1}] \tag{1}$$

Carbon content for individual types of organic amendments was collected through literature. These values were not always easily accessible and some estimates were based on less academic sources such as construction reports, websites, and online volume calculators (e.g. Fabian 2019; Swan 2020; Aqua Calc 2021). In case of several types of organic amendments added to the same field, the C content was determined for each amendment (*j*) and summed up to a total value of C added.

$$C\ from\ organic\ amendments = \sum_{i\,=n}^{n} total\ organic\ amendments_j[t\ ha^{-1}] * C_j content[\%] \tag{2}$$

Additionally, C content was calculated for manure from grazers, based on the time and number of grazers on the individual field. Carbon inputs from roots are neglected in this estimation. Through literature, an estimate was found for the amount of manure the specific grazing animal produces per day, together with the representative moisture and C content (Table 2). The moisture content was used to find the dry weight (dw) of the manure which was then used to calculate the C content.

Table 2: Daily amount of manure produced by different animals

Animal	Manure produced [kg day^{-1}]	Moisture content [%]	C content [% of dw]	Source
Cow	29	68	35.6	Herring 2014; Pettygrove 2010; Kimura *et al.* 2011
Horse	14	77	47.1	Fabian 2019; Chastain *et al.* 2014
Sheep	1.4	59	19	Ogejo *et al.* 2010; Thomsen *et al.* 2003; Jahanbakhshi & Kheiralipour 2019
Goat	1.6	78	19	Ogejo *et al.* 2010; Jahanbakhshi & Kheiralipour 2019; OMAFRA 2021

The above-mentioned values were multiplied with the number of grazing animals and the number of days the grazers had been on the field within the years 2015 – 2020 and divided by the size of the grazed area:

$$C\ from\ grazers\ [t\ ha^{-1}]\ = \frac{animals * manure\ [t\ day^{-1}] * dw * C\ content\ [\%\ of\ dw] * days}{area} \tag{3}$$

Finally, the total amount of C added to the field was determined by adding up C content from external inputs of organic amendments and from grazers:

$$total\ C\ added\ [ton\ ha^{-1}] = C\ from\ organic\ amendments + C\ from\ grazers \tag{4}$$

A crop diversity index (CDI) was calculated for the period 2015 – 2020 by multiplying the number of plant species in the total crop rotation with the average number of plant species per year, as suggested by Tiemann *et al.* (2015):

$$CDI\ =\ total\ species\ in\ rotation *\ average\ species\ per\ year \tag{5}$$

This index is based on a normal crop rotation of production fields. Crop rotation and annual number of species were estimated together with the farmer. In case of untouched fields, where the vegetation is not controlled by seeding or harvesting but purely by grazing patterns, the total amount of species was estimated from field pictures of the vegetation (e.g. *Figure 3*). For these fields the vegetation was primarily perennials, and the total number of species was therefore assumed to be equal to the number of species per year.

Soil disturbance was quantified as the number of continuous years without soil tillage. Consequently, a higher number depicts less disturbance. The study period of 2015 to 2020 resulted in a minimum value of 0 years and a maximum value of 6 years without tillage.

Figure 3: Vegetation picture from P14, perennial grazing field. Number of species estimated to 5 species (own photo 2021)

Information on share of legumes and share of perennials per field was collected in intervals of <10 %, 10 – 30 %, 30 – 50 %, 50 – 70 %, 70 – 90 % and >90 %. These were translated into the median of the interval: 5, 20, 40, 60, 80, and 95 for further statistical analysis.

2.3 Soil health indicators

The chosen soil health indicators (Table 3) consist of indicators easily assessed in the field, together with indicators requiring analytical laboratory facilities. While many studies only analyse the uppermost horizon or 10 cm, in the present study the profiles were analysed down to the C-horizon, as more than half of SOC stocks are found in depths of 20 – 80 cm (Rodale Institute 2014). For the sake of simplicity, in the statistical analysis only A-horizons were included. When possible, indicators were also measured for B- and C-horizons.

Physical, biological, and chemical indicators, together with parameters describing soil characteristics (Table 3) were included and carbon-related parameters were grouped together separately.

Table 3: Soil indicators and methods used for determination

Soil Indicators	Method determined in the field (F) or the lab (L)
Basic soil characteristics	
Profile description	Visual assessment (F)
Texture analysis	Hydrometer reading (L)
Bulk density (BD)	Core method (L)
pH	pHenomenal VWR MU 6100 L after ISO 1390:1994 (L)
Electric conductivity (EC)	pHenomenal VWR MU 6100 L (L)
Physical soil parameters	
Wet aggregate stability (WAS)	Cornell Rain Simulator (L)
Infiltration rate	One ring method on unsaturated soil (F)
Plant-available water (PAW)	Estimated from texture fractions
Penetration resistance	Penetrologger (F)
Chemical soil parameters	
Carbon-related soil parameters	
Total carbon (TC)	vario MAX cube elemental analyser (dry combustion) after ISO 10694 (L)
Organic carbon (TOC)	vario MAX cube elemental analyser (dry combustion) after ISO 10694 (L)
Inorganic C (IC)	Calculated from total and organic carbon
Active carbon (AC)	Colorimetric measurement of absorbance of $KMnO_4$ (L)
Microbial Biomass carbon (MBC)	Chloroform fumigation extraction (CFE) and multi N/C 2100 S direct injection TOC analyser (L)
Other chemical parameters	
C:N	Calculated from organic C and total N
Total nitrogen	vario MAX cube elemental analyser (dry combustion) after ISO 13878 (L)
Nitrogen and phosphorus loss	Leaching experiment with rain simulator; combustion and photometry (L)
Biological soil parameters	
Vegetation density	Visual assessment (F)
Root depth	Visual assessment (F)
Root abundance	Visual assessment (F)
Earthworm number	Visual assessment (F)

Basic soil characteristics

During the field work, detailed soil profile descriptions were conducted directly on-site, using a combination of two field guides: Guidelines for soil description (FAO 2006) and Bodenkundliche Kartieranleitung (*Manual for Soil Mapping)* (Ad-hoc-AG Boden 2005).

A soil pit of approximately 50 x 50 cm was dug, going as deep as needed to reach the C-horizon (*Figure 4*). First, general data like date and time, profile number, location on the farm, GPS coordinates, weather conditions, ambient temperature, landform and position within the landform, vegetation/ crops, surface characteristics, human influence, and land use were recorded. In the soil pit, horizons and their depth, the nature of the boundaries were recorded. For every horizon information on gravel and stone content, current soil moisture and temperature, consistency, porosity, colour, and mottles were gathered.

Figure 4: Soil profiles 3, 14, 17, and 21 (own photos 2021)

Additionally, for the A-horizon, root size and abundance, and number of earthworms were counted. Further, rooting depth and vegetation density were recorded, as well as pictures of the site, the soil pit and any extra information about the site and the soil that could help interpret the data later on were captured. Infiltration rate and penetration resistance were measured in close proximity of the pit. For every horizon possible, a minimum of three, but generally five samples were taken with cylinders of known volume in the field (*Figure 5*). The depth in which those were taken was noted. Further, disturbed samples from all horizons were taken, as well as cooled samples for microbial analysis, and lysimeters for leaching experiment.

Figure 5: Soil cylinders in an A-horizon (own photo 2021)

Parts of the recorded data are kept as background information about the profiles to help with interpretation. The rest was used for further calculations or processed to be used for statistical analysis. More detailed descriptions of these methods can be found below.

2.3.1 Texture analysis

The particle size distribution of a soil influences many, if not all other soil characteristics. Smaller particles have a higher specific surface area and are thus more chemically reactive. Generally, pore volume increases with smaller particle size. Texture is a soil characteristic that is comparably stable and does not change through management (Blume *et al.* 2018). It was analysed according to the lab compendium in soil science for students (Institutionen för mark och miljö; Biogeokemi och miljöanalys, SLU 2015).

The sand, silt, and clay fractions were translated into textural classes according to the Soil Survey of UK and Wales. This classification system was selected as it has the same size classification as commonly used in Sweden, with <0.002 mm for clay, 0.002 – 0.06 mm for silt, and 0.06 – 2 mm for sand and was part of the *soiltexture* package used for visualisation (Moeys 2018) in R version 4.0.4 (R Core Team 2021).

2.3.2 Bulk density, water content, dry matter

For the estimation of the soil's BD, the soil was removed from the cylinders sampled in the field and weighed before and after being air-dried until reaching constant weight. Bulk density $bulk\ density\ [g\ cm^{-3}] = \frac{dried\ soil\ [g]}{cylinder\ volume\ [cm^3]}$ (6) and water content $volumetric\ water\ content\ [\%] = \frac{moist\ soil\ [g] - dried\ soil[g]}{cylinder\ volume\ [cm^3]} * 100$ (7) were calculated as follows:

$$bulk\ density\ [g\ cm^{-3}] = \frac{dried\ soil\ [g]}{cylinder\ volume\ [cm^3]} \tag{6}$$

$$volumetric\ water\ content\ [\%] = \frac{moist\ soil\ [g] - dried\ soil[g]}{cylinder\ volume\ [cm^3]} * 100 \tag{7}$$

For dry matter, approximately 5 g of air-dried soil were weighed and dried at 105°C for a minimum of 6 hours, then transferred to a desiccator to cool down before determining the final oven-dried weight. The dry matter content $dry\ matter\ [\%] = \frac{air\ dried\ soil\ [g]}{oven\ dried\ soil\ [g]} * 100$ (8) was calculated as follows:

$$dry\ matter\ [\%] = \frac{air\ dried\ soil\ [g]}{oven\ dried\ soil\ [g]} * 100 \tag{8}$$

2.3.3 pH

The pH of a soil reflects its development and resulting chemical characteristics. It demonstrates how nutrients and contaminants behave in a soil, and its fitness as a medium for plant growth, habitat for soil organisms, and filter for pollutants (Blume *et al.* 2018). pH was measured according to ISO 1390:1994 with a pHenomenal VWR MU 6100 L.

The samples were air-dried and sieved to 2 m. Soils from organic horizons additionally went through an organic mill to produce a representative and homogeneous sample < 2 mm. 5 ml of soil and 25 ml of deionised water were mixed in 50 ml tubes and shaken for 5 minutes.

The tubes were left to rest for 2 hours before shaking them again for 5 seconds immediately before measuring.

2.3.4 Electric conductivity

Electric conductivity (EC) was measured as a proxy for the ecologically effective salt content. Increased salinity can result from a naturally occurring high salt concentration, improper irrigation practices (especially in arid climates), fertilisation with easily dissolvable salts like chlorides or nitrates, or the inordinate application of de-icing salts, and can harm plants by inducing nutrient deficiencies (Blume *et al.* 2018). Electric conductivity was measured with a pHenomenal VWR MU 6100 L following same procedure as for pH measurements.

Physical soil health parameters

2.3.5 Infiltration rate

Increased water infiltration can be a potential benefit of increased SOC. Higher infiltration is associated with reduced runoff and thus erosion and better soil aeration (Brown & Cotton 2011). It depends highly on the hydraulic conductivity of the soil surface, which can be impaired by slaked aggregates, siltation, and crusting. Following the method used by Van Eekeren *et al*. (2010), infiltration rate was measured as a rough estimate for soil compaction and water flow. This was done in-situ by placing 3 cylinders with a diameter of 18 cm around the soil pit. The vegetation was removed, and the time needed for 1 L of water to infiltrate an unsaturated soil was measured. Additionally, infiltration tests were performed on top of four mulching layers, classified as O-horizon for P8, P11, P12, and P13. An approximate infiltration rate was calculated as follows. Note that mL cm^{-1} equals mm:

$$infiltration\ rate\ [mm\ hour^{-1}] = \frac{infiltrated\ water\ volume\ [mL]}{infiltration\ time\ [sec] * area\ of\ cylinder\ [cm^2]} * 3600 \qquad (9)$$

Finally, an average of the three values per pit was calculated. However, this is a simplified field method that should rather be seen as an estimation than a precise investigation of infiltration processes.

2.3.6 Plant-available water (PAW) (Lærke)

Plant-available water was determined by estimates of textural porosity (Table 4) obtained from Saxton and Rawls (2006). Plant-available water was included to account for water as a potential limiting factor for plant growth. The textural class of the soil was determined by the sand, silt, and clay fraction from the texture analysis (Swedish classification system, described above), while it was classified by the international texture triangle (Brady & Weil, 2014). The direct conversion of texture fractures from the Swedish classification system to textural classes within the international system create a small risk of misinterpreting plant-available water of soils that are located on the border between two classes. The small risk is acceptable, since the PAW value is mainly another estimate for textural relations.

Table 4: Plant-available water (PAW) estimates based on textural classes according to Saxton & Rawls (2006)

Textural Class	Sa	LSa	SaL	L	SiL	Si	SaCL	CL	SiCL	SiC	SaC	C
PAW [% vol.]	5	7	10	14	20	25	10	14	17	14	11	12

Sa = sand, L = loam, Si = silt, C = clay.

2.3.7 Wet aggregate stability (WAS) (Alena)

Aggregation is one of the most important processes to stabilise SOC and thus increasing its MRT in the soil (Ussiri & Lal 2017). Wet aggregate stability (WAS) was determined following the CASH Manual from Cornell Soil Health Laboratory (Schindelbeck *et al.* 2016), using a Cornell Sprinkle Infiltrometer (*Figure 6*, left). It was assessed for 0.25- 2 mm and > 2 mm aggregates, respectively.

For statistical analysis, a mean value of WAS of 0.25 – 2 mm and > 2mm aggregates per profile was calculated, to combine them in one single variable. Mean WAS as it is used in the statistical analysis below is thus not a measured value but an indicator for aggregate stability.

Figure 6: Cornell Sprinkle Infiltrometer (left) and stable aggregates after rain simulation (right) (own photos 2021)

2.3.8 Penetration resistance (Lærke)

Penetration resistance of the soil was measured to analyse the compaction of the soil. It was measured nine times around the soil profile and an additional five times per quarter of the field using a Penetrologger (Eijkelkamp 2010) with cones of 1.0 cm^2 base area, 60° angle, and a penetration speed of 2 cm/s (*Figure 7*).

Data from the Penetrologger was extracted with the force unit [N] per cm depth. The resistance of the soil to penetration was converted to Megapascal [MPa], using the cone surface area S [mm^2] in accordance with the manufacturer (Eijkelkamp 2010).

$$\mathit{resistance\ to\ penetration}\ [MPa] = \frac{\mathit{force}\ [N]}{\mathit{cone\ surface\ area},\ S\ [mm^2]} \quad (10)$$

An average of the nine penetration measurements around the plot and an average for the 20 penetration measurements over the whole field were calculated for three depth intervals: 0 – 10, 10 – 20, and 20 – 30 cm (after Deru *et al.* 2018). The median of the nine penetrations around the plot in the depth of 10-20 cm was used for further analysis. The remaining results can be found in Appendix 5.

Figure 7: Penetrologger used in the field (own photo 2021)

Chemical soil health parameters

Carbon-related parameters

2.3.9 Total carbon (TC), total organic carbon (TOC) and inorganic carbon (IC)

Total carbon and TOC were measured after dry combustion with a vario MAX cube elemental analyser according to ISO 10694. Inorganic carbon is the difference between TC and TOC.

2.3.10 Active Carbon (AC)

Active carbon is the easily oxidisable SOM fraction and is a readily accessible energy resource for soil microbes that reacts faster to changes in soil management than TOC, which makes it a good indicator for soil health (Moebius-Clune *et al*. 2016). It was determined following the CASH Manual from Cornell Soil Health Laboratory (Schindelbeck *et al*. 2016).

2.3.11 Microbial biomass carbon (MBC) (Alena)

Microbial biomass carbon is defined as the living SOC fraction. It has a high impact on microbial driven processes and is a good indicator of biological activity in soils, responding quickly to stress and disturbances. Soil microbes are vital for the functioning of ecosystems, recycling energy and nutrients (Ramesh *et al*. 2019; Li *et al*. 2021). In terms of C sequestration, MBC is important firstly, because it reacts faster than other SOC fractions to disturbances. Secondly, microbes enhance the formation of aggregates which subsequently turn into more persistent forms of SOC, and thirdly MBC release extracellular enzymes into the soil that play an essential role in SOC turnover (Li *et al*. 2021).

MBC was determined with the chloroform fumigation extraction method, using a protocol by Shi & Spångberg (2019) from the Department of Soil and Environment at SLU after

Brookes *et al.* (1985) and Vance *et al.* (1987). The TOC analysis of all samples was done in a multi N/C 2100 S direct injection TOC analyser by catalytic high temperature combustion up to 950°C using Focus Radiation NDIR. The microbial biomass carbon can then be calculated as follows:

$$MBC = E / k \tag{11}$$

Where:

MBC… Microbial biomass carbon [µg C g^{-1} soil]

E … Soluble microbial carbon, which is the difference of organic carbon from fumigated samples and non-fumigated samples [µg C g^{-1} soil]

k … A coefficient that describes the efficiency of extraction, usually set at 0.45

2.3.12 Ratio of microbial biomass carbon to total organic carbon (MBC:TOC)

The ratio of microbial biomass C to TOC was calculated as percentage MBC out of TOC.

Other chemical parameters

2.3.13 Plant-available phosphorus and potassium

Phosporus and potassium (K) are essential plant macro-nutrients, with importance for plant processes such as e.g. photosynthesis, energy storage and cell division and enlargement. The extractability of P is dependent on pH and mineral composition, whereas K is only marginally affected by soil pH. Potassium is dependent on textural composition, it is poorly held by OM, and it leaches easily from sandy soils. Low nutrient values indicate poor availability to plants, while excessively high nutrient values indicate a risk of adverse environmental impact. Especially P creates a risk of eutrophication of waterbodies in the external environment (Moebius-Clune *et al.* 2016).

Plant-available P and K were determined with a double lactate extraction and ICP-OES measurement according to Ad-hoc-AG Boden (2005).

2.3.14 Total nitrogen

Nitrogen is an essential plant macro-nutrient, and its absence will restrict plant growth. This nutrient can be imported in the form of organic amendment or synthetic fertilizer, as well as biologically produced at farm-level using internal manure, plant residues or compost or by growing legumes and their symbiotic rhizobia with the ability to bind N_2 from the atmosphere and transform it to plant-available forms of N in the soil. The availability of N changes rapidly and is dependent on weather conditions, physical soil conditions, microbial activity, and the availability of OM (Moebius-Clune *et al.* 2016). The dynamic nature of N makes it relevant to look at total N, instead of plant-available N.

Total N was measured after dry combustion with a vario MAX cube elemental analyser according to ISO 13878.

2.3.15 Nutrient loss (Lærke)

The application of nutrients to agricultural soil is needed to provide essential macro-, and micro-nutrients to plants. However, excessive nutrient application can lead to poor plant growth or environmental degradation. Nitrogen and phosphorus from surface run-off and leached water of agricultural land are contributing to groundwater contamination and eutrophication of waterbodies (Moebius-Clune *et al.* 2016).

Lysimeters with undisturbed soil samples were collected from each sampling site. The vegetation layer was removed, a lysimeter was hammered vertically into the soil, and a lid was slid under to remove it without disturbing the sample. The samples were kept at field moisture level and cooled at 8°C one day prior to usage.

Before the simulation, 7-8 samples were placed in the rain simulator on a lysimeter base (collector) (*Figure 8*) with a mesh net in between to avoid OM in the leached water and a plastic skirt around to avoid extra water entering the collector. The upper 3 cm soil from the lysimeter rim were removed to avoid surface run-off. No additional treatment was applied to adjust for moisture content, thus the samples were kept close to field moisture level. The

Figure 8: Eight lysimeter samples placed in rain simulator at the Soil and Environment department at SLU (Own photo 2021).

rain simulator tank was filled in a solution ratio of 5 mL rainwater concentration[1] to 10 L volume. The irrigation interval had a total time of 6 hours and a stop time of 2 minutes between the 2 minutes irrigations with a precipitation rate of approximately 47-51 mm rainwater h^{-1}. 1.5 L bottles were attached to the collector tubes for collection of leached water. The same simulation interval was repeated once on the same soils, with exchanged water collection bottles, after a break of 24 hours from the starting time of the first simulation. The water was collected after the first and second simulation, cooled at 8°C before samples were well shaken and divided for analysis. Water samples from both simulation periods were measured at the SWEDAC-accredited Geochemical Laboratory at the Swedish University of Agricultural Sciences for N and P concentrations in the leached water.

Biological soil health parameters

2.3.16 Organic matter (OM)

Soil organic matter consists of microbial, plant and animal residues in various stages of decomposition, and other organic substances in association with inorganic substances (Ramesh *et al.* 2019). It feeds microbial activity, influences physical and chemical soil properties and is a vital part of nutrient cycling, as well as enhancing soil fertility and thus influencing crop yields and all soil ecosystem services (Barnwal *et al.* 2021). It is thus an important indicator of soil health.

Organic matter content was calculated using the Van Bemmelen factor, assuming that OM consists of 58% organic C (Nelson & Sommers 1982):

$$organic\ matter\ content\ [\%] = 1.724 * organic\ C\ content\ [\%] \tag{12}$$

[1] Rainwater concentration contains 0.0048 M $MgCl_2$, 0.0050 M KCl, 0.022 M NH_4Cl, 0.0030 M $Ca(NO_3)_2$, 0.032 M $NaNO_3$, 0.021 M H_2SO_4, 0.013 M HNO_3, 0.013 $(NH_4)_2SO_4$

2.3.17 Vegetation density

Vegetation density was measured in the field by placing a 50 x 50 cm frame on a representative place on the soil surface. The area covered by vegetation inside the frame was estimated by visual assessment as a percentage of the whole frame area. Vegetation density was included to measure for soil cover, and both crops and weeds were included in the estimation. Additionally, photos were taken at every field site with the frame for documentation (*Figure 9*).

Figure 9: Example pictures of vegetation cover from P7 (left), P21 (middle) and P24 (right) (own photos 2021)

2.3.18 Rooting depth and abundance

For the rooting depth the depth of the lowest reaching roots was measured vertically in each soil profile. The root density was determined by counting the number of roots < 2 mm and > 2 mm on a horizontal 10 x 10 cm area in the A-horizon. To calculate root abundance, the amount of medium and coarse roots > 2 mm was multiplied by 10 as recommended in the Guidelines for soil description (FAO 2006) and added to the amount of very fine and fine roots < 2 mm. Thus, medium or coarse root counts as 10 very fine or fine roots. Consequently, this is an artificial indication of root abundance integrating the size of the roots and is not equal to the actual number of roots.

2.3.19 Earthworm number

Earthworms provide essential ecosystem services and improve soil structure by e.g. burrowing, mixing, aerating, and recycling nutrients (Briones & Schmidt 2017). Through the introduction of deep-rooted plant species, the application of irrigated compost, and increased earthworm numbers, C stabilisation in higher depths might be promoted (Rodale Institute 2014).

Figure 10: Earthworm counting in the field from P12 (own photo 2021)

Earthworms in the A-horizon were counted in the process of digging the soil pit (*Figure 10*) and profile description after a simplified version of the method by Stroud & Bennet (2018). Again, this is a simple estimate, but it can be seen as a good approximation of macrofaunal activity in the soil. Additionally, notes were taken on other soil fauna when visible.

2.4 Statistical Analysis

Data analysis was performed in R version 4.0.4 (R Core Team 2021). Unless specified, functions were included in the base packages. Only A-horizons were considered in statistics.

The analyses included a principal component analysis (PCA), to detect associations between the measured variables. Further, the first principal components were extracted and used as response variables for MLR with the management indicators as predictor variables. Ultimately, analyses of variance (ANOVA) were calculated for different models and combinations to further explore the dataset.

2.4.1 Principal Component Analysis (PCA) (Alena)

As a preparatory step, single missing or erroneous negative values were replaced by the mean of the overall values for the indicator, as PCA requires one value per row and column. This does not influence the outcome of the PCA but enables to incorporate indicators with single missing values. These included one value for MBC/TOC, penetration resistance and TIC respectively and four values for both leached N and P.

A subset was created to reduce the number of indicators for the PCA. Excluded variables were electric conductivity for its limited explanatory value, as well as TC and TIC, as only the TOC fraction is used in the analysis. Nutrients were also excluded in this step, as a separate regression was performed on total N, N loss and P loss. The final variable selection included BD, pH, AC, MBC:TOC, infiltration rate, PAW, penetration resistance, root abundance, root depth, earthworm number, vegetation density, TOC, C:N and mean WAS, as well as operation mode for grouping.

All data was saved in tidy data format via the *tidyverse* package (Wickham *et al.* 2019), and was centered and scaled before the PCA was run. Score, loading and biplots were created for different combinations of PC1, PC2 and PC3, as well as a loading and score matrix. The *compositions* package (Boogaart *et al.* 2021) was used for the biplot. PC1 and PC2 were extracted to be used for further analysis.

A heatmap with soil health indicators and profiles was made using the *pheatmap* (Kolde 2019) and *RColorBrewer* (Neuwirth 2014) packages.

2.4.2 Multiple Linear Regressions (MLR) and analysis of variance (ANOVA) (Alena)

Two MLR models with PC1 and PC2 as response variables and the management indicators as predictors were computed. However, when plotting Bayesian information criterion (BIC) vs. number of predictors and with the help of *regsubsets* from the *leaps* package (Lumley 2020), it could be concluded that a reduced model of the form

$$PC = a * amount\ of\ C\ added + b * years\ without\ tillage \quad (13)$$

performed similarly with less predictors. Also, models with and without interactions of the predictors were compared, where the first showed higher significances and was therefore used for further analysis. Plots were made using the *ggplot2* package (Wickham 2016).

Type II- ANOVAS for the two MLR models were calculated with the *car* package (Fox & Weisberg 2019) to find significances of the indicators in the models. Type II- ANOVAs were also made for MLR models with single soil health indicators and amount of C added + years without tillage. Although these models do not stand for themselves in the present analysis, they serve for interpretation. Shapiro-Wilk tests were applied to check the null hypothesis of a normal distribution of the ANOVA residuals, as well as normal Q-Q plots to visually check for normality of residuals, residuals vs. fitted plots to check for homoscedasticity (or constant variance) and predicted vs. actual value plots. These can be found in Appendix 3 and Appendix 4. Outliers were identified with the *rstatix* package (Kassambara 2021). Moreover, estimated marginal means of soil health parameters and the PCs were calculated with the *car* (Fox & Weisberg 2019) and *emmeans* (Lenth 2021) packages to see the variance of single parameters between the groups. Boxplots showing means and standard deviations between groups were made using the *ggpubr* package (Kassambara 2020) and can be found in Appendix 2. Type II- ANOVAs were further applied to multiple linear regressions on nutrient indicators, followed by a Shapiro-Wilk test for normal distribution. In the absence of normal distribution, a Kruskal-Wallis test was performed to check for differences in the means of the farm categories

3 Results

3.1 Quantitative literature review

The number of searches for the term *regenerative agriculture* in popular academic search engines increased in the last 10 years (*Figure 11*): slowly with annual search results below 100 between 2010 and 2015, and exponentially until 2020. The majority of the search results were found in Google Scholar.

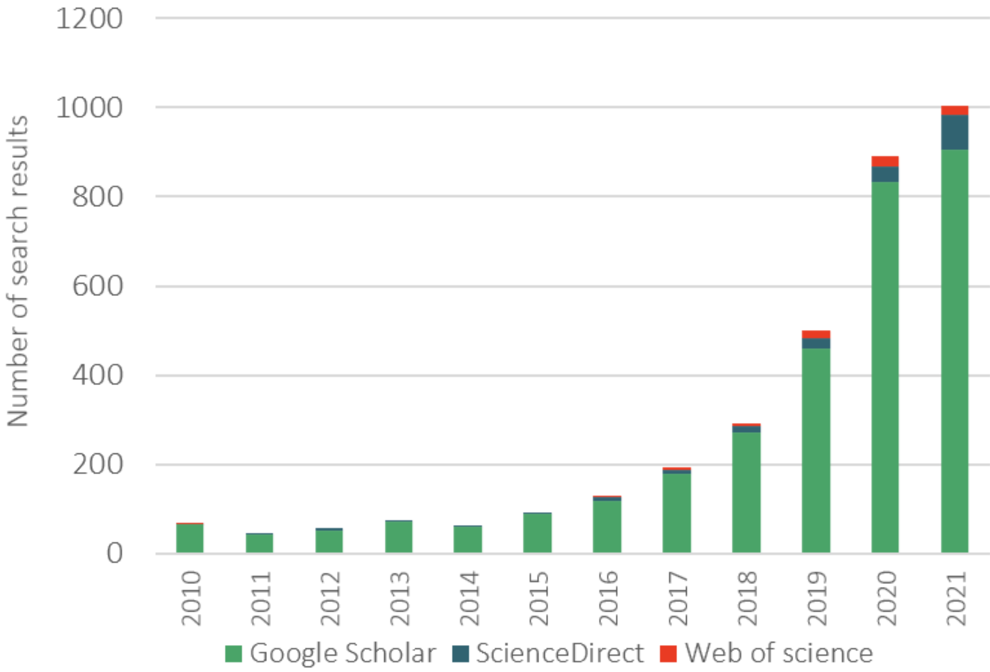

Figure 11: Academic search engine results for "regenerative agriculture" 2010 – 2021, compiled in September 2021

A similar trend can be observed in *Figure 12*, where the search interest of "regenerative agriculture" is shown via Google Trends. The figures are a sample of Google search data that are normalized to 100, which means that 100 represents the highest interest in the topic in the given time period. It is thus rather a measure of the popularity of terms than a display of search numbers (Rogers 2016).

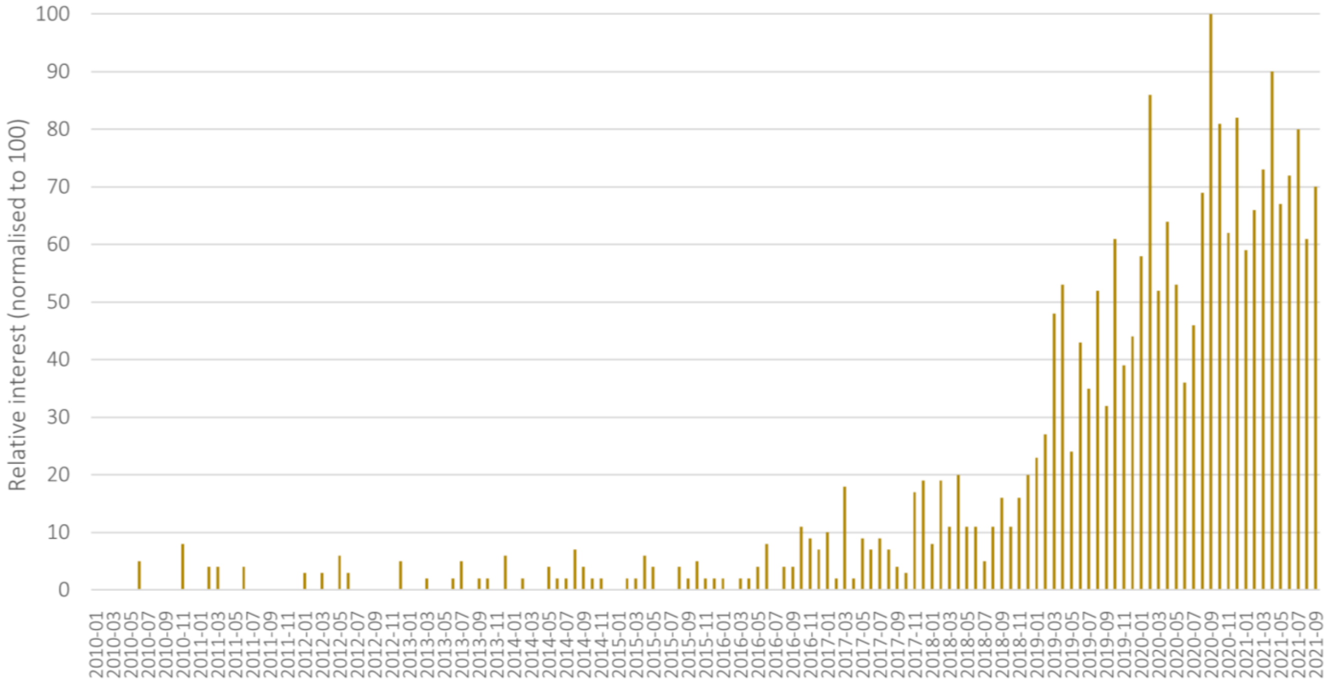

Figure 12: Monthly Google Trends data of "regenerative agriculture" 2010 – 2021, Zcompiled in September 2021

Thus, it can be concluded that the term has gained substantial attention since 2016 in both the academic and non-academic field.

3.2 Qualitative literature review

Regenerative agriculture is a concept that is not universally defined in a field with many stakeholders, interests, and understandings. In addition, it takes place in an almost infinite number of different contexts that all have their own inherent challenges on an environmental, social, and economic level. However, there are a few main subjects at the core of the debate around RA that will be touched upon. We do not claim to explore the topic in its integrity due to its bare complexity and the fact that the conversation around it is evolving quickly in both the scientific and mainstream field.

3.2.1 Contemporary definitions and understandings of RA

The focus of RA in contrast to sustainability is not the reduction of harms but net-positive outcomes (Robinson & Cole 2015), however there are varied perceptions of RA. Two main dichotomies could be identified in most definitions. First, there are the two contrasting views that RA either is a set of practices (e.g. Lacanne & Lundgren 2018), that can be applied individually or in combination, and on the other side is the view of RA as a holistic system where all actions are intertwined and that also includes many more aspects than the growth of crops. Second, there are definitions of RA that are process-based and others that are outcome-based, and combinations thereof (Newton *et al.* 2020). However, most proponents state an extension of sustainability as a cornerstone of RA (Hermani 2020). A third group of definitions comes from authors that refuse to define the term *regenerative agriculture*. Soloviev & Landua (2016) for instance state that this would put an end to the development of RA and thereby go against the whole concept of regeneration. Instead, they offered a framework to distinguish between different levels of the development of RA. Similarly, an interactive definition website to continuously update the definition of RA was created as a participatory project by Terra Genesis International (Hermani 2020).

Contemporary academic literature (Burgess *et al.* 2019; Lal 2020; Newton *et al.* 2020; Schreefel *et al.* 2020) acknowledges that there is no uniform definition of RA. By analysing core themes of RA, they conclude on definitions that are broad, dynamic and encompassing of more than one agricultural system. RA is often described in opposition to conventional or industrial farming (Lal 2020; Burgess et al 2019) by proponents of holistic interpretations of the term. Toensmeier (2016) and Project Drawdown (2020) define RA as an umbrella term for various land practices, where the C sequestration potential has been scientifically discussed.

Hermani (2020) names two main strands within RA, a techno-economic and an agroecological-ruralist movement. The first is often characterized by large agribusinesses that are not aiming for a paradigm shift in agriculture and aspire to keep up their production. The latter is pursuing a more fundamental (and maybe radical) restructuring of food systems. This argument is carried forward to divide between a camp that is aiming for a holistic, ecosystem-centric view vs. the application of single practices.

Many US-American corporations like General Mills, Lush cosmetics, Unilever, and One Planed Business for Diversity (OP2B), a business corporation including Nestlé, Danone and L'Oréal, are using RA as a promotion strategy. Starting from about 2017, RA has become a new buzzword for many companies, with a rather reductionist approach of applying single practices in an unaltered system, often without clear and binding standards (Beste 2019; Hermani 2020; Giller *et al.* 2021). While they are applying practices that are considered regenerative, the implementations miss out on interactions and complexity that will be elaborated later on. Keeping definitions open and dynamic can be a way of contributing to a continuous development of the understanding, practicing, and expansion of RA (Soloviev & Landua 2016), however it can also be a two-edged sword, enabling the co-option of the term by large corporations.

Giller *et al.* (2021) argue that the large variety of context-specific policies, agroecosystems, food and farm systems tackle different issues. Hence, no one specific set of practices or meaningful problem definition can be made to address all challenges alike. Newton *et al.* (2020) further formulate three main issues with *regenerative agriculture* being a largely undefined term, in respect to researchers, consumers and public administration or corporations. First, verifying claims about the impact of RA can be challenging for scientists without clear terminology. Secondly, labelling and marketing can be misleading for consumers. Thirdly, policies, laws, and (public) incentives to support RA are difficult to argue for without a widely accepted perception of the concept. This underlines that a definition can evolve and differ in context of its user.

There are however some common denominators that most RA stakeholders agree on, where the most important outcomes are

- C sequestration
- Increasing soil fertility
- Enhancing biodiversity and resiliency

and the most common practices are

- Addition of OM through manure, compost, green manures, etc.
- No-till, reduced tillage or conservation tillage
- Cover crops or other permanent soil cover
- Integration of livestock and crops (Elevitch *et al.* 2018; Newton *et al.* 2020).

Other practices or principles that are part of many definitions are diverse crop rotations, integration of more perennials, inclusion of agroforestry and tree crops, maintenance of living roots in the soil, residue management, and reduced external inputs. Less often, but also mentioned, are the restoration of natural habitats, and a focus on localism or regionality. A dividing factor is the debate about whether organic methods are inherent to RA. While many argue that synthetic fertilizer, pesticide, and insecticide use cannot be part of regenerative systems, proponents of more reductionist approaches of RA, argue that minimum soil disturbance and thus C sequestration is only possible with synthetic inputs (e.g. Giller *et al.* 2015; Regenerative Organic Alliance 2018). In response to the discordance about synthetic inputs, the Rodale Institute that initially coined the term *regenerative agriculture*, now refers to it exclusively as *regenerative organic agriculture* (Rodale Institute 2014).

Other stated outcomes and co-benefits are improved watersheds and water resources, enhanced ecosystem services and health, closed nutrient loops, reduced GHG emissions, same or higher farm productivity, improved animal welfare, better social and economic wellbeing of communities and rural livelihoods, improved food access, security and nutritional quality, circular systems and reduced waste (Rodale Institute 2014; Elevitch *et al.* 2018; Al-Kaisi & Lal 2020; Newton *et al.* 2020; Giller *et al.* 2021).

Rodale Institute (2014) argues that through RA farming becomes a "knowledge intensive enterprise", instead of a "chemical and capital-intensive one (ibid)", which calls for a shift in mindset and in whole food systems rather than the isolated application of practices that could sequester C. The strongest and most unifying principle that differentiates RA from other alternative agricultures is however the focus on SOC for C sequestration and improved soil health.

Many of the above-mentioned practices are also found in conventional or other farming systems and are generally considered *good agricultural practices* (Giller *et al.* 2015). Often, other alternative agricultural systems are openly included. For example, Terra Genesis International includes the design perspective from permaculture and agroecology (Hermani 2020). Agroecology is often incorporated due to its high potential in sequestering C aboveground, and when integration of animal or closed nutrient cycles are included in the definition it often relies on holistic management practices (Soloviev & Landua 2016). Giller *et al.* (2021) argue that the reframing of other alternative agricultures through RA leads to confusion instead of clarification in the public debate and deflects from more essential challenges. However, RA might have the potential to bridge the ideological gap between different agricultural camps, and to unite them under the premise of soil health and C sequestration. Some of the below-mentioned farming systems may be seen as one among others within RA, with increased SOM as their intersection. Bossio *et al.* (2020) point out that RA, organic farming, agroecology, climate smart agriculture, agroforestry and permaculture are not mutually exclusive systems and can have significant positive impacts on SOC in certain geographies.

3.2.2 A brief history of alternative agricultures

Throughout the last century, various movements towards alternative agriculture and food systems have emerged. Different issues are taken on, some more fundamentally and all-encompassing and others within the existing industry. RA inherited large parts of its meaning today from agroecology, the organic movement, and recent findings in soil science. The question arises whether and how RA is different from other agricultural systems, how do they overlap and why this concept is met with such enthusiasm recently. Evaluating the relevance of RA in the landscape of alternative agricultures requires the knowledge of their history and evolution.

3.2.2.1 Organic agriculture (Alena)

Organic agriculture as defined by the International Federation of Organic Agriculture Movements (IFOAM) General Assembly (2008) "relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects". This means that it refuses synthetic inputs like synthetic fertilizers, pesticides, herbicides, and additives as well as genetically modified seeds. The focus lies on site-specific ecosystem management to prevent pests and diseases and maintain soil fertility (FAO 2021c) and is based on the four principles of health, ecology, fairness and care (IFOAM 2021).

In the beginning of the 20th century, different visionaries from around the world contributed to the founding of the organic movement with various approaches, including Rudolf Steiner, Albert Howard, Ana Primavesi, and Jerome Rodale. (Arbenz *et al.* 2017) call this period Organic 1.0, where a first shared understanding of the interrelatedness between food production, ecosystem health and human health evolved.

Staying within this narrative, Organic 2.0 was grounded in the 1970s with the formation of IFOAM – Organics International. With it came the implementation of standards and certifications and considerable growth of the organic industry around the globe in the coming years (Arbenz *et al.* 2017). In this period, many of the radical ideas that Organic 1.0 was founded on have been watered down through the conventionalisation of organic farming. Often, capital went into fewer but bigger organic producers, and the necessity of clear production standards made a progressive socio- political movement developing into an institutionalized industry that has to obey to existing paradigms. New actors were joining, who had a higher interest in a growing market than in the founding ideologies of the movement (Robinson 2009).

While in the beginning organic agriculture was supply-driven, the consumer demand increased, and consequently two very different strands of organic producers emerged. On the one side, a so-called 'conventional' strand with high- capital, specialized ventures that aimed at export; rather representing a modification of industrial agriculture than the transformation envisioned by the pioneers of Organic 1.0. On the other side is an 'artisanal' strand that is characterized by smaller scales, higher product diversity and local sales, arguing that the re-invention of food systems includes not only the farm economy and ecology but also the social and political dimension (Robinson 2009).

The EU organic label that came into place in 2010 is an example for the furthering of the industrialization of organic agriculture, where unsustainable practices can be common. In a recent report on organic certification labels by (Greenpeace (2018), the EU organic label scored 4 out of 5, rated "trustworthy", but is criticized for lower standards and only demanding a minimum level compared to other organic certification schemes in Europe. For instance, a processed organic product may contain up to 5% non-organic components after EU regulations and 53 different additives are allowed. Other organic labels like *Demeter*, *Bioland*, or *Naturland* do not allow any share of non-organic components and further restrict the use of many additives. The Swedish KRAV label complies with the EU organic label and partly goes beyond EU standards (The KRAV Association 2020).

Organic 2.0 is where we still find ourselves at present, with about 1% of the global food market being certified organic, however many smallholder farms are organic by default without being certified. For instance, 7.7% of the agricultural land in the EU were farmed organically in 2018 (Mathews & Mitschke 2020). However, pressing challenges like CC and soil degradation underline the need for more than what is done in the organic movement currently. Strategies brought forward by IFOAM - Organics International for the future of organic farming are united under the name Organic 3.0. Going beyond the definition of minimum requirements of Organic 2.0, the "new" Organic wants to be innovative, holistic, accountable, and regional, without abandoning the original principles. The aptitude of the organic sector to impact global issues like CC mitigation and adaptation, access to land, water and seeds, soil fertility, genetic and cultural diversity, gender equality, and accessibility to both traditional and scientific expertise is highlighted.

What is communicated as new is a striving towards "dynamic and continuous improvement [...], adopt[ing] leading-edge concepts that bring substantial change to solve major social and environmental issues (Arbenz *et al.* 2017)", including i.a. awareness and relationship building, systematic use of indigenous knowledge, and the use of precision farming, intensified crop and livestock breeding to avoid genetic engineering. Without giving up existing institutions and certifications, stakeholder-driven initiatives should contribute the reformation towards increased sustainability and expansion of the sector (Arbenz *et al.* 2017).

Dinis *et al.* (2015) state clearly that organic farming is not necessarily synonymous with sustainable agricultural practices, especially because of the conventionalization of the market. They divide the movement in organic and deep organic farming, the latter complying with core organic values to a higher degree.

3.2.2.2 Agroecology (Alena)

The term agroecology first appeared in scientific publications in the 1930s and initially described a scientific discipline. In the 1980s different agricultural practices came up under the same name, often connected to social movements that emerged opposing industrialized agriculture after the Green Revolution. Agroecology stays present in different contexts and scales around the world and today refers either to a scientific discipline, an agricultural practice or a socio-political movement (Wezel *et al.* 2009).

In science, the scale of agroecology has evolved from plot or field size in the 1930s and over time expanded to the farm, then to the landscape, and finally to food systems in the 2000s (Wezel *et al.* 2009). In the 21st century, agroecology is summarized as the ecology of food systems, investigating all steps in food production, processing, marketing, access, consumption, and benefits for all actors. A transdisciplinary and participatory approach, with a clear focus on the social and economic dimension of food systems, and food sovereignty is the core of agroecology. It is characterized by bottom-up, regional and context-specific concepts, regarding autonomous producers with practical (traditional) knowledge as the agents of change (Gliessman 2020). Agroecology puts emphasis on enhanced functional biodiversity in the spatial and temporal dimension to maintain production and profitability. This also involves utilizing ecosystem functions to the highest degree possible and enhancing biological regulation (Francis & Wezel 2015; Gliessman 2020).

3.2.2.3 Permaculture (Alena)

The term permaculture is a portmanteau of the words *permanent* and *agriculture* and was coined by David Holmgren and then professor Bill Mollison, who met in the 1970s at the Environmental Design School of Hobart in Australia (Permaculture Society of the Philippines n.d.) and together published their initial work *Permaculture One* in 1978. In a more recent publication Holmgren defines permaculture as "consciously designed landscapes, which mimic the patterns and relationships found in nature, while yielding an abundance of food, fibre, and energy for provision of local needs (Holmgren 2002a)". Thus, there are two main elements: first, the imitation of natural ecosystems for a human use, and second, the optimisation of the system so that yields can be accomplished with minimal effort and ecosystem functions are extended beyond their ordinary output (Krebs & Bach 2018). Further, permaculture sees land use systems as intricately linked with social systems and draws upon the ethical principles of care for earth, care for the people and fair share (Holmgren 2002b).

Today, permaculture is considered a global grassroots network based on a site-specific holistic design process and eco-mimicry (Morel *et al.* 2019). Ferguson & Lovell (2014) specify four components of permaculture: the international movement, the worldview, the design approach, and the set of best practices. It provides resources for an agroecological transition but is rarely referred to by scientific literature or itself refers to scientific literature. The lacking attention from the scientific community is attributed to an "idiosyncratic use of scientific terms, [and] the spreading of scientifically unproven claims (Krebs & Bach 2018)" by some practitioners. The use of pseudo-scientific theories on and oversimplification of social and ecological systems is also criticized. However, community-based research and cooperations with scientific institutions are arising increasingly, resulting in more publications about permaculture in peer-reviewed journals in the last years (Morel *et al.* 2019).

Permaculture in its practical execution has many analogies with other alternative farming systems, namely organic agriculture, biodynamic agriculture, agroforestry, and agroecology. They all strive towards a resource-efficient, pesticide-free farming approach with biological regulation, high biodiversity and local nutrient cycling (Krebs & Bach 2018).

Specific to permaculture is the focus on the design process, rather than on distinctive techniques (Morel *et al.* 2019). Krebs & Bach (2018) underline that most methods used in permaculture have not been newly invented, but available methods are investigated and adopted.

3.2.2.4 Conservation Agriculture (Alena)

The 1930s Dust Bowl in North America was the cause of massive soil and water degradation that was intensified by large-scale mechanised tillage. It triggered no-till, minimum tillage, ridge tillage and similar approaches to tackle soil erosion and C efflux by wind (Mitchell *et* *al.* 2019). In the 1960s and 1970s, highly effective herbicides, injection of fertilisers and direct seeding were introduced to agriculture that alleviated the need for tillage. On top of that, the US-government started incentivising no-till systems and herbicide-resistant GMO crops came onto the market in the 1990s, further disseminating the movement towards reduced tilling (Giller *et al.* 2015).

Today, especially in the Americas and Australia, conservation agriculture is popular on large, highly mechanised farms. According to the European Conservation Agriculture Federation ECAF, about 3.3% of arable land and permanent cropland in Europe is managed as conservation agriculture, where Sweden has one of the lowest adoption rates with 0.6%. For comparison, Finland has the highest rate with 21.3%, but most European countries lie below 10% (ECAF 2021).

Conservation Agriculture is based on three main principles: minimum soil disturbance (or no-till), the maintenance of a continuous soil cover, and crop rotations with a diversification of plant species. By doing so, it is claimed that overall soil quality is improved: biological processes are nurtured that help to increase soil OM, soil aggregation, water retention, and nutrient use efficiency and reduce soil erosion and water evaporation. This has positive effects on soil flora and fauna and in turn can improve and uphold crop production (Giller *et al.* 2015; Mitchell *et al.* 2019; FAO 2021b). Advantages besides soil protection are lower production costs in comparison with conventional tillage agriculture through savings in fuel and labour. These factors are often the major driving forces of conservation agriculture, not necessarily increasing yields as often assumed. Conservation agriculture leads to an accumulation of SOC close to the surface as the soil is not mixed, however the overall effects on soil C sequestration remain vague. When legumes are part of the crop rotations, they could help to sequester C at greater depths (Giller *et al.* 2015).

Conversely, benefits of tillage can be the handling of biotic stresses like weeds, pests and crop diseases. The iterating reliance on chemical weed management in conservation agriculture promotes the emergence of herbicide-resistant weeds (Giller *et al.* 2015). So-called strategic tillage can help to mitigate this, as well as soil compaction, reduced water infiltration, runoff of soluble nutrients and vertical stratification that might arise with long-term no-till (Wortmann & Dang 2020). Outside of mechanised large-scale agriculture, conservation agriculture is implemented to a lesser extent. Discontinued or interrupted implementation of conservation agriculture due to lacking farmer support can be a problem, as benefits only arise after several years of continuous conservation agriculture (Pulido-Castanon & Knowler 2020). Especially for smallholder farmers the competition for soil residues between feed for livestock and mulching is a limiting factor. Also, hand weeding can be an additional burden, that in the context of low-income countries often is carried by women (Giller *et al.* 2015). Further, the lacking availability of technologies like seed drills and expensive herbicides are constraints for the adoption of no-till by smallholders in Africa and Asia (Lal 2004).

While traditionally conservation agriculture and organic agriculture oppose each other on account of an extensive use of herbicides in conservation agriculture, there are also organic minimum or non-inversion tillage systems that deal with stresses without synthetic inputs. These practices rely on the meticulous integration of crop rotations, cover crops and undersowings to suppress weeds and fix nutrients, organic mulches and surface composting for nutrient supply- all to build SOM, microbial biomass and mycorrhizal networks. Such techniques should boost soil fertility, as it is described as "not the result, but rather the prerequisite for no- or minimum tillage (Junge *et al.* 2020)", but could also partly counteract the reduction of labour that is a major driver behind conservation agriculture especially for large-scale farmers. Deep ripping, subsoiling or other technical solutions might be required with certain soil types to prevent subsoil compaction, which can be a result of no-till (ibid.).

There is a large body of scientific literature on conservation agriculture and its effects today. However, the principles that are applied remain rather general and do not scrutinise the ruling paradigm of industrial agriculture. Mitchell *et al.* (2019) argue for a more flexible and creative application of the core concepts of conservation agriculture in a way that they "mimic regenerative natural ecosystems (ibid)" and underline that there are no one-size-fits-all solutions. They also state that conservation agriculture will act as an important tool for sustainable intensification of agricultural production worldwide. The interventions of conservation agriculture so far have contributed to slowing but not stopping (or reversing) soil and ecosystem degradation (Mitchell *et al.* 2019). Nevertheless, some of the applied practices are essential for more sustainable farming approaches. The distinction of conservation agriculture and regenerative agriculture is not clear, some authors state that the latter is the combination of the first and holistic grazing, sometimes with organic principles. Others argue that while conservation agriculture wants to preserve the current state of the soil, regenerative agriculture wants to improve it (Hermani 2020). Burgess *et al.* (2019) conclude that conservation agriculture can be seen as one among other systems withing regenerative agriculture.

3.2.2.5 Holistic Management / Holistic Grazing (Alena)

Holistic management and holistic grazing are concepts that were established by the biologist Allan Savory in the 1970s, even though similar ideas have already come up in the 1920s (Nordborg & Roos 2016). He gained substantial prominence in 2013 after giving his TED talk *How to fight desertification and reverse climate change*. Savory's claims were widely applauded but also harshly criticised for exaggerating and lacking scientific evidence. Holistic management is also oftentimes advocated by proponents of RA.

Grazing management in general has three goals: first, higher productivity and species diversity by letting key species rest, second, lower grazing selectivity and third, more uniform animal distribution (Briske *et al.* 2008; Nordborg & Roos 2016). Holistic management is a decision-making and planning framework "to work with the web of complexity that exists in nature [to balance] key social, environmental, and financial considerations (Savory Institute 2021)" that is centered around holistic grazing. Holistic grazing is based on the approach of rotational grazing, a grazing management method where it is assumed that grazing livestock packed in herds and moved often to imitate 'natural grazing' of wild herbivores that try to evade predators can regenerate degraded land. Savory is claiming that this method should sequester C to pre-industrial atmospheric CO_2 levels. While these are grand claims that could not be confirmed, holistic grazing can be an example of good grazing management which could sequester approximately 0.35 t C ha^{-1} $year^{-1}$ on grasslands (Nordborg & Roos 2016).

3.2.2.6 Agroforestry (Alena)

According to World Agroforestry (ICRAF), "agroforestry is the interaction of agriculture and trees, including the agricultural use of trees (ICRAF 2021)". Trees provide many benefits in natural ecosystems, above all ecological stability. The specifications in combination with agriculture can be manyfold, including trees on farms, agriculture in and along forests and tree-crop production, e.g. cocoa or coffee. Agroforestry promotes the formation of a system that consists of a wide variety of niches that stabilise the ecosystem and render it biologically diverse (Leakey 2017b). Trees can provide livestock fodder, fuel, food, fertilisation, timber, medicine, shelter, shade or other ecosystem services. Beyond this, they are also of socio-cultural, aesthetic and religious value. Moreover, animal husbandry is oftentimes integrated into agroforestry systems (ICRAF 2021).

Agroforestry dates back to prehistoric times but scientific investigations only arose in the 1970s and focused on the tropics (Ramachandran Nair 2013; Udawatta *et al.* 2017). However, it can be practiced everywhere where trees or other woody perennials grow (Newman 2019) and is not restricted to specific geographic areas. In some definitions it relates to the welfare and reduction of poverty in rural communities and focuses on smallholder production (Leakey 2017b).

Traditionally, practices in agroforestry include intercropping with trees, shaded perennial cash crops, silvopasture, windbreaks and the establishment of trees for land rehabilitation and regeneration in fallow periods. The multipurpose use of trees can provide long-term concepts for CC mitigation, reduce loss of biodiversity, increase food security (Ramachandran Nair 2014) as well as restore degraded soils and sequester C below and above ground, making it a next-best alternative to C sequestration in native forests (Ollinaho & Kröger 2021). It is also referred to as an approach to sustainable intensification, especially in the tropics (Leakey 2017a). Ollinaho & Kröger (2021) further delineate social benefits like preventing rural exodus, malnutrition, CC risks and the economic takeover of few agribusinesses.

The positive impacts of agroforestry have been researched and underpinned likewise by academia and international organisations. Agroforestry also stands out as a field where participatory research with "real-life" practitioners is customary. However, the focus remains on farm-level practices and large-scale investigations like the influence on transitions within the global food system are yet to be made. There is also a risk of co-option of the term by large-scale agribusiness and drivers of forest degradation (Ollinaho & Kröger 2021).

3.2.2.7 Climate-Smart Agriculture (or Climate-Resilient Agriculture) (Alena)

Climate-smart agriculture represents a set of strategies and guiding actions to transform agricultural systems in order to ensure food security in a changing climate. It is an iterative process that aims at overcoming challenges connected to CC and finding ways of sustainable transitions (Lipper *et al.* 2014; Steenwerth *et al.* 2014). There are three main objectives in climate-smart agriculture: "sustainably increasing agricultural productivity and incomes; adapting and building resilience to CC; and reducing and/or removing GHG emissions, where possible (FAO 2021a)". One integral part is the identification of synergies and trade-offs between different objectives above as well as the support of the prioritisation process by assessing different technologies (Lipper et al. 2014). Thus, climate-smart agriculture is outcome-based and focuses on CC adaptation and mitigation. Evidence-based strategies and coordinated efforts between farmers, researchers, the private sector, civil society and policy makers shall help to meet the need for food, fuel, and fibers (Lipper *et al.* 2014). The scale of action ranges from smallholders to transnational coalitions (Steenwerth *et al.* 2014).

3.2.2.8 Carbon Farming (Lærke)

There are several, sometimes conflicting, definitions of Carbon Farming. Toensmeier (2016) describes Carbon Farming as "a system of increasing C in terrestrial ecosystem[s] for adaptation and mitigation of climate change, [to] enhance ecosystem goods and services and trade carbon credits for economic gains." The book is one of the prominent books in linking C sequestration research to RA practices (Hermani 2020). Generally, *carbon farming* is a term for practices that mitigate and sequester C, including active (IPCC 2019) or co-beneficial (Toensmeier 2016) adaptation to CC. Some expanded definitions include C offsets, where C sequestration is rewarded by e.g. higher product prices or by selling credits to emission entities (Toensmeier 2016). Carbon offsets have the potential to enhance practices that increase C sequestration, and co-beneficially improve other ecosystem services, but have often proven to encourage monoculture plantations instead, causing decreases in biodiversity, substituting natural landscapes, and potentially decrease the C sequestration dependent on the substituted land use (Lin *et al.* 2013).

Carbon farming practices include the increase of C in biomass above and below ground. The basic strategy is to enhance net primary production (NPP) and net ecosystem production (NEP) to increase the photosynthetic flow of atmospheric CO_2 to biomass C, and further increase SOC and SIC to sequester C in the soil (Lal *et al.* 2018). While there is no universal practice to create a positive C budget, identification of context-specific practices is necessary. The basic strategy is to maintain continuous soil cover, replace harvested nutrients, enhance soil structure and rhizosphere processes, and improve eco-efficiency by reducing general losses (e.g. soil erosion, C loss, or nutrient leaching) (ibid). Examples of such practices include integration of perennials and woodland, increased crop diversity, cover cropping, no-till or conservation tillage, agroforestry, improved fertiliser use, addition of organic amendments and biochar (Lal 2004; Bates 2010; IPCC 2019). These practices have the potential to increase other biological factors (e.g. microbial activity) and thus enhance ecosystem services such as increases in biomass productivity, water purification, reductions in energy and fertiliser use, and increases in biodiversity (Bates 2010). In general, the practices mentioned in carbon farming and RA are similar, but carbon farming has a more narrow, thus more detailed focus on quantification of C sequestration for the individual practices.

3.2.3 Debates in RA

3.2.3.1 Context! Or: REgeneration of what? (Alena)

When talking about regeneration, one must ask what is to be REgenerated. In the context of RA it is mostly soil, and more precisely SOC. The semantics of the word imply a state of degradation, it holds an inherent notion that the object should develop into how it used to be before it had been deprived. Accordingly, regeneration can only take place on land that has been degraded, hence the previous land use is pivotal to RA. Implementing RA on grassland or forest and thus converting it to cropland is pointless – even if it is to be a sustainable agriculture, it cannot be regenerative. The conversion from a natural to a managed ecosystem always decreases C stocks, and causes gaseous emissions (Lal *et al.* 2018).

Furthermore, an important aspect that is often overlooked in RA discussions are the wide variety of starting points, local environments, and scales of operation. No panacea for C sequestration exists, and biophysical, social, economic and cultural considerations have to be taken instead of blindly prescribing individual practices to all agricultural contexts (Lal *et al.* 2018; Giller *et al.* 2021). Further, the storage capacity of SOC highly depends on soil characteristics like soil and horizon depth, texture, mineralogical composition, available water capacity and nutrient reserves, as well as landscape characteristics like terrain, position, and drainage, and historic C losses from the soil. Activities that build organic C in one soil might be ineffective in another soil (Lal *et al.* 2018; Bossio *et al.* 2020).

3.2.3.2 Some dichotomies: Organic vs. conventional, single practices vs. holistic (Alena)

We identified two main unresolved questions in the definition of RA, namely the use of chemical inputs and the use of single practices in an unchanged system.

Firstly, there is a disagreement regarding the rejection of synthetic inputs being a precondition for RA. An argument is that occasionally herbicides are necessary to avoid tillage, with no-till being seen as one of the main impact points of RA. Hermani (2020) observes that, if RA is understood like this, it might be more attractive for conventional farmers that feel too restricted by the standards and ideologies of organic agriculture. RA thus might be able to bridge the gap between organic and conventional farmers with conservation tillage as a smallest common denominator. However, many proponents agree with the Rodale Institute (2014) that organic and regenerative farming are closely intertwined and the latter cannot exist without the first. Further, “organic” is protected globally, whereas “agro-ecological” and “regenerative” are not, providing the opportunity for misuse and greenwashing with a vague concept (Beste 2019). The term *regenerative organic agriculture* has thus been coined to make a clear distinction between the two RA fractions (Rodale Institute 2014; Giller *et al.* 2021).

Second, for some practitioners every practice that could potentially increase SOC is perceived as regenerative. This can be compared to conventionalised organic farming where often little emphasis is put on the complex interactions of an agroecosystem and socio-political impacts. The main interest of many new actors in RA is the exploration of a new market in an unchanged industrialised agricultural doctrine. Here, the more general question arises whether global agribusiness can be allies moving towards more sustainable food systems (Hermani 2020). Another doubt is whether RA can exist in large scale agriculture, fulfilling its promises concerning diversification, enhanced ecosystem services, and resiliency. Further, social subjects like food sovereignty and the impact on rural communities which according to some actors are a vital part of RA are rarely addressed by large agribusinesses using the term. However, RA holds the potential for a fundamental redesign of food systems by changing the narrative of food production to centering soil health and SOC instead of yields.

3.2.3.3 Greenwashing and certification systems (Alena)

Regenerative is the new *sustainable* in terms of marketing for many corporations. As to date there is no collective understanding of RA, it is threatened to become a buzzword, being co-opted by businesses, and facilitating greenwashing. It is easy for companies to use it in their own interest without being held accountable for specific actions or outcomes. Danone, Unilever, General Mills, and Bayer/Monsanto are only some of the businesses that are trying to depict themselves as innovative leaders of the movement (Koehn 2021). Certification schemes could help with the development of clear frameworks for RA that avoid misuse and co-option of the term. On the other hand, it could also lead to increased tensions between opposing interpretations of RA (Hermani 2020).

Toensmeier (2016) argues that national governments will be slow to promote carbon farming and/ or RA. In the meantime, certification systems could incentivise farmers in the transition towards more climate-positive farming practices, as well as remunerate those that already apply them. Newton *et al*. (2020) add to the discussion that there can be process-oriented and outcome-based certifications, the first being more common but involving a certain amount of trust towards the producer. Outcome-based certifications are more robust and raise less concerns towards the actual effects of the applied practices. While in outcome-based certification systems farmers need to prove specific improvements, which might make them more popular with consumers, assessments might be expensive and make products less accessible. The Europe-based platform Climate Farmers advocates for an outcome-based scientific evaluation of regenerative practices (Climate Farmers 2021). Organisations that currently develop certification schemes are for example the Sustainable Agriculture Network, the Regenerative Organic Alliance (ROA), and a certification programme developed by the Savory Institute (Toensmeier 2016; Newton *et al.* 2020).

The most advanced is the US-based Regenerative Organic Certification (ROC) by the ROA which is led by the Rodale Institute and includes board members from companies and organisations like the Fair World Project, Dr Bronner's, Patagonia, and Textile Exchange (Regenerative Organic Alliance 2021). On top of the USDA organic label, it has three main criteria, namely soil health, animal welfare, and social fairness. Their long-time goals are to tackle the "climate crisis, factory farming and fractured rural economies (ibid)" and the certification involves three levels, working together with other certification systems like *Demeter, Naturland* and *Fairtrade* to avoid overlaps (Regenerative Organic Alliance 2018).

Several authors (e.g. Al-Kaisi & Lal 2020; Lal 2020; Newton *et al.* 2020) also add to the discussion that farmers should be incentivised for ecosystem services like C sequestration, improvement of water resources, and strengthening biodiversity through payments. In Europe, the EU Soil Framework Directive failed to enter into force, however the New Soil Strategy is currently in progress. In the New Common Agricultural Policy of the EU at least 25 % of direct payments have to be paid for so-called "eco schemes", under which carbon farming is mentioned (European Commisssion 2021a, b, c).

3.2.3.4 Roots in Indigenous traditions (Lærke)

In many interpretations, RA openly states to be rooted in indigenous practices and cultures (Toensmeier 2016). While intercropping, polycultures, and agroforestry are seen as new trends in modern agriculture in Western cultures, these are practices that have existed for hundreds of years within indigenous agricultural practices. Practices like intercropping, agroforestry, rotational animal grazing, and legumes for N-fixation are historical and contemporary common practices within American Indigenous communities (Heim 2018).

Agricultural practices originating from indigenous and local knowledge can contribute to overcoming the combined challenges of CC, food security, biodiversity conservation and land degradation (IPCC 2019). Observing and learning from what makes traditional agroecosystems more resilient can help climate-adapted farm designs (Altieri *et al.* 2015). It is important to acknowledge that the adaptation of indigenous knowledge onto the Western perception of agriculture to date is far from the cultural mindset of indigenous communities. The statement that RA and permaculture are the solution to the climate and ecological crisis have been criticised by more than ten indigenous community leaders in an online statement shared on different environmental blogs (Angarova *et al.* 2021). The critique raises awareness to a fundamental misunderstanding of indigenous cultures. While practices like intercropping, agroforestry, and N-fixating crops are adapted by RA, the more philosophical worldview is ignored. The authors raise the opinion that adoption of individual traditional indigenous practices to a food system which is permeated with over-consumption, RA and permaculture is contributing to the continuous erasing of indigenous history. Deeper cultural change must be included to address and realise the need for a collective healing of the world, based on systemic changes. According to indigenous leaders, the main issue with RA and permaculture is the understanding of nature as something we have to mimic (biomimicry) to optimise agriculture, whereas indigenous languages even often lack the word for nature because they view humans as part of nature, where the land owns the people instead of to the other way around (Angarova *et al.* 2021).

While RA has the potential to optimise the agricultural pattern within our western culture, indigenous communities are still mostly excluded from the discussion. Not only consumption patterns would have to be changed, but adaption of policies addressing disproportionality of land rights and barriers to participation in sustainable land management will be essential (IPCC 2019).

3.2.3.5 Science and practitioner collaboration

A rising enthusiasm for RA emphasises that agronomists need to engage in the public debate and learn to better communicate their appraisals on the topic (White & Andrew 2019; Giller *et al.* 2021). While farmer-to-farmer communication of success stories can be a very potent mean of catalysing change (Rosenzweig *et al.* 2020), there seems to be a lot of criticism from the side of science as many advocates for RA or related practices have been proven to exaggerate and fallaciously upscale their field-scale results (e.g. Nordborg & Roos 2016; McGuire 2018). Based on such claims, some scientists reject RA completely, while others acknowledge the exaggeration without turning down the general message, and call for researchers to view it as an opportunity to investigate new approaches towards agricultural systems (Toensmeier 2016; Hermani 2020).

Sustainability problems cannot be solved by science alone but need the interaction of many stakeholders and their interests. Farmers with a tendency to search for traditional practices have been found to be more sceptical and misunderstanding the diversity in academic research (Fairweather 1999). White & Andrew (2019) call it a "clash of cultures" between "orthodox soil scientists" and "alternative" practitioners, where both sides have the same goal of improved soil and land management but lacking mutual understanding.

More practice-oriented research is needed, where a closer relation between scientists and practitioners can enable mutual learning and insights. This raises the question, whether contemporary scientific methods, especially in agronomic sciences provide appropriate tools to investigate all impact levels of alternative agricultures. For many years, research on sustainable agriculture was performed on individual plot, field, or farm level, while many environmental issues associated with agriculture, biodiversity, water quality and CC, are manifested at larger scales. Sustainability has different levels of scale, and while studies on field or farm level can answer questions on agronomic and micro-economic sustainability, a study on communities will be more suitable to answer questions on social sustainability

(Robinson 2009). This also means that research should deter from only looking at single practices, but focus on interactions of several practices as applied in local contexts and on real-life farms (Robinson 2009). Rodale Institute (2014) criticises the sometimes reductionist methods of agricultural sciences and underlines that the advocated practices are not meant to be implemented or judged in isolation. Nevertheless, feedback from scientists to practitioners about the measurable impact of their practices and their possible future applications is indispensable and will enable further improvements and proliferation. Some methods promoted by practitioners however do not yet have scientific underpinnings, which also needs to be communicated aptly. Altogether, this debate would highly profit from communication experts within the different interest groups that can translate findings in an understandable manner to all stakeholders (White & Andrew 2019). Initiatives like "4 per 1000" introduced at COP21 are in need of a close liaison between farmers, land managers, policy makers and the academic community worldwide (Singh *et al.* 2018).

3.3 Management Information (Lærke)

Management practice values (Table 5) show that wide ranges were represented within the analysed farms. Between all fields, management intervals varied from no C added to a maximum of 139.1 t C ha^{-1}, P12 and P19 had very high C added values of 100.4 t C ha^{-1} and 139.1 t C ha^{-1} respectively, compared to the mean of 27.21 t C ha^{-1}. These two fields were both vegetable fields with a permanent mulch layer, additionally biochar was added to P19. Crop diversity values ranged between monoculture with an index value of 1 to a broad variety of crops and an index value of 74 for untouched fields. For tillage practices within the timeframe 2015 – 2020, farms with 0, 2, 3, 5 or 6 years without tillage were part of the analysis. Looking at specific crop distribution, the spread of the values was smaller for legumes than for perennials. Legumes included values up to 50-70 %, whereas all intervals were present for perennials.

Table 5: Quantified management information for individual fields

Farm field	Type	Category	Total C added [t C ha^{-1}]	Years without tillage	Crop Div. Index	Legumes [% of species]	Perennials [% of species]
P1	Farm	R	5.9	6	7.5	10-30	30-50
P2	Farm	C	10.4	0	1	<10	<10
P3	Farm	R	6.8	6	5	10-30	<10
P4	Farm	R	6.8	6	7.5	10-30	30-50
P5	Farm	R	18.9	5	74	50-70	50-70
P6	Farm	C	7.5	0	3	10-30	10-30
P7	Farm	T	16.2	2	10	10-30	10-30
P8	Garden	R	42.3	5	18	<10	<10
P9	Farm	C	0	2	9	<10	<10
P10	Garden	T	60.5	6	6.75	<10	<10
P11	Garden	T	66.1	6	1	<10	<10
P12	Garden	T	100.4	6	4.8	10-30	10-30
P13	Garden	T	32.9	6	4	<10	<10

P14	Farm	T	0.3	6	25	<10	70-90
P15	Farm	R	0.6	6	36	<10	>90
P16	Farm	R	0.6	6	36	<10	10-30
P17	Farm	R	3.0	5	74	50-70	70-90
P18	Garden	T	17.0	6	36	10-30	70-90
P19	Garden	T	139.1	6	4.5	<10	<10
P20	Farm	T	0.3	6	16	<10	70-90
P22	Farm	T	0.6	6	16	<10	70-90
P23	Farm	R	8.4	6	36	10-30	>90
P24	Farm	R	7.6	3	7.5	50-70	50-70
P25	Farm	R	16.3	6	36	10-30	>90
Min			0.0	0	1	<10	<10
Max			139.1	6	74	50-70	>90
Mean			27.21	4.77	21.14	17†	95†
Median			8.00	6.00	9.5	5†	41†
Std dev			35.18	1.95	20.85	5†	20†

† = estimated mean based on interval values, R = regenerative, T = transition, C = control.

Divided into the farm categories, control, transition, and regenerative (Table 6), it becomes clear that in the control group only the lower range of management practice values are present, as determined by the categorisation of the farms. For amount of C added, the control group has the lowest average, whereas transition farms include the highest values. The control group further includes the highest intensity in tillage and lowest index values for crop diversity, compared to transition and regenerative farms. Tillage values do not differ distinctly between regenerative and transition farms, whereas regenerative farms have a higher mean of crop diversity, compared to transition farms.

Table 6: Management information by farm categories: control, transition, and regenerative

	Control			Transition			Regenerative		
	Amount of C added [t ha^{-1}]	**Years without tillage**	**Crop div. index**	**Amount of C added [t ha^{-1}]**	**Years without tillage**	**Crop div. index**	**Amount of C added [t ha^{-1}]**	**Years without tillage**	**Crop div. index**
Min	0	0	1	0	2	1	1	3	5
Max	10	2	9	139	6	36	42	6	74
Mean	6	1	4	43	6	12	11	6	31
Median	8	0	3	25	6	8	7	6	36
Std dev	5	1	4	48	1	11	12	1	25

3.4 Soil Parameters

3.4.1 Basic Soil Characteristics

The 24 plots of this study belong to five textural classes: sand, loamy sand, sandy loam, sandy clay loam, and clay loam (*Figure 13*) classified after the Soil Survey of England and Wales (Moeys 2018).

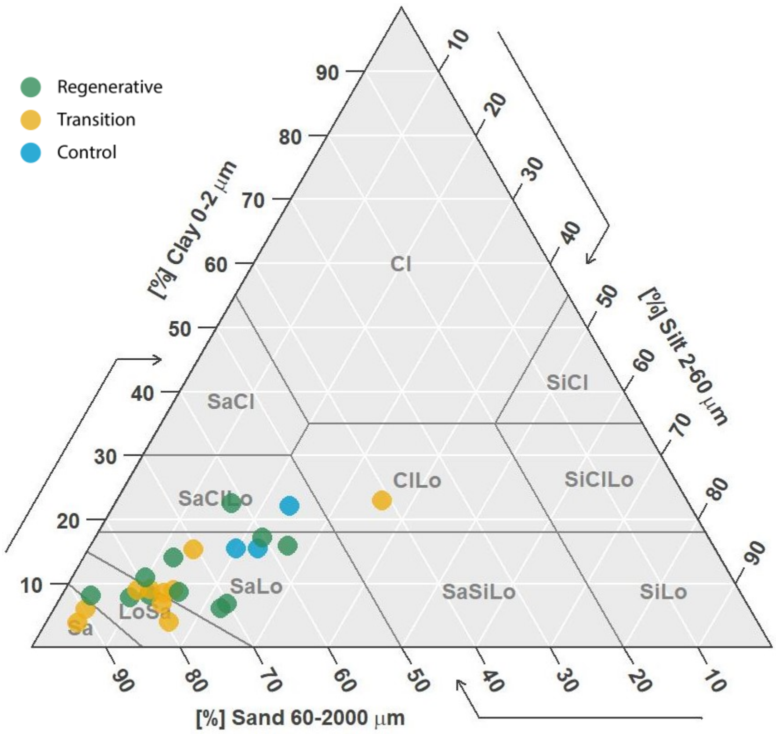

Figure 13: Soil texture displayed in texture triangle after the Soil Survey of UK and Wales.

Sand, silt, and clay content varied from 41.1 % to 91.8 %, 4 % to 35.9 %, and 3.9 % to 23, respectively within all 24 plots (Table 7).

Table 7: Soil texture classes based on the Swedish size classes

	Sand (60 – 2000 µm) [%]	Silt (2 – 60 µm) [%]	Clay (< 0.2 µm) [%]
Min	41.1	4	3.9
Max	91.8	35.9	23
Mean	72.28	16.11	11.6
Median	76.00	14.5	8.9
Std dev	12.14	7.72	5.74

The grouped results by the three defined farm categories (Table 8) revealed that only control fields with sandy loam and sandy clay loam are included in this study. The only clay loam was P7, a transition plot containing the lowest sand fraction of all plots. In general, the control plots were less sandy and more clayey than transition and regenerative plots.

Table 8: Soil texture by farm categories

	Control			Transition			Regenerative		
	Sand [%]	**Clay [%]**	**Silt [%]**	**Sand [%]**	**Clay [%]**	**Silt [%]**	**Sand [%]**	**Clay [%]**	**Silt [%]**
Min	54.1	15.5	19.9	41.1	1.08	4.3	57.4	6.1	4.0
Max	64.6	22.1	23.8	91.8	9.32	35.9	87.9	22.5	26.7
Mean	60.1	17.7	22.2	76.6	4.06	13.9	72.7	11.5	15.8
Median	61.6	15.5	22.9	79.2	2.08	13.8	73.7	8.7	15.6
Std dev	5.41	3.81	2.04	13.93	3.38	8.80	9.76	5.22	7.10

A summary of values for BD, pH and EC can be found in Table 9. Bulk densities ranged between 0.58 g cm^{-3} and 1.63 g cm^{-3}. The forest plot P16 and vegetable plots P12, P13, P19 had BD values below the expected range for cultivated loamy soils (Brady & Weil 2014). The lowest BD value came from P12, a shallow soil profile which had been built up considerably through the addition of organic amendments over the last years. The same profile also shows the highest TOC content with 9.32 % (Table 11). Basic chemical soil parameters, like pH and EC results were found to comply with expected values for arable soils, with a pH range of 5.5 – 8 for optimal nutrient uptake (Moebius-Clune *et al.* 2016) and between 1.00 and 1.8 g cm^{-3} for bulk density, together with values below 1,00 g cm^{-3} for organic soils (Brady & Weil 2014). The pH values range close to neutrality, while an average of 7.41 fit to the more calcareous soils found on Gotland. Inorganic C values are similarly high for Swedish soils. Electric conductivity values were all below 2 g cm^{-3}, which is generally assumed to be the lower growth limit for sensitive plant (Brady & Weil 2014)[2].

Table 9: Basic soil characteristics

	Bulk density (BD) [g cm^{-3}]	**pH**	**Electric conductivity [µS cm^{-1}]**
Min	0.58	6.2	53
Max	1.63	8.1	745
Mean	1.31	7.4	245
Median	1.38	7.6	195
Std dev	0.26	0.5	176

3.4.2 Physical Soil Parameters

Results for physical soil parameters are presented in (Table 10). Plant-available water matched the expected range for sandy soils (Table 4) since it was directly calculated from measured sand and clay content. One exception is profile P7, where the PAW content of 14 % matched a loamy soil, according to its lower sand content of 41.1 % and higher clay and silt content of 23 % and 35.9 % respectively. Wet aggregate stability had relatively high values compared to other agricultural soils according to (Moebius-Clune *et al.* (2016). It was

[2] Note that 1 µS cm^{-1} = 0.001 dS m^{-1}

generally higher in aggregates > 2mm, with a mean value of 81.8%. P12 showed an exceptionally high WAS in both fractions of above 98%, and P4, P13, P19, and P23 also have values > 80% for both particle sizes. The lowest values for large aggregates were found on P1, P6 and P25. P8, a regenerative plot, had comparatively low values in both classes with 38.3 % and 69.2 % for small and large aggregates respectively. Comparing WAS across farm categories (Appendix 1), the lowest mean was found in the control group. Infiltration rates were > 100 mm h^{-1} and thus highest for the vegetable fields from gardens, including P8, P10, P11, P12, P13, and P19. These plots all had a layer of mulch on top of the measured A-horizon. Additionally, results of infiltration rates for the mulch layer were 758.5 mm h^{-1}, 879.8 mm h^{-1}, 1804.1 mm h^{-1}, and 2122.1 mm h^{-1} for P11, P13, P8, and P12, respectively. The penetration resistance was between 0.42 MPa and 3.85 MPa, with no clear pattern between farm categories or land use. For penetration resistance, values are missing from plot 13.

Table 10: Physical soil parameters

	WAS (0.25 – 2 mm) [%]	WAS (> 2 mm) [%]	Infiltration rate [mm h^{-1}]	PAW [% Volume]	Penetration resistance [MPa]
Min	38.3	64.7	8.3	5	0.42
Max	98.7	98.9	253.0	14	3.85
Mean	68.7	81.8	96.9	9	1.89
Median	69.1	83.3	69.2	10	1.85
Std dev	16.5	9.4	72.0	2.09	0.73

3.4.3 Chemical Soil Parameters

Carbon-related parameters are presented in Table 11. The majority of TOC values ranged from about 11 to 41 g kg^{-1} soil, however there were some values > 80 g kg^{-1} soil in the transition plots P12, P13, and P19. Total organic carbon was lower in the control plots with 15 g kg^{-1} soil than in the regenerative and transition plots with 24 g kg^{-1} soil and 41 g kg^{-1} soil, respectively. Transition had a high mean for IC but also a large standard deviation of 34 g kg^{-1} soil A similar pattern could be seen for AC, where the highest results were found within transition plots, with values > 2800 mg C kg^{-1} soil in the vegetable plots P12, P13, P18 and the grazing plot P19. Microbial biomass was very high in P12 with 1248.1 µg C g^{-1} soil, and > 800 µg C g^{-1} soil, in P13 and P25. Relating MBC to TOC showed a different pattern, with the highest ratios in regenerative plots P5, P15, and P23 being > 0.6 and one high value of 0.7 on control plot P2. C:N ratio had the lowest mean for the control group and the highest for the transition group. It was lowest in P7, a transitional fodder field and highest in the vegetable fields P8, P10, P13, and P19, except for P18, a transitional grazing field.

Table 11: Carbon-related soil parameters

	Organic C † [g kg^{-1} soil]	IC † [g kg^{-1} soil]	Active C † [mg kg-1 soil]	Microbial biomass C † [µg C g-1 soil]	MBC:TOC [%]	C:N
Min	10.8	0.0	1907	146	0.46	6.95
Max	93.2	34.1	2928	1248	3.96	12.54
Mean	31.6	9.7	2447	510	1.90	9.57
Median	22.2	4.3	2455	447	1.63	9.33
Std dev	23.7	9.9	283	249	0.96	1.38

† Related to dry weight

Other chemical soil parameters are presented in Table 12. Total N had the lowest mean for the control group and the highest for the transition group. Similarly, total N mean values were 3.2 g kg^{-1} soil for most fields, where outliers were P12 and P13 with 9.5 g kg^{-1} soil and 6.9 g kg^{-1} soil respectively, as well as the transitional vegetable field P19 with 7.3 g kg^{-1} soil. Nitrogen loss was the highest for the control group and lowest for the transition group after the first leaching simulation. After the second leaching simulation, the N loss was the highest for the transition group and lowest for the control group (Appendix 1). The range of P loss was broader than for the N loss, whereas the means of the P loss were much lower compared to the N loss for both simulations. Contrary to the pattern of N loss, the second simulation had higher values of P loss than the first simulation.

Table 12: Chemical soil parameters

	Total N [g kg^{-1} soil]	N loss 1st simulation [µg L^{-1}]	N loss 2nd simulation [µg L^{-1}]	P loss 1st simulation [µg L^{-1}]	P loss 2nd simulation [µg L^{-1}]
Min	1.2	799	1000	37.7	21.8
Max	9.5	157000	124000	46200	52400
Mean	3.2	38824	21126	5223	6389
Median	2.3	19050	6305	457	438
Std dev	2.1	43762	28369	10255	11649

3.4.4 Biological Soil Parameters

Biological soil parameters are presented in Table 13. Results for vegetation density for all soils range from 0 % to 100 %. The same range was present for transition and regenerative farms, whereas only farms with a vegetation density ≤ 40 are represented in the control group (Appendix 1). The mean for vegetation density of 47 % for transition farms was lower than for regenerative farms with a mean of 76 %. One control farm P2 and three vegetable fields P8, P10, and P11 had a vegetation density of 0%. Root abundance values > 60 all include big roots, which are counted the same as 10 small roots (see 2.3.18). The forest plot P16 and plot P22 next to a forest has root abundance values > 100. Both the ranges and averages were higher for regenerative and transition farms, compared to control farms. For root depth and earthworm counts there was not much difference detected in the average between the farm categories, but the range of values is greater for regenerative and transition farms compared to the control (Appendix 1).

Table 13: Biological soil parameters

	Vegetation density [%]	Root abundance [†]	Root depth [cm]	Earthworm number [#]
Min	0	10	15	0
Max	100	130	55	26
Mean	57	54	37	8
Median	65	50	39	5
Std dev	42	32	9	7

† index value

3.5 Principal Component Analysis (PCA) (Alena)

In a biplot of the PCA (*Figure 14*) scores and loadings are represented by dots and arrows respectively. In this biplot PC1 is represented on the x-axis and PC2 on the y-axis. Control plots had values between 0 and 1 on PC2 and between -1 and 0 on PC1. Regenerative plots

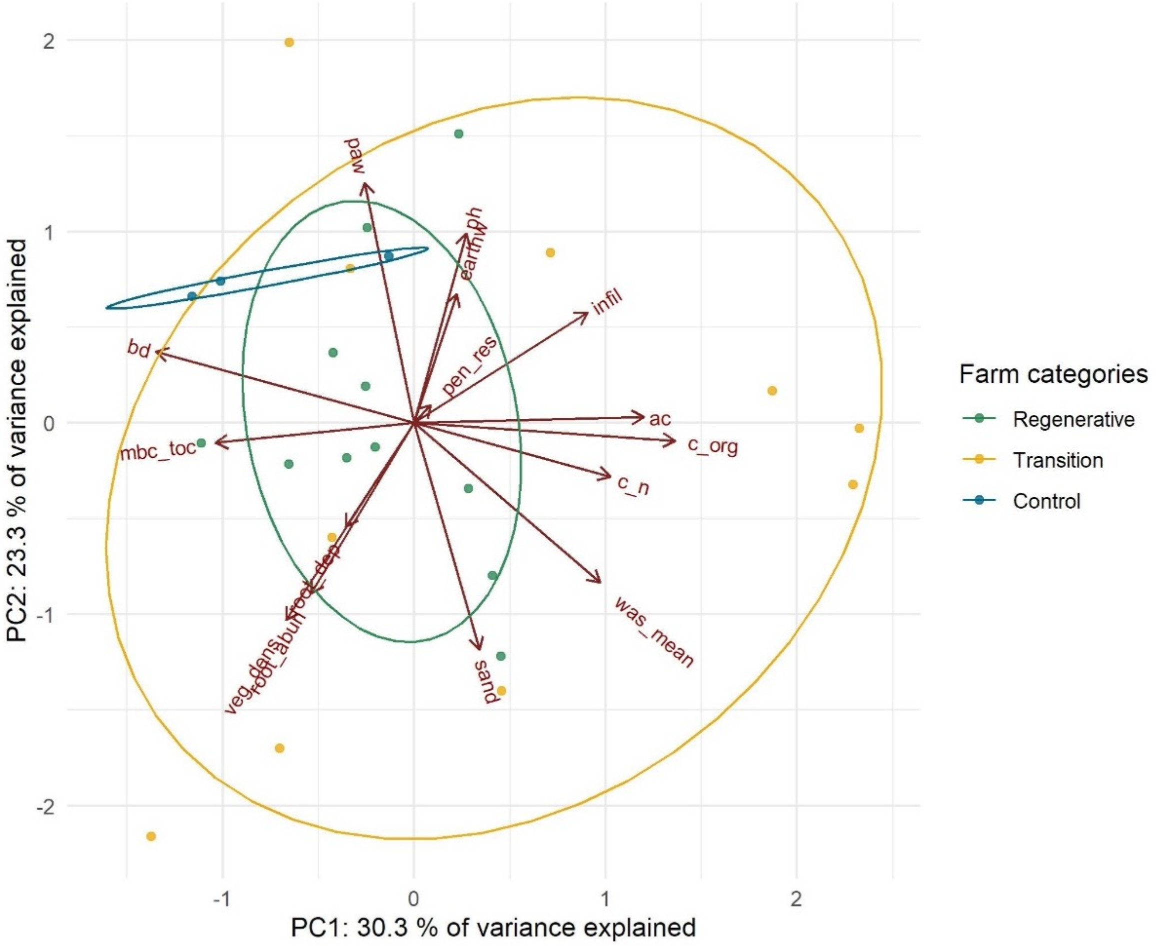

Figure 14: Biplot of principal component analysis with PC1 and PC2, showing loadings and scores, grouped in farm categories.

ac = active carbon, paw = plant-available water, bd = bulk density, c_org = organic carbon, c_n = C:N ratio, earthw = number of earthworms, infil = infiltration rate, mbc_toc = MBC:TOC ratio, pen_res = penetration resistance, ph = pH, root_abun = root abundance, root_dep = root depth, sand = sand fraction, veg_dens = vegetation density, was_mean = mean wet aggregate stability.

were found in the middle on both PCs, mostly around -1 to 1. Transition plots were more scattered, some scoring low on PC2 and around 0 on PC1, while others scored high on PC2 and around 0 on PC1, another group scored high on PC1 and around 0 on PC2.

Overall, the biplot shows that root abundance, root depth, and vegetation density are strongly negatively related to earthworm number and pH, and BD and MBC:TOC are negatively related with C:N, TOC, and AC. Another strong negative relation can be found between sand and PAW. Penetration resistance only has low loadings on PC1 and PC2 but very high loadings on PC3 with 0.5, as can be seen in Table 14.

PC1 and PC2 are characterised by two groups of variables (Table 14). PC1 is mainly described by BD and MBC:TOC with a negative impact and AC, OC, C:N ratio, mean WAS and infiltration rate with a positive impact. PC2 is influenced negatively by pH, earthworm number, PAW, and positively with vegetation density, root abundance and depth as a dense cluster as well as sand and WAS.

Table 14: PCA loadings matrix for PC1 – PC3

	PC1	PC2	PC3
bd	-0.42265970	0.13336961	-0.048894208
ph	0.08556345	0.35342778	0.215960751
ac	0.37597570	0.01116596	0.067439978
mbc_toc	-0.32530646	-0.03715280	-0.196341873
infil	0.27845754	0.20666228	0.157460550
paw	-0.08127696	0.44862714	0.265456842
pen_res	0.02658776	0.03357183	-0.527789263
root_abun	-0.16833689	-0.31745696	0.168468282
root_dep	-0.11213957	-0.19402523	0.484591409
earthw	0.06940450	0.24051428	-0.393326792
veg_dens	-0.21003984	-0.36834875	0.195240262
sand	0.10668687	-0.42282711	-0.264785539
c_org	0.42706862	-0.03388097	0.085033735
c_n	0.32116472	-0.10055824	-0.006490911
was_mean	0.30402861	-0.29782274	0.025099643

ac= active carbon, paw= plant-available water, bd= bulk density, c_org= organic carbon, c_n = C:N ratio, earthw= number of earthworms, infil= infiltration rate, mbc_toc = MBC:TOC ratio, pen_res= penetration resistance, ph= pH, root_abun= root abundance, root_dep= root depth, sand= sand fraction, veg_dens= vegetation density, was_mean= mean wet aggregate stability

As shown in Table 15, PC1 accounts for 30.3 % and PC2 for 23.3 % of variance in the dataset. This amounts to a cumulative proportion of variance of 53.5 % explained. Considering also PC3 which is mostly described by penetration resistance and number of earthworms, a cumulative proportion of variance of 63.5% could be reached.

Table 15: Importance of principle components

	PC1	PC2	PC3
Proportion of variance explained	0.3025	0.2328	0.1153
Cumulative proportion	0.3025	0.5353	0.6505

The heatmap shows correlations between the 15 soil health indicators and 24 plots (*Figure 15*). Turquoise fields show positive correlations while yellow-brown fields show negative correlations. There are some strong correlations in the upper left-hand corner where infiltration, C:N, AC, and TOC cross with P12, P13, P19. Another cluster of positive correlations can be found with root depth, root abundance and vegetation density and P17, P24, P14, P22, P16 and P18. Negative correlations are found with pH, PAW and P14 and P22, as well in the upper right-hand corner with BD, MBC:TOC, root depth and abundance and vegetation density and P12, P13, P19, P8 and P10.

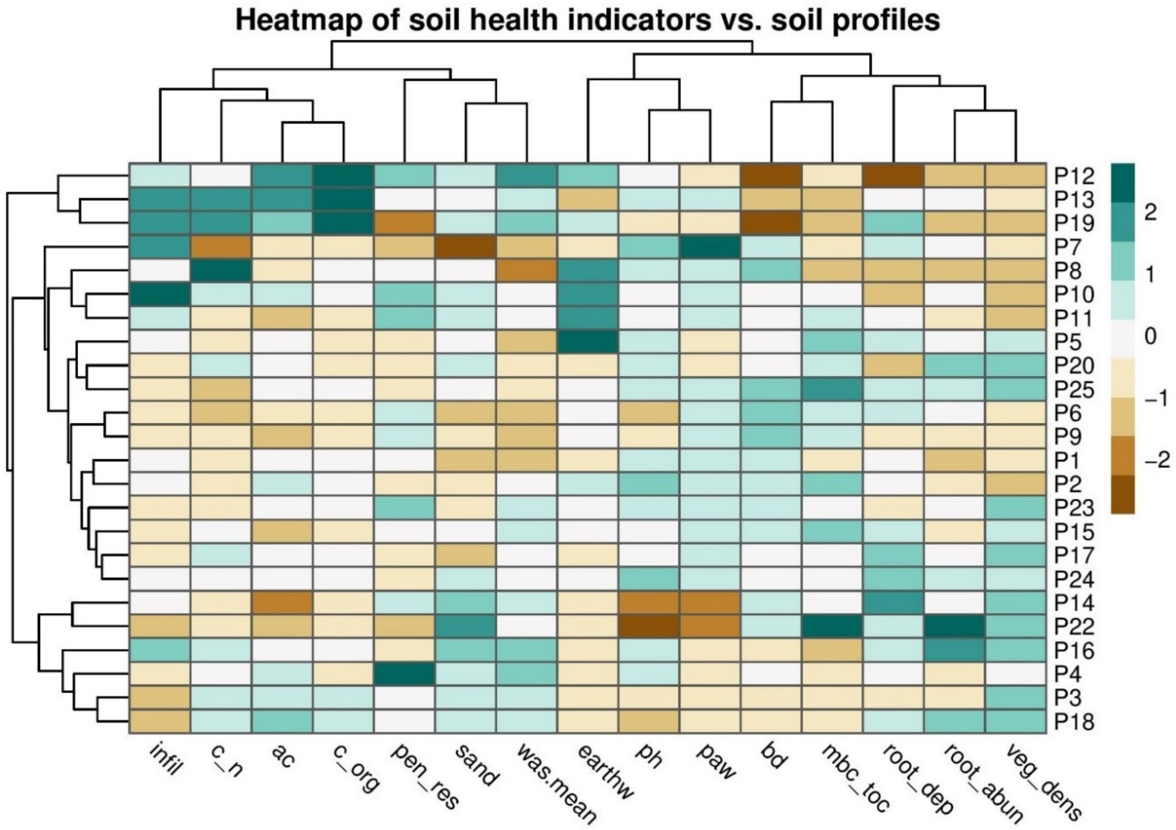

Figure 15: Heatmap showing correlations between soil health indicators and the soil profiles.

ac= active carbon, paw= plant-available water, bd= bulk density, c_org= organic carbon, c_n = C:N ratio, earthw= number of earthworms, infil= infiltration rate, mbc_toc = MBC:TOC ratio, pen_res= penetration resistance, ph= pH, root_abun= root abundance, root_dep= root depth, sand= sand fraction, veg_dens= vegetation density, was_mean= mean wet aggregate stability

3.6 Multiple Linear Regression (MLR) and Analysis of variance (ANOVA) (Alena)

The ANOVA of the MLR with PC1 as response variable and amount of C added + years without tillage as predictors shows an adjusted R^2 of 0.55, which signifies that about 55% of the data variability are explained by the regression line. A strong statistical significance ($p < 0.001$) of amount of C added and no significance of years without tillage with PC1 can be observed (Table 16). PC2 as a function of years without tillage and amount of C added could explain about 25% of the data variance, where years without tillage has a p-value < 0.01 and amount of C added had no statistical significance (Table 16). Note that explanatory variables could potentially be important in both regressions even though the components are non-correlated.

Table 16: ANOVA of multiple linear regressions with PCs and amount of C added + years without tillage

	ANOVA		adjusted R^2	Shapiro-Wilk (residuals)
	c_add	till		
PC1	***		0.55	p= 0.36
PC2		**	0.25	p= 0.95

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ° $p < 0.1$; c_add = amount of carbon added [t ha^{-1}], till = years without tillage

The function f(c_add | till) = PC1 can be expressed with the respective regression coefficients as follows:

$$PC1 = -2.01 + 0.04 * c\_add + 0.20 * till$$

It can thus be concluded that a high amount of added C and to a small degree more years without tillage positively impact PC1. Further, it can be interpreted that soil health indicators that had strong loadings on PC1 will be impacted by high amounts of C added and increased years without tillage. However, the influence on the single indicators has to be interpreted related to their positive or negative loadings on the PCs. These trends can also

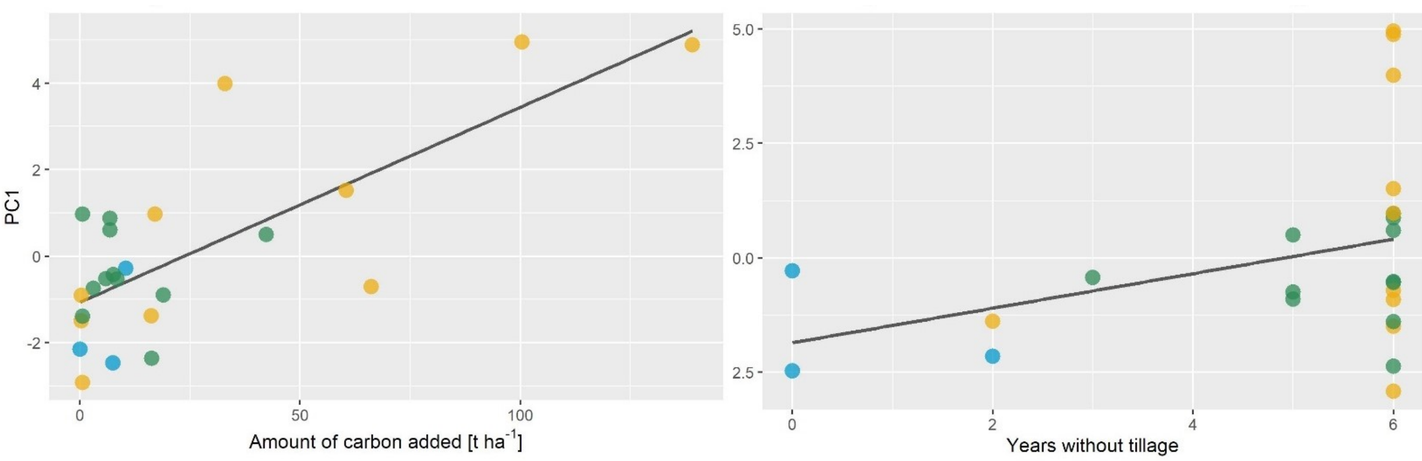

Figure 16: Separate visualisation of the linear regression model with PC1 and amount of carbon added (left) and years without tillage (right)

be observed visually in the graphs (*Figure 16*) of the linear regression models of PC1, with the amount of C plotted to the left and years without tillage plotted to the right.

In the MLR with PC2, it can be observed that amount of added C has a slight positive influence on PC2 (*Figure 17*), but as seen in Table 16, it is not significant. An increase in years without tillage has a negative influence on PC2, which can be construed as a negative

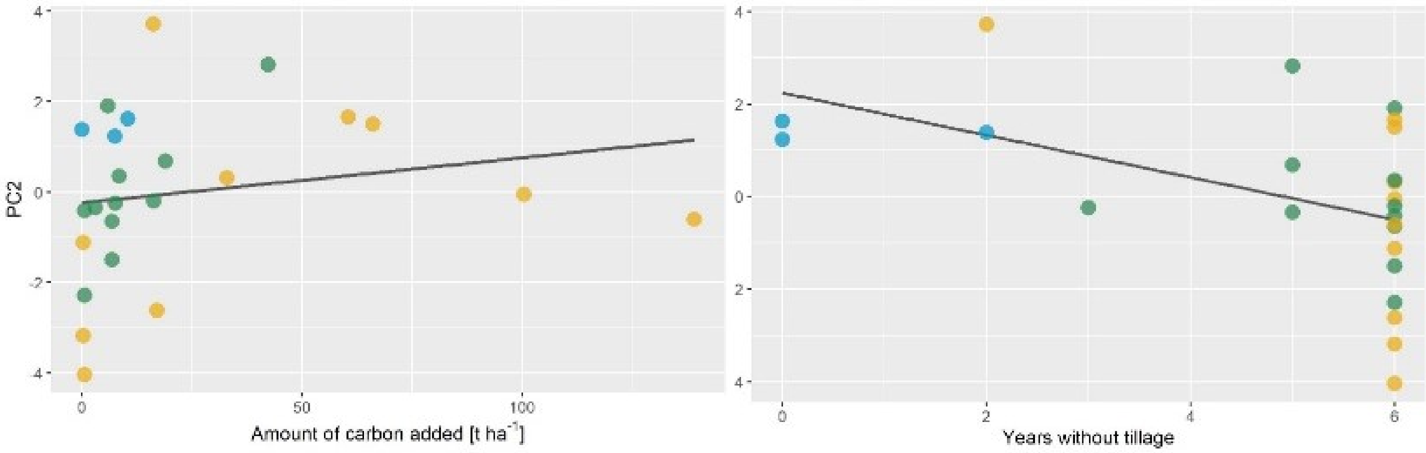

Figure 17: Separate visualisation of linear regression models with PC2 vs. amount of carbon added (left) and years without tillage (right)

influence on soil health indicators that had positive loadings on PC2, and a positive influence on indicators that had negative loadings on PC2. The function f(c_add |till) = PC2 can be expressed as:

$$PC2 = 2.18 + 0.02 * c_{add} - 0.52 * till$$

In the plots showing estimated marginal means (EMMs, also called least-squares means, (*Figure 18*) of PC1 and PC2 the data was grouped into the three farm categories as explained in the Methods chapter. EMMs are based on a linear model and not on the raw data. The blue bars show the confidence intervals for the EMMs whereas the arrows show the pairwise comparisons between the groups. Overlapping arrows suggest that there are no significant differences between the farm categories, which can be observed for PC1 and PC2. Nevertheless, some non-significant trends can be identified visually. The control group shows a lower EMM value than transition and regenerative for PC1 and a higher value

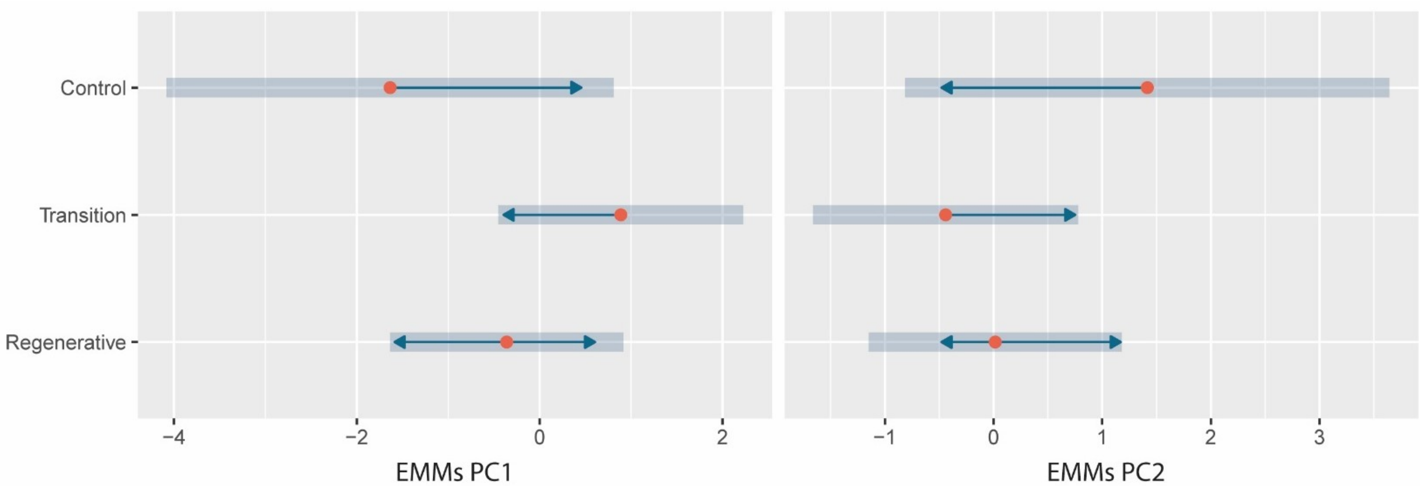

Figure 18: Estimated marginal means for PC1 (left) and PC2 (right) grouped by farm categories

for PC2. The transition group has the highest mean value for PC1 and the lowest for PC2 and the regenerative group has slightly lower and higher means respectively than the transition group for PC1 and PC2.

Similar results were found in groupwise comparisons of the EMMs of single soil health indicators. ANOVAs comparing the single health indicators, soil characteristics and PCs according to the farming groups did not show many significant differences, except for BD, infiltration, and vegetation density with $p < 0.1$ and WAS of large aggregates > 2mm with $p < 0.05$. However, significant differences were found in the ANOVAs with the management indicators crop diversity (p= 0.044), amount of C added (p= 0.061), years without tillage (p= $2.6 * 10^{-6}$) and share of legumes (p= 0.056) which supports that the management can be categorised in the three farm groups. Nevertheless, these significances are not very high, which will be further reflected on in the discussion.

Multiple linear regressions were conducted for single soil health indicators as response variables and amount of C added + years without tillage as predictor variables for interpretation (Table 17). Amount of C added has a very high significance level regarding BD, TOC, and vegetation density, a high significance level regarding infiltration rate and a mild significance level regarding AC, root abundance and number of earthworms. Tillage has a p-value < 0.01 with vegetation density and sand, together with mild significances regarding WAS and PAW.

Table 17: ANOVA of multiple linear regressions with single indicators vs. amount of C added + years without tillage

	c_add	till	adjusted R^2
bd	***		0.4721
ac	*		0.1837
mbc_toc			0.0430
infil	**		0.3396
c_org	***		0.4891
c_n		°	0.2292
was_mean		*	0.2181
ph			-0.0709
paw		*	0.1646
root_abun	*		0.1811
root_dep			-0.0475
earthw	*		0.1957
veg_dens	***	**	0.6277
sand		**	0.3053

*** p < 0.001, ** p < 0.01, * p < 0.05, ° p < 0.1; c_add = amount of carbon added [t ha^{-1}], till = years without tillage

3.7 Total nitrogen and nutrient Loss (Lærke)

For all N-related MLRs, the Shapiro Wilk test had a p-value higher than 0.05 (Table 18). Thus, the data are considered normally distributed, and the ANOVA test was applied to check for statistical significance. ANOVA results for all N-related MLRs showed p-values < 0.001 and were strongly statistically significant. Shapiro-Wilk tests on P loss after both simulations showed p-values < 0.05 and the data were thus not considered normally distributed, thus the MLRs for P losses were not accepted. Kruskal-Wallis tests were performed to compare P losses between the farm categories control, transition, and regenerative. The p-value for P losses after the first and second simulation was < 0.05 and no significant difference was found between the groups indicating that P losses in our investigated soils are not related to the included management indicators and no further analysis is performed.

Table 18: Significance test of MLRS with nutrient indicators vs amount of C added.

	ANOVA	Shapiro-Wilk	Kruskal-Wallis
n_tot	***	p= 0.09	
n_loss_6	***	p = 0.0526	
n_loss_12	***	p= 0.2235	
p_loss_6		p= $3.79 * 10^{-8}$	p= 0.4581
p_loss_12		p= $9.05 * 10^{-8}$	p= 0.7665

*** p < 0.001, ** p < 0.01, * p < 0.05, ° p < 0.1, n_tot = total N, n_loss_6 = N loss after 1st simulation, n_loss_12 = N loss after 2nd simulation, p_loss_6 = P loss after 1st simulation, p_loss_12 = P loss after 2nd simulation.

The linear regression between total N as the response variable and amount of C added as the predictor can be expressed as follows:

$$total\ nitrogen\ [\%] = 0.20 + \ 0.004 * \ c_{add}$$

This regression model had an adjusted R^2 of 0.48. The regression is visualised in *Figure 19* and show that an increase in amount of C added will result in an increase in total N in the soil.

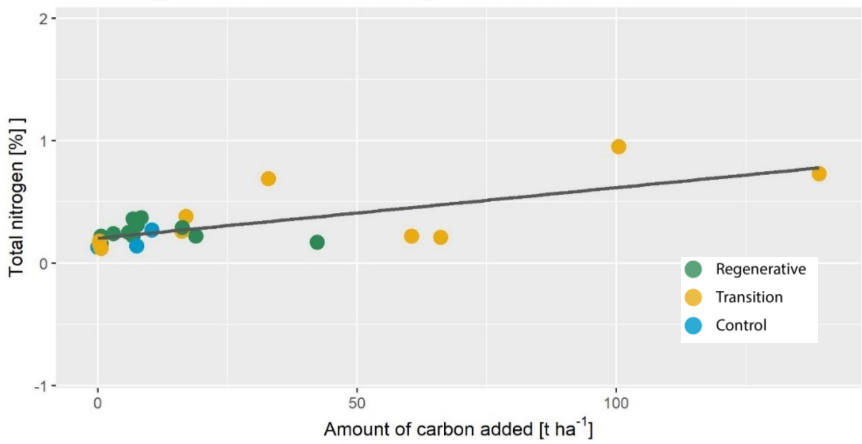

Figure 19: Visualisation of the linear regression model with total nitrogen and amount of carbon added

When looking at N loss after the two rain simulations the linear regression model with amount of C added was expressed as follows:

$$nitrogen\ loss\ (1^{st}\ simulation)[\mu g\ L^{-1}] = \ 14228 + 971.6 * c_{add}$$

$$nitrogen\ loss\ (2^{nd}\ simulation)[\mu g\ L^{-1}] = 2464 + 685.4 * c_{add}$$

The linear regression models had an adjusted R^2 value of 0.54 and 0.59 for N loss after first and second simulation, respectively. An increase in amount of C added results in a relatively high increase of N loss for both simulations. The intercept of 14228 and the slope of 971.6 is higher for the first simulation and result in a higher increase in the late N loss, when the same amount of C is added (*Figure 20: Visualisation of the linear regression model with total nitrogen after first simulation and amount of carbon added* and *Figure 21: Visualisation of the linear regression model with total nitrogen after second simulation and amount of carbon added*).

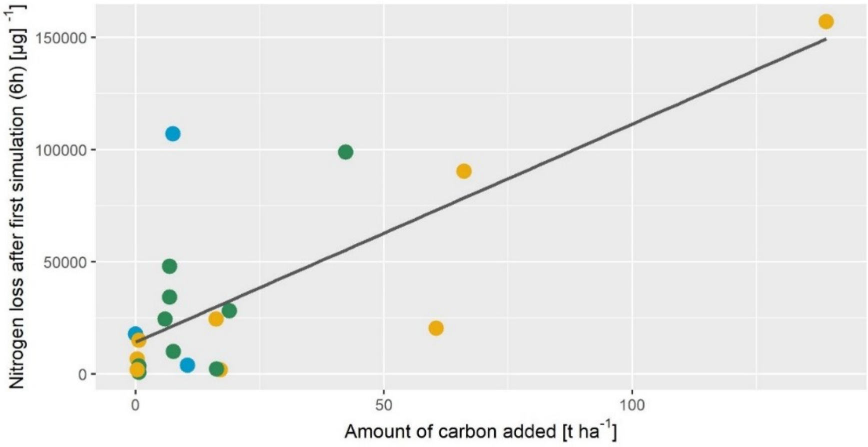

Figure 20: Visualisation of the linear regression model with total nitrogen after first simulation and amount of carbon added

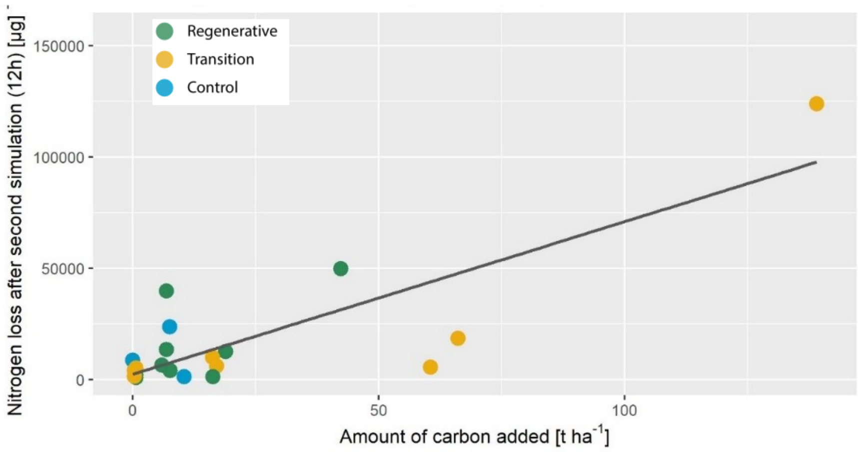

Figure 21: Visualisation of the linear regression model with total nitrogen after second simulation and amount of carbon added

4 Discussion

First, it will be elaborated how the design of this study emerged from an abstract idea into an on-farm study (4.1). After, the results of the soil health study on Gotland will be discussed and put into perspective with findings from other studies (4.2). The parameters will be examined in terms of absolute and relative values within groups, statistical significance, response to management, relation to soil health and C sequestration, and possible future implications for improved soil management. The goal was to extract key parameters and develop recommendations for future evaluations of soil health in RA (4.2.17). At last, the limitations of this study will be discussed (4.2.18).

4.1 Designing a study on regenerative agriculture

4.1.1 Discussion of our definitions (Lærke)

By introducing a holistic definition together with our working definition, we aim to involve the reader in our deeper understanding of RA, which go beyond what we were able to measure within the scope of this study. We hereby, try to broaden and be transparent about the context of which we evaluate RA as a promising sustainable agriculture.

Our working definition is a combination of a process- and outcome-based definition. We base our study on the soil health as the studied outcome, represented by chosen soil health indicators. The aggregated soil health is then used as a means to evaluate the fields, which are defined by their management practices.

The process-based perspective of our definition consists of the management practices and is a limited selection of our perception of RA, which helped us to narrow down and define which fields to include in the study. In case of a purely process-based definition, the last part of our definition would have been excluded. In our literature of RA, we found that the focus on soil health and SOC was a primary distinction between RA and other alternative agricultures. By excluding this perspective, we would no longer be able to claim that this study is about RA. From studies on single indicators, it is possible to get an idea about the outcome of the studied management practices, but due to the complexity of agroecosystems it is uncertain whether practices excluded in the RA definition will negatively interfere and change the outcome (Newton *et al.* 2020); therefore, an evaluation of RA should not exclusively include whether specific practices are involved or not, but be paired with an evaluation of the outcome of these practices in combination.

Where practice-based definition has been the base for organic certification systems, the RA certification is aiming to change focus to outcome-based definitions, as discussed above. In this way, farms would be held accountable to deliver results on the claims. If an outcome-based definition would be applied exclusively in this study, it would consist of the last part of our working definition: *restoring soil health and/ or sequester C*. A definition like this would demand an introduced limit for what level of soil health or C sequestration would be accepted as regenerative. Hereby, fields would not be studied as regenerative from the beginning but be defined as regenerative based on the outcome of the soil health study. We would limit the evaluation of RA to only include farms with high scores on our soil health study, in which case we would ignore an important discussion on why some indicators are showing unexpected low values or unexpected negative correlations.

It is important to emphasise that this definition is a working definition, which is not aiming to work as a universal definition for RA. Our definition is limited to carefully selected management practices even though our perception is that many more practices can be included in RA. While a narrow working definition is useful for the tangible perspective of RA, it should be adjusted to local environments, climate, soil characteristics, mineralogical composition, and state of the land, to incorporate the most appropriate local management practices.

4.1.2 Study design (Lærke)

4.1.2.1 On-farm research

On-farm research design can help answer questions in direct collaboration with the agricultural practitioners, but requires attention to high variability (Nielsen 2010). On-farm research is often conducted on a bigger field scale than experimental field-sites, increasing the risk of within-field variability, such as differences in moisture content, elevations, or shadow and sun exposure. This study included a complex diversity of management, firstly by looking at four management indicators included in our working definition, but further complicated by involving the necessity of the farmers intention to enhance the soil health and/ or soil C sequestration. The same attempt to include attention behind management choices would not be possible on neatly designed field-site studies.

Furthermore, management choices on an actual working farm are often influenced by more complexity than a well-planned field-site, which has the advantage of designing the site with a clear objective. For "real-world" farms, changes of landowners, consumption patterns or sustainable trends can cause sudden changes in management practices. To avoid background noise from previous management choices, historical information of the farm should be included to the possible extent. Our group of transition farms consists of many farmers who have recently taken over or started the management of the field area from scratch, not all knew or could collect information from previous landowners. Hopefully, the collaboration with the farmer for this study has created a base for the initial collection of management information which can be further developed for future studies on soil health status and the development of RA on Gotland. On-farm research in general has the advantage of working in close contact with the farmers, which presents the opportunity to get a 'real world' view across a wide range of sites, practices, soils, and crops (Nielsen 2010; Brown & Cotton 2011).

4.1.2.2 Management information and representation within farm categories (Lærke)

The results for management information compared between the farm categories control, transition, and regenerative did not always show the patterns expected and no significant differences were detected in the means of the soil health indicators between the groups. But a general trend of control farms having lower values were still detectable (Appendix 1 Appendix 2). The behaviour of management indicator results can mainly be explained by two interfering causes.

First, the intentional management practices, which our groups are defined by ends up determining the outcome. This is for example the case when years without tillage are lower for the control group. Here the lower values are a result of intensive tillage being a chosen management practice defining the fields as control fields.

Second, the results can further be affected by the land uses represented in the individual farm category. The study included a few different agricultural land uses, including vegetable, grazing, and fodder fields, due to an understanding of RA as something that can be applied on any field as long as the management increases the soil health from the previous state of the land. Detailed management information was collected parallel to the soil samples resulting in the final categorisation of the farms being carried out after soil sampling, while soil analyses were being conducted. An overrepresentation of vegetable fields in the transition category are the explanation behind the high mean for amount of C added in this category. The vegetable fields in the transition group all included a frequent and thick mulch layer. In the regenerative category, an over-representation of untouched grazing fields resulted in a high mean value for the crop diversity index. Grazing fields include a mixture of perennials, resulting in average species per year to be equal to the total species in rotation, resulting in a high crop index value.

In soil health studies, a baseline or control group has to be included to enable the identification of management effects (Bünemann *et al.* 2018). Preferably, a control group for RA should represent the soil health status before RA management practices were applied. The aim of the greater SLU project is to study one of the farms from the baseline year of 2020 and relate them to future measurements from the exact same plots on the farm. Conventionally managed fields were included to mimic the state of our fields before RA management was applied, to be able to study potential advantages within RA, already now. But due to the low number of control fields, the lack of textural representation of more sandy soils, and the lack of vegetable and grazing fields in the control group, additional control plots should be included in future studies. The aim is for the analysed farms to be included in future studies and contribute to the understanding of the development within RA over a longer timeframe. Another possibility would involve comparison to unmanaged fields instead of conventional fields following the approach of Williams *et al.* (2020), where the aim is to compare with the desired state of the aggregated soil health of natural ecosystems. This would neglect the important connotation of the word *regenerative*, which implies that the current agricultural state should be restored.

The four included management indicators were carefully selected based on RA literature together with their claimed C sequestration potential. Reduced or no-till, crop diversity, and addition of organic amendment and C to the soil are included in the most common practices of RA (see section 3.2.1). Additionally, we included the percentage of legumes included in the crop rotation of the field. Legumes have a high potential in increasing soil health through their N-fixing ability, thus increasing the C sequestration potential of the soil through altered C:N ratio and promotion of microbial biomass C (Kumar *et al.* 2018). A field-site study conducted by Al-Kaisi & Kwaw-Mensah (2020) found that switching from conventional tillage to no-till could on average sequester 57 ± 14 g C m^{-2} yr^{-1} and enhanced complexity of the crop rotation compared to monoculture could on average sequester 20 ± 12 g C m^{-2} yr^{-2}. Management indicators, which were considered, but ended up being excluded to keep the working definition simplified for the scope of the thesis was amongst others percentage of perennials and use of pesticides. Most farms included a high percentage of perennials, through ley crop rotations and the use of pesticides was present on all control farms and three of the regenerative farms. The discussion on whether pesticides should be included or excluded in RA is still to be resolved. Results from crop diversity are very high for perennial fields compared to the conventional controls. Since the crop diversity index is based on a method to compare production fields it might not be the best method for inclusion of perennial fields.

4.2 Results from soil health study

Generally, one should avoid overinterpretation of the single soil health indicators, as only values from a single sampling round in April 2021 are available. They represent the soil health of the chosen fields only in this specific moment and are a baseline for future observations. The present dataset will hopefully bring more detailed insights after repeated measurements and analyses in the coming years within the greater RA project at SLU. However, in the absence of a chronosequence, control plots were integrated in the study to ascertain if trends between regeneratively and conventionally farmed plots can be already observed today.

4.2.1 The impact of texture

The control group in this study only included fodder fields, which limits its comparative value with vegetable and grazing fields. Further, the control groups only represent the fields with a significantly higher clay content ($p= 0.087$) of 15.5 – 22.1 %. Clayey soils generally have a higher OM content than silty or sandy soils with the same C influx and same climatic conditions. This is induced by two main stabilising processes: Firstly, a generally higher amount of aggregates favoured by a higher clay content shields C complexes from microbial decay and secondly, clay minerals, Fe- and Al-(hydr-)oxides adsorb OM and impede microbial decay (Blume *et al.* 2018). In a long-term field experiment in Ultuna, Sweden it was observed that the silt fraction builds up stable microaggregates and was thus interpreted as a medium-term C sink, whereas the clay fraction contained the oldest and most stable organic C fraction (Kandeler *et al.* 2005). Singh *et al.* (2018) even suggest the addition of clay to sandy soils as a management practice to increase SOC. Further, it is reported that reduced tillage has stronger positive effects on SOM in finer-textured soils as the SOM is less protected in coarse-textured soils and depends on the regular addition of OM to the soil (Giller *et al.* 2009).

We see the trend that soil health parameters for transition and regenerative fields have higher, but not significantly higher values in comparison to the control farms. This could be attributed to the higher amount of clay throughout the control group which emphasises that the trends in differences between the groups might be underestimated in the statistical evaluation.

For example, mean TOC levels, mean WAS, active C, infiltration rates were non-significantly higher in both regenerative and transition than in control, even though the clay content in control was higher. This could indicate that significant differences could possibly be observed if soil textures between the groups were less heterogenous.

4.2.2 PC1 and related indicators

In the MLR with PC1, amount of C added had a very high significance of $p < 0.001$, but years of tillage did not show any significance. PC1 is mainly characterised by SOC-related variables. This concurs with the findings of Giller *et al.* (2009) and Gómez-Muñoz *et al.* *(2021*) that tillage alone only has a minor effect on the amount of C stored in the soil. Increases in SOM mainly appear due to higher biomass production and retention, which might be influenced by reduced tillage, and higher OM inputs.

Combining the loadings on PC1 with the MLR results, it can be concluded that higher C additions are associated with higher organic C, AC, C:N, and mean aggregate stability. Furthermore, there is a negative relationship between BD and MBC:TOC with increased C amendments. PC1 thus represents all direct indicators of C contents included in the study.

4.2.3 PC2 and related indicators

The MLR between years without tillage and PC2 shows a negative relation, thus a lower tillage intensity will cause a lower PC2 value. pH, infiltration rate, PAW, and number of earthworms have a positive relationship with PC2, which means that reduced tillage intensity results in a decreased value for these indicators. On the other hand, root abundance, root depth, vegetation density, sand, and WAS have a negative association with PC2, thus a reduced tillage intensity results in a higher value for these indicators.

4.2.4 Multiple linear regressions with single indicators

The results from the PCA and therefore the loadings of indicators on PC1 and PC2 largely corresponded to the MLRs with single indicators and amount of C added + years without tillage as predictors. However, some of the indicators were influenced by another management factor than presumed by the PCA. Mean WAS showed high loadings on both PC1 and PC2, but only had a low significance for tillage, and no significance for C added in the individual MLR. Root abundance and number of earthworms had a higher loading on PC2, which is mainly connected to tillage, but in the individual MLR had an association with C added. It can thus be concluded that the aggregation of the indicators through the PCA detected trends, and facilitated interpretation of a diverse dataset. However, it also reduced the degree of details that can be interpreted in the MLR.

4.2.5 Microbial biomass carbon, total organic carbon, and active carbon

Total organic carbon varied from around 1 % up to 9.35 % which is in the expected range for agricultural soils (Blume *et al.* 2018). In the PCA, TOC had a strong loading of 0.42 on PC1, underlined by a p-value < 0.001 in the ANOVA with only TOC and amount of C added. Total microbial biomass for agricultural soils measured with the chloroform fumigation method gives values of 100 – 1000 µg C g^{-1}, where the amounts are decreasing with depth in a soil profile (Blume *et al.* 2018). This complies with the measured values of MBC in our study, except for P12 which had a higher score of 1248.1 µg C g $soil^{-1}$.

On average, the ratio between microbial biomass C and total organic C values amounts to about 2 – 3 % with a possible range of 0.9 – 6 % (Kandeler *et al.* 2005). MBC:TOC in this study was mostly within the expected range. A large part of the microbial biomass is associated with fine silt and clay rather than larger particle size fractions (Kandeler *et al.* 2005). While it showed a higher trend on average for the control group, mean total MBC values were non-significantly higher in both the regenerative and transition group (see boxplot Appendix 2). These results must be considered in relation to total organic C, which was highest in transition, then regenerative and lowest in the control group. One of the highest MBC:TOC ratios, as well as a higher-than-average MBC value were found in P2, a control plot where organic material had been added shortly before sampling. Higher MBC:TOC ratios are generally observed after the application of organic fertiliser, after which the level decreases back to a level that depends on soil characteristics, soil biota, climate, land use and management practices (Dilly 2005; Ramesh *et al.* 2019). This could be a reason for the distortion in the trend between groups here, as both other control farms had lower-than-average TOC and MBC values.

Active carbon has very high values throughout all soil profiles, with the lowest value in P14 with 1907 mg C kg^{-1} which according to Moebius-Clune *et al.* (2016) scores exceptionally high. However, absolute values can be neglected in this case, as we are mainly interested in the relation to other soil health indicators and management impacts and the data had been centered and scaled before the PCA. Active C is positively related with TOC, WAS and MBC, which are all represented in PC1. Active C is non-significantly lower in the control group than in regenerative and transition, which is probably attributed to more organic amendments in the latter. The main strategies to increase AC in soils are increased OM inputs through amendments, forage and cover crops, and keeping living roots in the soil for large parts of the year (Moebius-Clune *et al.* 2016). It is thus no surprise that PC1 which is highly related to amount of C added is influenced by AC with a loading of 0.38 (see Table 14).

4.2.6 Carbon to nitrogen ratio (C:N)

The carbon to nitrogen ratio in soil affect microbial activity and structure and thus are an important factor for OM decomposition, C sequestration and soil health in general (Ussiri & Lal 2017). C:N ratios in our soils were between 6.95 and 12.54, the common range for arable surface horizons is expected to be between 8 and 15 (Brady & Weil 2014). Most plots were within the expected range, with only P6 and P7 with a ratio < 8. Both fields are experimental fields managed by the same farmer that receive synthetic amendments, and P7 also is amended with biochar and plant residues. According to the MLR with PC1, a higher C:N ratio was also accomplished by more C additions. An explanation for this could be the type of organic amendments that were added to the soils. In an experiment with OM amendments from urban organic wastes, a higher C:N ratio for control soils was explained by a low degree of degradation and high C:N content of inputs. Farmyard manure for instance contains straw that has a high C:N ratio and would be degraded slowly (Paetsch *et al.* 2016). As the sampling took place in April before the main vegetation season, organic amendments that had been added before the winter might not have been completely degraded yet, or new mulch in the case of some vegetable patches had only recently been added. Jagadamma & Lal (2010) argue that SOM decomposition induced by soil disturbance in combination with N enrichment in SOM can lead to a lower C:N ratio. Paetsch *et al.* (2016) report a lower C:N ratio for silty and clayey soils which aligns with our findings of the control group having a lower C:N ratio being composed of less sand than regenerative and transition.

Organic carbon and total N are closely related, and both can decrease drastically through conversion of forest or grassland into agricultural land. However, a regular input of manure or compost can prevent humus losses and, depending on initial conditions, might also increase total N and organic C contents in agricultural soils up to 25 % (Blume *et al.* 2018), which might be an effect observed within RA. The decay of materials with a small C:N ratio like dead soil bacteria, leguminous roots or grass and leguminous cuttings releases N that is then available for plant uptake (Blume *et al.* 2018). Further, manure, fresh green materials and compost with a high N content can help to lower the C:N ratio (Moebius-Clune *et al.* 2016).

4.2.7 Wet aggregate stability

Mean WAS was found to have positive loadings on PC1 and hence according to the MLR increased with higher C amendments. It is higher than the control group in both transition and regenerative plots, however not statistically significant. The reason for the lacking significance could again be the distortion of the texture distribution between the groups, as the soils in the control group have a higher clay content than almost all other soils. Both amount of C added, and total organic C were slightly higher for regenerative and transition fields. Recent additions of OM to a soil are primarily found in larger aggregates. However, macroaggregates are also more susceptible to destruction by agricultural disturbances like tillage, compared to microaggregates. The subsequent decomposition and C loss is especially high in the larger aggregate fraction (Blume *et al.* 2018). In the statistical analysis only the mean WAS was included, but it would be interesting to compare the values of small and large aggregate stability in a few years and see if the large aggregates have become more stable in relation to the small aggregates with more C additions and less tillage. Lal (2014) and Ramesh *et al.* (2019) state the formation and protection of stable aggregates and the incorporated SOC as one of the most important strategies for a positive ecosystem C budget. Aggregated soil organic C is physically protected as it is part of an anaerobic environment and less prone to degradation by microbial activity (Ramesh *et al.* 2019). Thus, soil characteristics that facilitate aggregation of soil particles into stable complexes have a higher capacity to store SOC (Lal 2014).

4.2.8 Bulk density & infiltration rate

Bulk density has a strong negative loading of -0.43 on PC1, which suggests a lower BD with higher C amendments. Infiltration rate on the other hand impacts PC1 positively and thus has the opposite relationship with C additions. Higher bulk densities and slightly lower infiltration rates can be seen on average in the control group which aligns with their higher clay content as soil texture has a substantial impact on both BD and infiltration rate.

Mulumba & Lal (2008) report no clear effects of mulching on BD in their study and describe that there are mixed findings in scientific literature about whether mulching increases or decreases BD. González *et al.* (2010) and Brown & Cotton (2011) however report significantly lower BD for compost application, on an experimental site and working farms respectively. In accordance with our findings, Brown & Cotton (2011) also measured higher infiltration rates compared to the control with compost additions in their experiments. Higher infiltration reduces surface runoff, inducing increased water use efficiency. However, the effects of mulching vary depending on soil type, initial soil properties, the type of amendments, climate and land use (Mulumba & Lal 2008).

4.2.9 Vegetation density

Vegetation density had negative loadings on both PCs, the loading on PC2 being stronger than on PC1. The negative relationship with PC1 can be explained, as many of the farms with the highest amount of C added are vegetable fields with annual vegetation and a mulch layer on top. Since the visual assessment of vegetation density was performed in April 2021 a minority of these vegetable fields had sprouted yet. The result for vegetation density would be higher for these fields if the analysis was conducted later in the season. Thus, no clear causal relationship with amount of C added in terms of soil health can be stated.

Figure 22: Soil surface of P6, a control field with low vegetation density (own photo 2021)

For PC2, a strong negative loading of -0.37 was computed, which in combination with the MLR signifies that more years without tillage lead to a higher vegetation density. This is cohesive with field observations of tractor tracks and ploughed topsoils on fields where vegetation density was low as can be seen for example in *Figure* 22.

Again, mean values of vegetation density were higher in transition and regenerative than in control, however with highest values in regenerative. This is due to the fact that some of the fields in the regenerative group have been under a reduced or zero tillage regime for many years and mainly consists of grazing and fodder fields with either undisturbed natural vegetation or perennial ley crops.

4.2.10 Root depth & abundance

We found that less intense tillage would cause an increase in both root abundance and root depth, which is to be expected when the root system is less disturbed. The grazing fields P16 and P22 were identified as outliers with high root abundance values. These plots where placed within and next to a forest respectively. Roots found in the belonging profile were thus quite big and caused the high value in abundance. Since the same root development would not have been possible if tillage had been present, these values were kept in the analysis. The vegetable field P12 was identified as an outlier with a low root depth of 15 cm. The same profile had a high penetration resistance of 2.66 MPa and the whole profile was shallow (15 cm) as the vegetable garden was established on a previous gravel area.

Due to exclusion of penetration resistance for the deeper horizons it was not possible to study if a possible occurrence of a hard tillage pan layer in the fields is limiting root depths, which has otherwise been detected in soil compaction studies (e.g. Materechera & Mloza-

Banda 1997). A study on the penetration resistance for the deeper soil-layers were possible with the collected data but beyond the timeframe of this thesis.

4.2.11 Earthworms

According to our model, tillage is found to have a negative effect on earthworm abundance in the fields. Earthworm number has a loading of 0.24 on PC2, suggesting that less intensive soil cultivation is expected to increase the earthworm population (Briones & Schmidt 2017). Even though conflicting results have also been documented, these have often been attributed to site-dependent differences in soil properties, such as climatic conditions and agronomic operations (e.g. fertilisation, residue management, and chemical crop rotation). Briones & Schmidt (2017) performed a quantitative meta-analysis to study the cause of inconsistent evidence and found a mean increase in earthworm population of 137 % and 127 % for no-tillage and conservation agriculture, respectively, compared to conventional ploughing.

Three quarters (18 out of 24) of the studied fields belonged to the group of farms with 6 years without tillage and both high and low values of earthworms were found in this category. P5 is an outlier due to its high number of 26 earthworms found in the profile. P5 is a rotational fodder and grazing field with long-term reduced tillage and is therefore expected to have high earthworm counts. However, this field was ploughed in 2020, which is one of the more recent tillage years for the studied fields with reduced tillage. A possible explanation can be that plant residues has been distributed into the soil and made more available for the earthworms. Other high values (~20) were found for P8, P10, and P11, which are all vegetable fields with a surface layer of mulch. This emphasises that the presence of plant residues is interfering with the causation of tillage on earthworm counts.

For earthworm number the highest loading of -0.39 was however found for PC3. This indicates that other relationships not analysed in this study are present between management and earthworms. Earthworms contribute to ecosystem services such as soil structure maintenance, humus formation and nutrient cycling (Blouin *et al.* 2013) and an increase in population size or biomass weight can be used as an indicator for structural improvement, e.g. for water infiltration.

4.2.12 Texture and plant-available water

Sand content is negatively related with reduced tillage, which is most likely due to the high clay content in our control group. Texture cannot be influenced by management in the short term and thus should not be interpreted in this way in the analysis. Sand content was kept in the PCA to see associations with other soil health indicators. PAW also has a negative relation with reduced tillage, which disagrees with the 44 % increase Blanco-Canqui & Ruis (2018) found for no-till. Since PAW is a theoretical value calculated directly from the texture, the value would also not change with management in the short term.

4.2.13 Infiltration rate and wet aggregate stability

Infiltration rates and WAS has already been explained with a positive relation to increased amounts of C added. These indicators were also higher with reduced tillage intensity according to loadings of 0.21 and 0.30 on PC2, respectively. These results correspond to observed positive correlations between structural changes and conservation tillage found by e.g. Abdollahi *et al*. (2017). A meta-analysis conducted by Blanco-Canqui & Ruis (2018) found that no-till increased water infiltration in 15 out of 24 cases with between 17 % and 86 % and increased WAS in 31 out 42 cases, with the wide range of 1 to 97 % increases compared to conventional tillage. WAS changes were primarily confined in the upper 10 cm of the soil. The results from this study indicate that in 24 % of the cases no-till might not have an impact on WAS and the combination with C added to the plots with reduced tillage have possibly emphasied the significance between tillage and WAS. The greater infiltration rates can be caused by multiple mechanisnms, such as increased residue cover within no-till systems, increased pore size and continuity, and structural changes caused by increased SOM content.

Crop diversity was excluded in the final MLR with PC1 and PC2 as it did not show any significance in the process of model selection, but studies have found that cover crops increase both infiltration rate and WAS (Abdollahi *et al*. 2017; Blanco-Canqui & Ruis 2018). Many of the analysed fodder fields with reduced tillage in our study had ley crops in the years without tillage, and the influence of cover and ley crops might have emphasised the relation with tillage. Since cover cropping is generally included within RA practices, this does not diminish the positive impact of RA on soil health.

4.2.14 Soil penetration resistance

Penetration resistance of the soil was in the end excluded from the interpretation of soil health in this study. It was mainly explained by PC3, whereas only PC1 and PC2 were analysed. Looking at the results for penetration resistance individually, no clear patterns were found. Other studies found that soil penetration resistance was reduced by minimised tillage and increased mulch layers, both individually and in combination (Kahlon *et al.* 2013). Penetration resistance in our study was only analysed for the depth of 10-20 cm. Past studies of penetration resistance in 0-10 cm depth have shown more significant reductions (Kahlon *et al.* 2013), however this layer was excluded in our study due to high variability of the top layer within the individual fields.

4.2.15 Nutrient analysis (Lærke)

Improved nutrient circulation is often included as a secondary effect in RA (see section 3.2.1). To check for these claims, together with the potential negative influence on the external environment, an additional nutrient analysis of N content, N loss, and P loss was conducted. Giller et al. (2021) raise the awareness that agronomic perspectives with the emphasis on one benefit of soil health, such as C sequestration often neglect and even have negative effects on other functions.

No significance was found for MLRs with P losses in relation to our management practices, indicating that P is controlled by other factors than management.

Nitrogen on the other hand, showed significant positive relations between increased amount of C added and N content in the soil as well as N loss from the field. Increased N content in the soil is normally an indicator of healthy soils, due to increased nutrient availability for plants and microbial life. Whereas increased N content also increases the risk of exceeding the soils capacity to retain the mobile fractions of the nutrient. Thus, allowing them to leach into water environments with the risk of causing eutrophication or groundwater pollution.

The only predictor showing significant associations to the nutrients were the amount of C added, possibly due to the uncontrolled addition of N amount as a site-effect of adding organic amendments. A comprehensive study on the influence of different types and amounts of legumes or perennials could be relevant for the discussion on nutrients in RA but was beyond the scope of this thesis. The potential of cover crops is the most emphasised agronomic strategy to help reduce environmental pollution from nutrient loss. Through sequestering N in growing biomass, compared to fallow ground, cover crops have been found to reduce excess soil inorganic N (Behnke et al. 2020).

4.2.16 Concluding thoughts on the statistical analysis (Alena)

Two main observations could be made through the interpretation of the statistical analysis:

Firstly, C-related indicators that were united in PC1 were highly influenced by the amount of C additions. The farms in the transition group often showed higher values for C-related parameters than the regenerative group. This adheres to findings in long term studies that C sequestration is highest in the early years of transition, but that this rate is temporary and will slow down eventually. SOC at some point will reach a new equilibrium with the improved management that is projected to be lower than the natural vegetation. However, this suggests that a rapid drawdown of CO_2 from the atmosphere to the soil is possible (Rodale Institute 2014; Giller *et al.* 2021). Nevertheless, the likeliness of other limitations for plant growth, and thus SOC sequestration, like higher temperatures, droughts or other extreme weather events, will increase in the face of CC and continuously challenge practitioners and scientists alike (IPCC 2019). Whether the higher values in the regenerative group are due to C saturation, their management or other circumstances is beyond the limit of this study and might become clearer once a data timeseries is available.

Secondly, soil health values for transition and regenerative fields generally showed a higher trend than the control group, however, it was not statistically significant in most cases. This can partly be explained by the heterogeneity of textural classes across farm categories, which is a major limitation in this study. On the other hand, the fact that despite such high variance in land use, texture, management practices and operation time, positive trends could be detected, is a good outcome. This might suggest that it is possible to study soil health in more complex and diversified settings than it is done in many scientific studies to date. We recommend other soil and agricultural scientists to "think outside the natural scientific box" and consider studying more real-life farms that apply regenerative practices, so that in the near future it will be possible to verify whether they are successful in specific contexts.

4.2.17 Key parameters & recommendations on the evaluation of RA

SOC is the primary focus of RA and thus an evaluation of C-related indicators is essential for the contemporary discussion on and development of the role of agriculture as part of the solution for the climate crisis. Furthermore, many co-benefits of soil health are a result of improved SOC and microbial activity, as touched upon in the introduction.

From the basic soil characteristics, texture and BD were used in the statistical analysis. Whereas the first was important for context, the latter was an interesting indicator that was related to C inputs. Dry matter, pH and electric conductivity might be handy background values for more detailed interpretation, but do not necessarily need to be repeatedly measured for this study. Water content is highly variable depending on climate and weather and did not add any value to the statistical analysis but could be helpful to explain other phenomena. Infiltration rate was only approximated by the used method which could be upgraded in the future. Wet aggregate stability, active C, MBC, TOC, and C:N ratio were the parameters that responded the most to management and are all related to important soil processes, as well as C inputs and present the core of this study. Rooting depth and abundance, as well as number of earthworms were not as clear in their relationship to the examined management practices but are nevertheless interesting in their role as soil biological indicators and might show trends in coming sample campaigns.

Vegetation density and penetration resistance were not as relevant indicators for the soil health evaluation and could be considered to be excluded in future studies. Vegetation density was highly affected by agricultural land use, thus it was not a good indicator for soil health in the spring, where vegetable fields did not have vegetation yet but on the other hand were covered with a thick mulching layer. Since vegetation density varies with crops and grazing intensity, without adjusting for these factors, vegetation density cannot be evaluated as a soil health indicator. Penetration resistance is an important indicator for compaction induced by tillage, but a bigger study would be relevant to be able to evaluate the trend in penetration resistance over time or with depth.

Additional parameters that could be taken up in future investigations for this project are C inputs into the soil from roots, more precise estimations of the soil water regime, yields, nutrient use efficiency, soil protein content, and GHG emissions.

4.2.18 Limitations of this study

We acknowledge that the scope of this thesis had some limitations in the experimental setup. First, the analysis is based on a single sampling round and hence is only depicting the soil health status at the exact moment, with the specific moisture content and weather conditions for the time of field sampling. Relating the results to literature and theory behind the single indicators allow us to make assumptions on general soil health trends based on the single sample round. If values are to be compared to this study, samples should preferably be collected in spring as well. Sampling collected in other seasons could be relevant for examining seasonal differences in soil health trends.

Second, the time frame of six years for the collected management information was relatively short. Mulumba & Lal (2008) analysed tillage effects 11 years after the experiment was initiated and Kahlon *et al.* (2013) studied the individual and combined effect from tillage and mulching 22 years within an experiment. This limitation was met by including fields at different states of RA, including transition farms and focus on the immediate response to change in management towards RA practices.

Third, to be able to compare indicators, the analysis was narrowed down to the A-horizons, though samples from other horizons were collected. A whole study on the differences between soil health responses in different layers would be relevant for evaluating the ability of RA to sequester more stable SOC and further improve soil structure in the deeper layers of the soil. This will be especially relevant for studies of long-term effects of RA.

Fourth, a detailed analysis of single indicators and their interactions was not possible due to time limitations of this thesis. Hopefully the collected data will be helpful for future studies, or for more in-depth explorations.

Lastly, by presenting two different definitions we acknowledge that our own perception of RA had to be narrowed down to enable studying soil health through a soil scientific lens.

5 Conclusion

A large potential is held in RA to be part of the solution to climate change and land degradation. This is also represented by the increased use of the term since 2015. While many alternative agricultural approaches with sustainable aspects exist today, and many of the promoted practices in RA have been deployed before, the concept of regenerative agriculture differs through its primary focus on increasing the SOC pool of degraded land.

Regenerative agriculture is however highly complex, and no magic bullets that can be applied in every situation exist. This thesis presented a holistic definition to emphasise that RA should always be viewed in relation to the current state of the land that is to be regenerated. Further, socio-economic, cultural and other factors play into the game, that cannot be generalised for a uniform definition. Thus, we advocate for a context-dependent, dynamic and ever-evolving definition of the concept.

To be able to study RA in the context of improved soil health, a more practical, process-based working definition was presented, with a focus on management. It should be emphasised that a definition for the present purpose should precede every study on RA, to clarify the framework of evaluation. This study was conducted as an on-farm study, making it possible to study complexity in management, but also complicated representation of diverse levels of management intensity. For further studies, more detailed and earlier collection of management information could improve the selection of and comparison between conventional and regenerative agriculture.

A relation between RA management and specific soil health indicators was found through statistical analyses. A general trend could be detected with higher values for the transition and regenerative groups compared to the control group, however no statistically significant difference was present for mean values. The designation of control groups can be improved for further studies on RA on Gotland. Especially the representation of control groups within textural classes matching the transition and regenerative groups are of importance. Instead, the individual effect from management practices described by a multiple linear regression had a statistically significant relation to some soil health indicators. High significances were found for BD, infiltration rate, TOC, and vegetation density, and lower significances for AC, mean WAS, PAW, root abundance and number of earthworms. Increased addition of C and reduced tillage intensity had a higher influence on the investigated soil health indicators than increased crop diversity and share of legumes. The measurements need to be continued to confirm the current and detect future trends.

Finally, it is important to keep the debates about RA and its practices diverse and open to avoid the reduction to one definition or certification for all. However, clarifying the meaning of RA for every context and use is important for policy decisions and to avoid co-option and greenwashing.

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Appendix 1

Individual soil health indicators grouped by farm categories

Bulk density and wet aggregate stability by farm categories: control, transition, and regenerative

	Control			Transition			Regenerative		
	BD [g cm^{-3}]	WAS (0.25 – 2 mm) [%]	WAS (>2mm) [%]	BD [g cm^{-3}]	WAS (0.25 – 2 mm) [%]	WAS (>2mm) [%]	BD [g cm^{-3}]	WAS (0.25 – 2 mm) [%]	WAS (>2mm) [%]
Min	1.42	44.15	64.72	0.58	46.50	74.58	1.14	38.29	66.22
Max	1.63	68.86	84.41	1.49	98.68	98.86	1.62	94.53	89.34
Mean	1.55	56.25	72.72	1.20	71.81	87.04	1.39	69.32	79.58
Median	1.59	55.75	69.03	1.37	76.20	88.06	1.38	69.32	81.43
Std dev	0.11	12.36	10.35	0.33	16.04	7.75	0.14	17.52	8.37

BD = bulk density, WAS = wet aggregate stability

Plant Available water, infiltration, and penetration by farm categories: control, transition, and regenerative

	Control			Transition			Regenerative		
	PAW [% volume]	infil rate [mm h^{-1}]	pen res [MPa]	PAW [% volume]	infil rate [mm h^{-1}]	pen res [MPa]	PAW [% volume]	infil rate [mm h^{-1}]	pen res [MPa]
Min	10	44.38	1.58	5	8.31	0.42	7	15.53	1.34
Max	10	68.82	2.45	14	253.02	2.70	10	170.45	3.85
Mean	10	55.26	2.03	8	131.48	1.77	9	70.73	1.92
Median	10	52.57	2.06	7	127.43	1.92	10	63.85	1.82
Std dev	0	12.44	0.44	3	91.42	0.84	2	43.84	0.74

PAW = plant-available water, infil rate = infiltration rate, pen res = soil penetration resistance

Vegetation density and root abundance and depth by farm categories: control, transition, and regenerative

	Control				Transition				Regenerative			
	veg dens [%]	root abun	root dep [cm]	earth worm [#]	veg dens [%]	root abun	root dep [cm]	earth worm [#]	veg dens [%]	root abun	root dep [cm]	earth worm [#]
Min	0	25	30	5	0	10	15	0	0	10	25	1
Max	40	50	40	10	100	130	55	20	100	120	50	26
Mean	27	37	36	7	47	59	37	8	76	51	39	8
Median	40	35	37	7	30	50	39	4	90	50	40	5
Std dev	23	13	5	3	47	36	12	8	33	32	8	8

veg dens = vegetation density, root abun = root abundance, root dep = root depth, earthworm = earthworm number

Organic, active, and microbial biomass carbon by farm categories: control, transition, and regenerative												
	Control				Transition				Regenerative			
	TOC [g kg^{-1} soil]	AC [g kg^{-1} soil]	MBC [g kg^{-1} soil]	MBC: TOC [%]	TOC [g kg^{-1} soil]	AC [g kg^{-1} soil]	MBC [g kg^{-1} soil]	MBC: TOC [%]	TOC [g kg^{-1} soil]	AC [g kg^{-1} soil]	MBC [g kg^{-1} soil]	MBC: TOC [%]
Min	11.0	2.04	0.36	0.56	10.8	1.91	0.26	0.28	15.7	2.11	0.15	0.17
Max	23.4	1.63	0.69	0.70	93.2	2.93	1.25	0.60	37.2	2.68	0.91	0.71
Mean	15.2	2.32	0.41	0.62	40.6	2.49	0.57	0.45	24.2	2.45	0.46	0.50
Median	11.1	2.30	0.28	0.58	20.8	2.49	0.45	0.43	22.4	2.47	0.45	0.50
Std dev	7.1	0.01	0.24	0.08	33.8	0.39	0.30	0.10	06.0	0.15	0.21	0.17

TOC = total organic carbon, AC = active carbon, MBC = microbial biomass C, MBC:TOC = ratio between microbial biomass carbon to total organic carbon

C:N, and total and inorganic carbon by farm categories: control, transition, and regenerative									
	Control			Transition			Regenerative		
	C:N	TC [g kg^{-1} soil]	IC [g kg^{-1} soil]	C:N	TC [g kg^{-1} soil]	IC [g kg^{-1} soil]	C:N	TC [g kg^{-1} soil]	IC [g kg^{-1} soil]
Min	7.9	12.3	1.3	7.0	12.8	0.0	8.1	16.4	0.7
Max	8.7	38.3	14.9	12.3	127.2	34.1	12.5	57.4	25.9
Mean	8.4	21.1	5.9	9.9	49.6	10.7	9.6	32.8	8.5
Median	8.5	12.6	1.5	10.0	30.15	4.3	9.5	29.1	6.7
Std dev	0.4	14.9	7.8	1.6	41.9	12.6	1.2	12.0	8.3

C:N = ratio between organic carbon and total nitrogen, TC = total carbon, IC = inorganic carbon

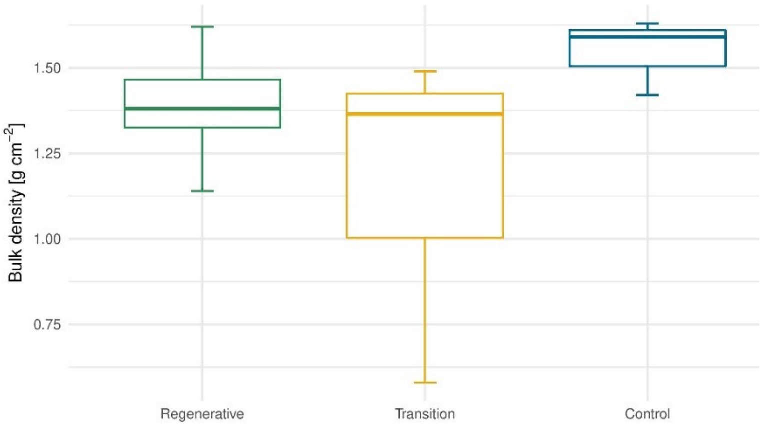
Total nitrogen and nitrogen loss after 6- and 12-hour simulations by farm categories: control, transition, and regenerative									
	Control			Transition			Regenerative		
	Total N [g kg^{-1} soil]	N loss 1st simu [μg L^{-1}]	N loss 2nd simu [μg L^{-1}]	Total N [g kg^{-1} soil]	N loss 1st simu [μg L^{-1}]	N loss 2nd simu [μg L^{-1}]	Total N [g kg^{-1} soil]	N loss 1st simu [μg L^{-1}]	N loss 2nd simu [μg L^{-1}]
Min	1.3	3990	1340	1.2	1800	1590	1.6	799	1000
Max	2.7	107000	23800	9.5	157000	124000	3.7	98900	49900
Mean	1.8	42930	11297	4.0	39668	21886	2.6	27800	14531
Median	1.4	17800	8750	2.4	17650	5808	2.4	24500	6530
Std dev	0.7	55914	11445	2.9	55424	41584	0.7	31232	17962

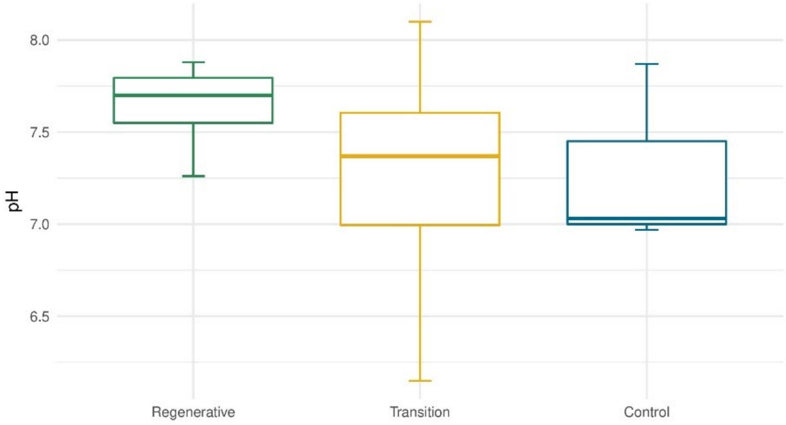
Total N = total nitrogen, N loss 1st simulation = nitrogen loss after first simulation of 6 hours, N loss 2nd simulation = nitrogen loss after second simulation of 6 hours

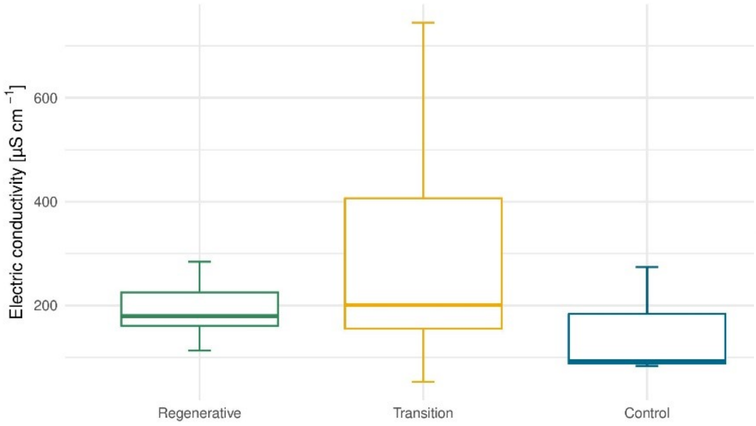
Appendix 2

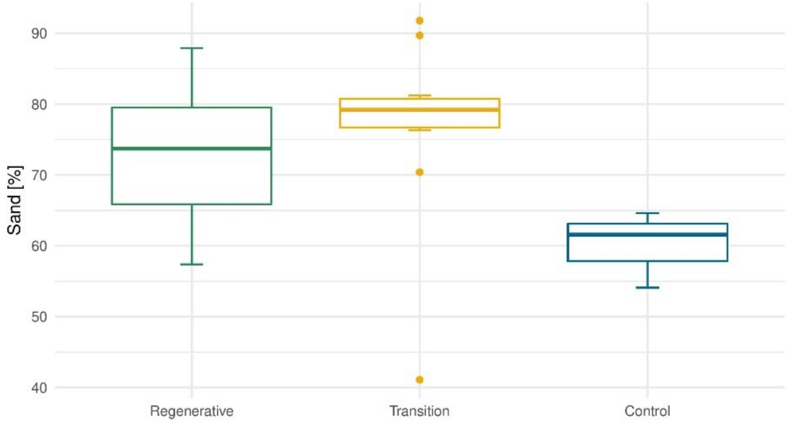
Boxplots of soil indicators grouped by farm categories

Basic soil characteristics

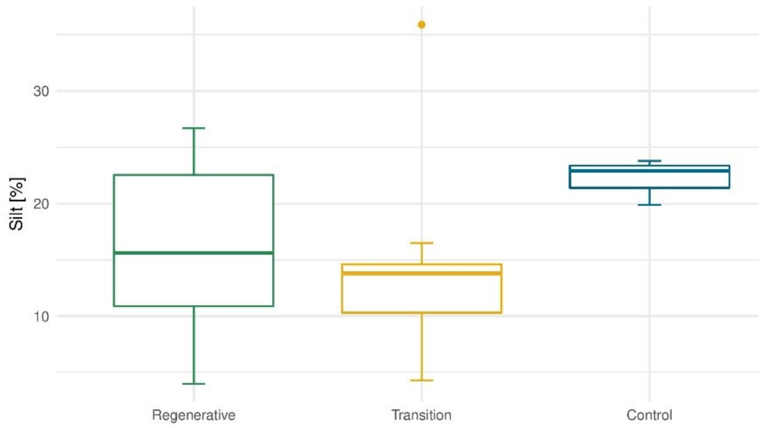


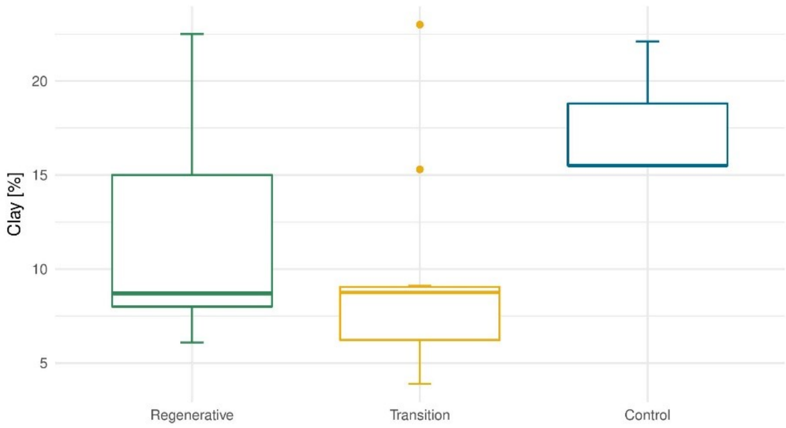


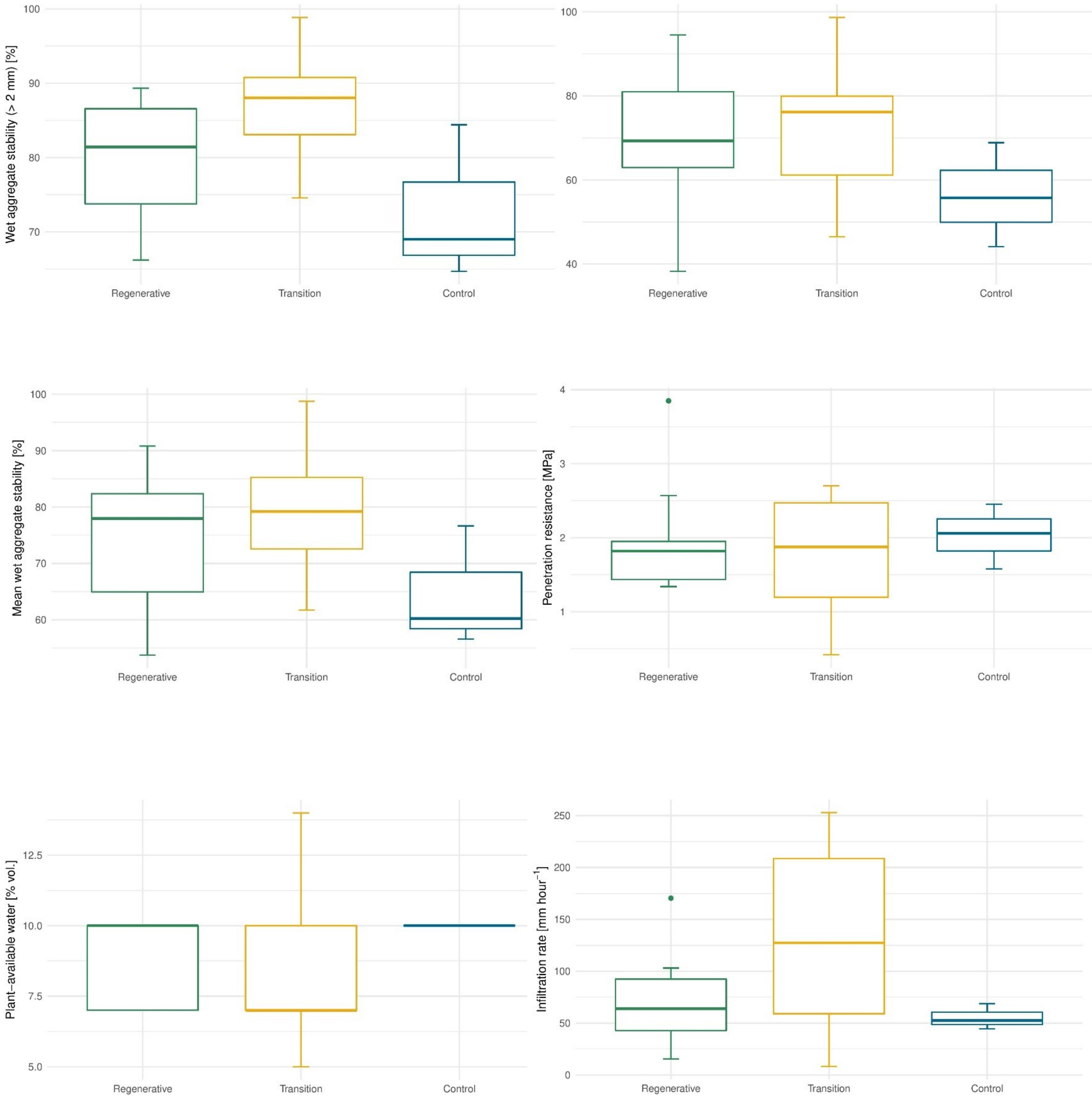




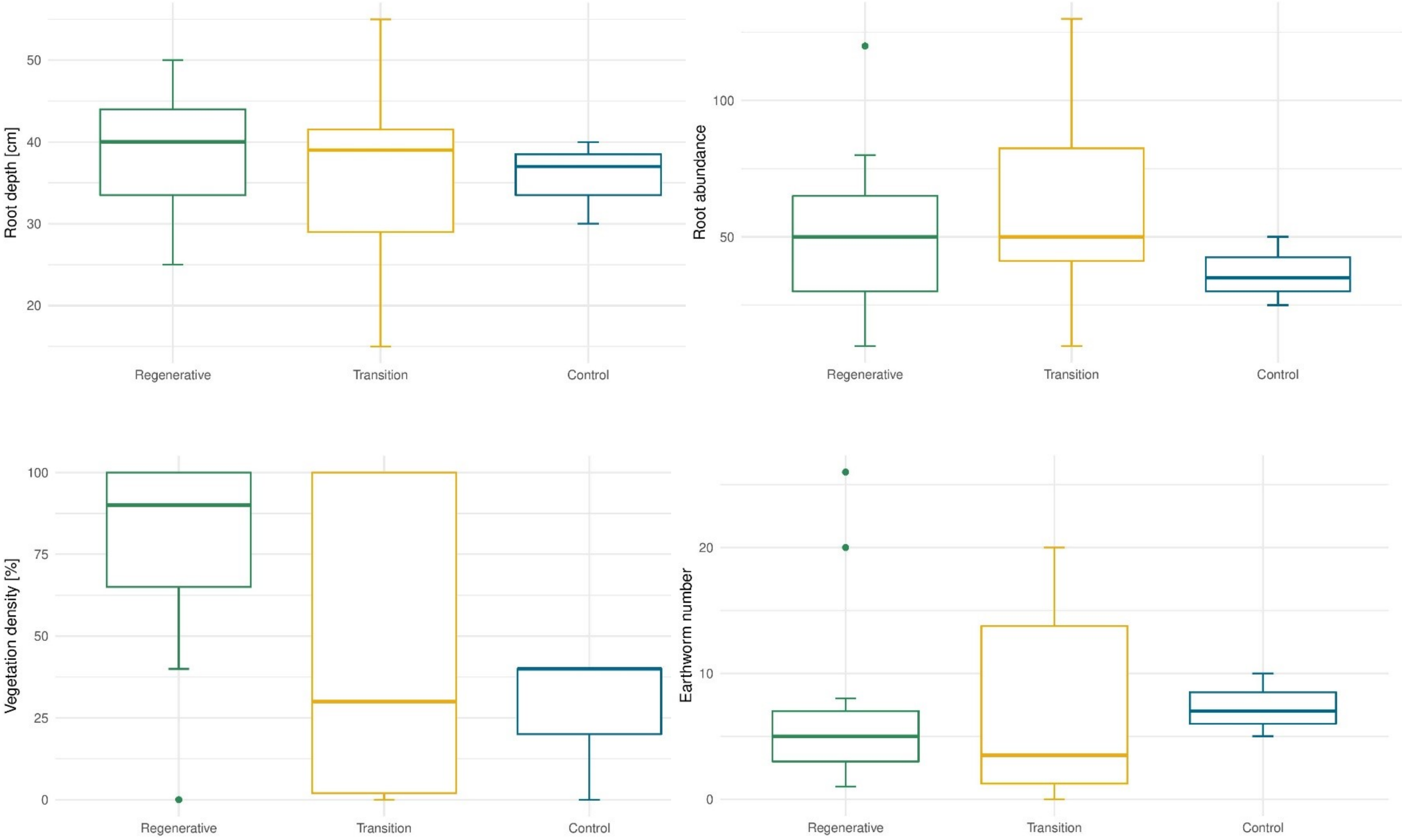
Physical soil parameters



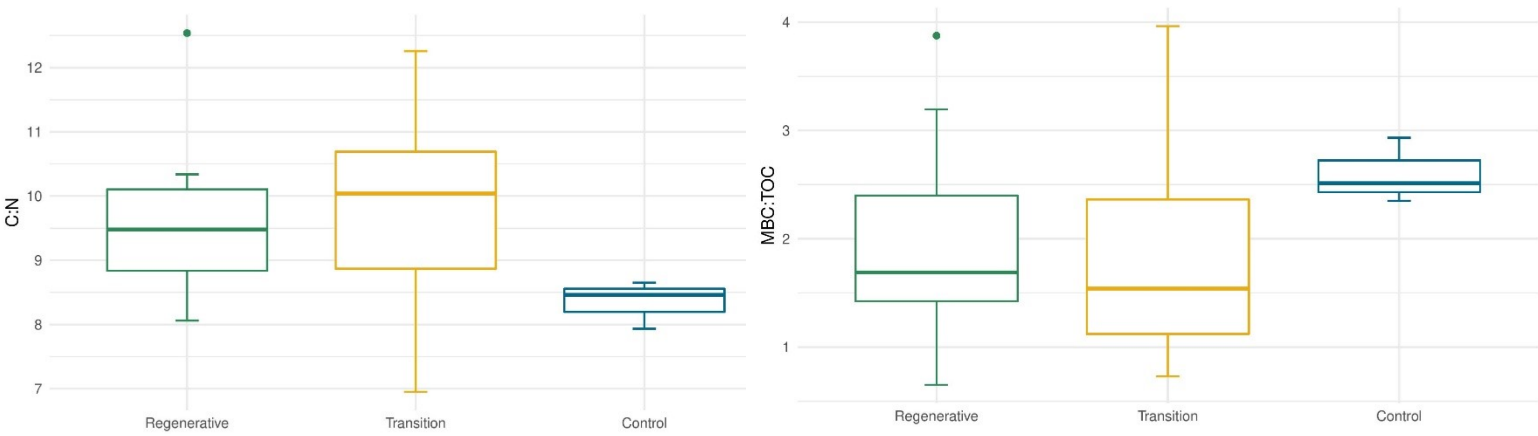


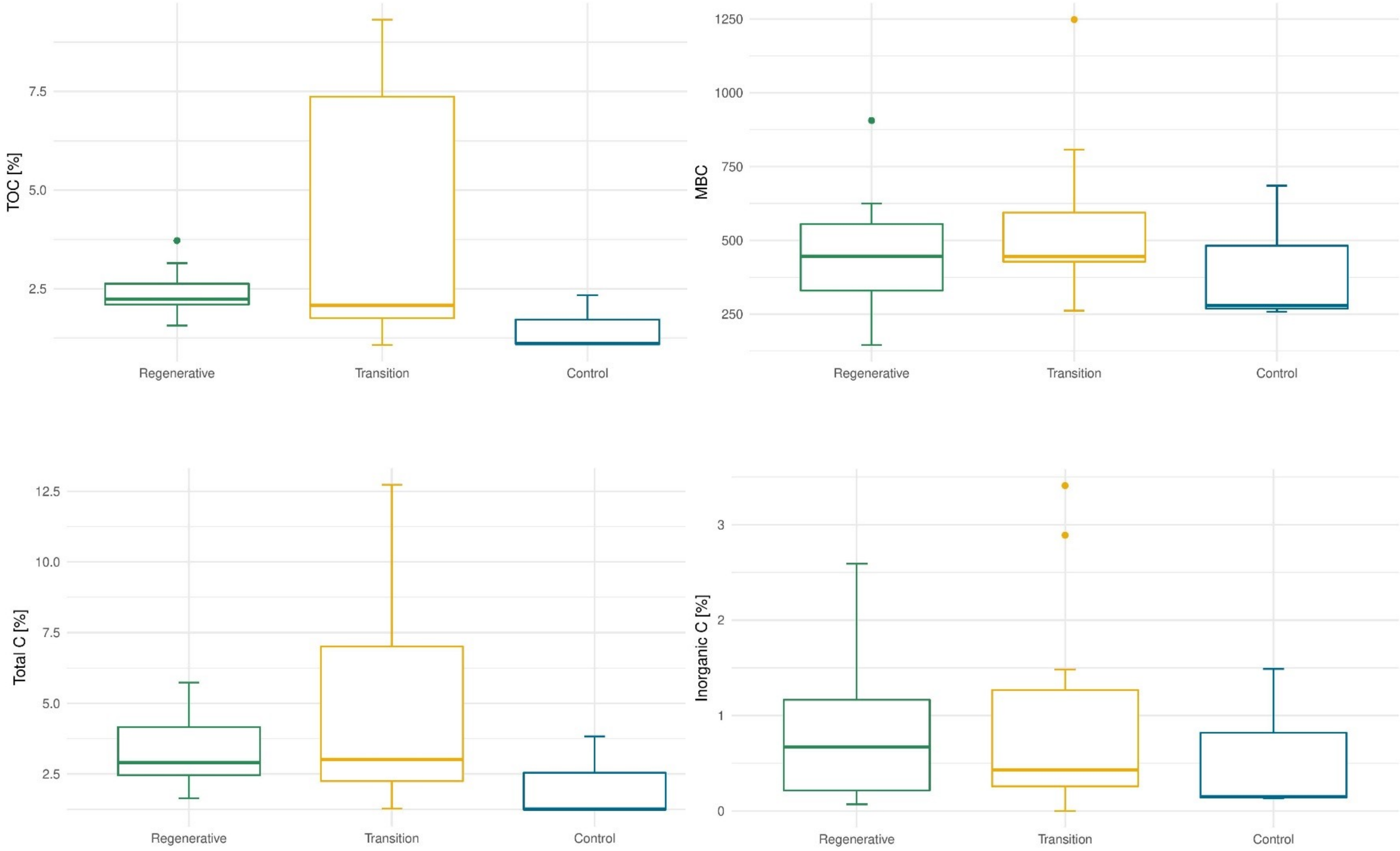

Wet aggregate stability (> 2 mm) [%]
Mean wet aggregate stability [%]
Penetration resistance [MPa]
Plant−available water [% vol.]
Infiltration rate [mm hour^{-1}]
Regenerative
Transition
Control

Biological soil parameters

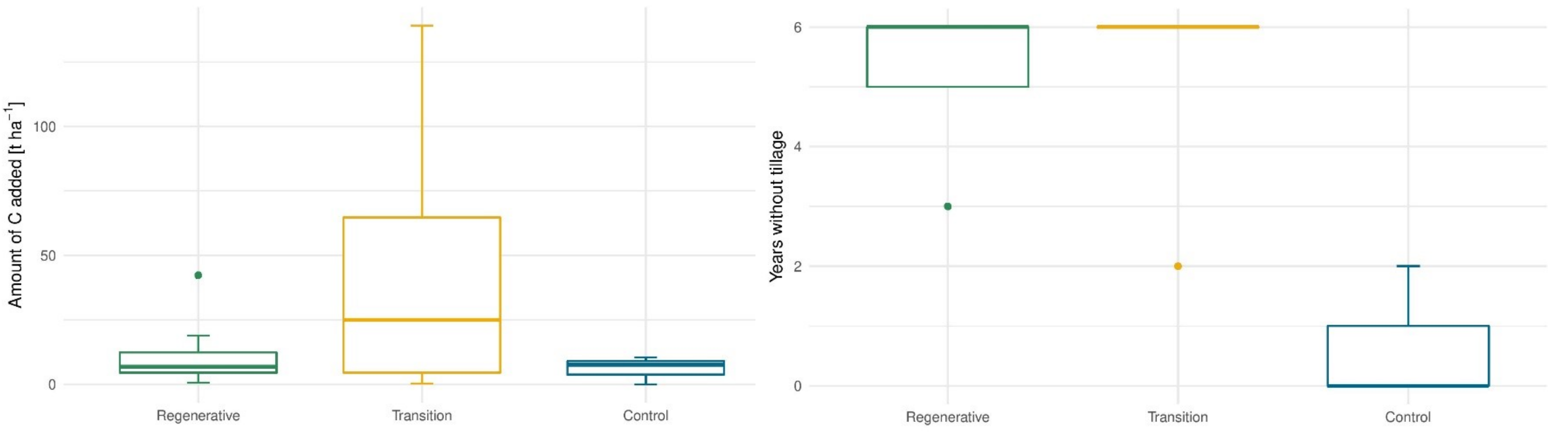


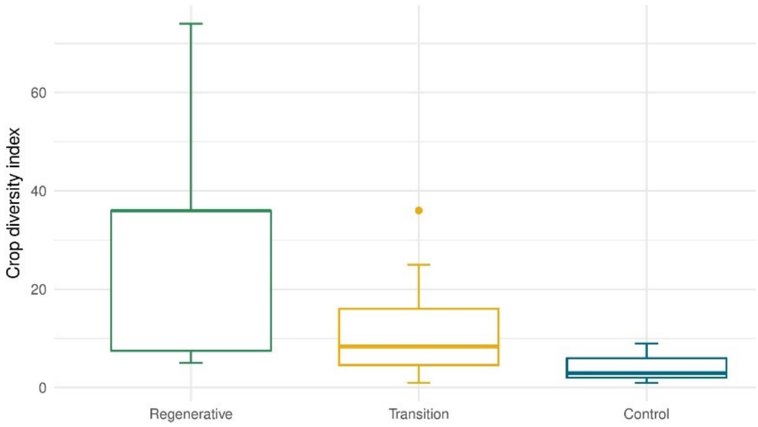
Carbon-related soil parameters

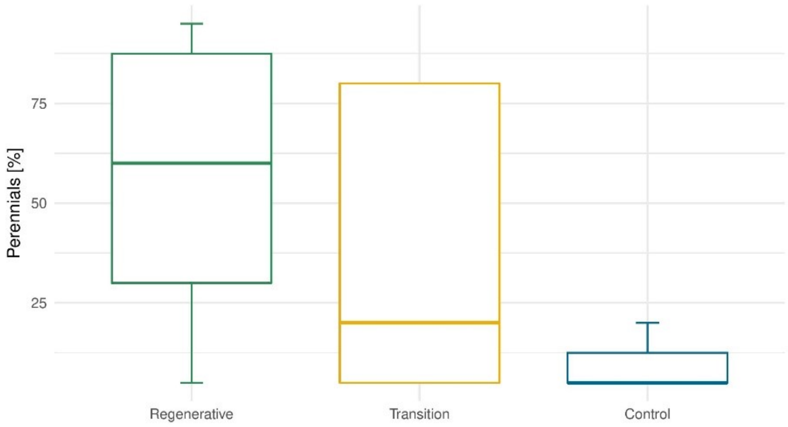


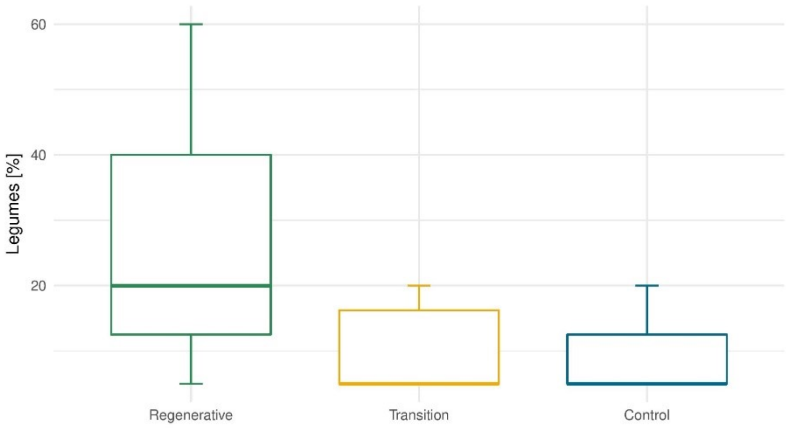


Management indicators



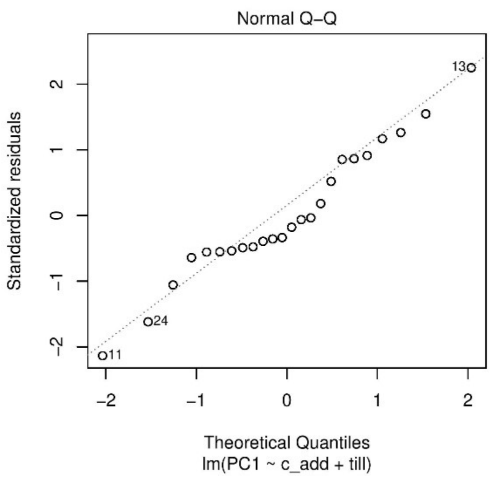


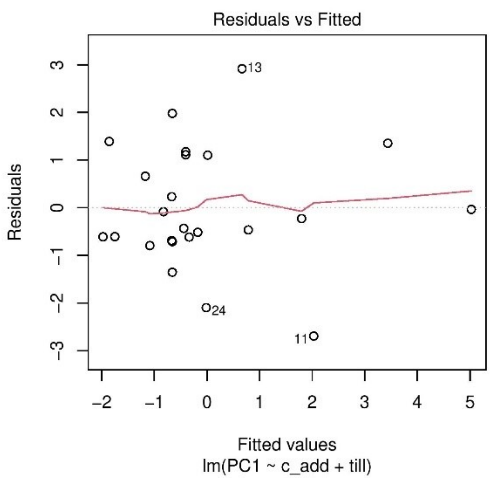


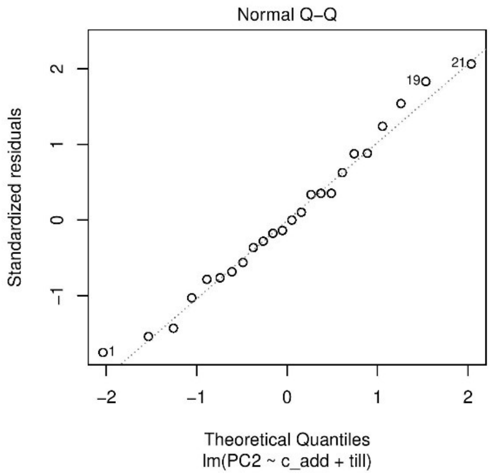


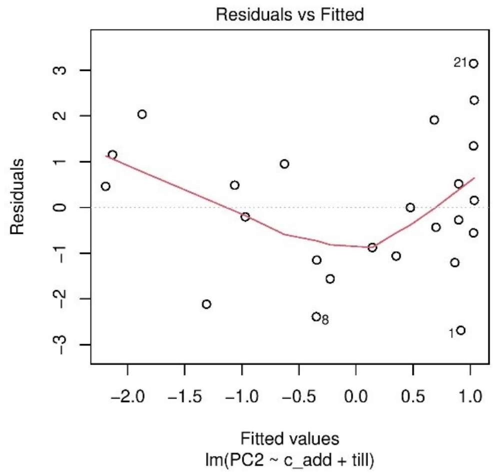
Appendix 3

Normal-QQ and Residuals vs. Fitted plots



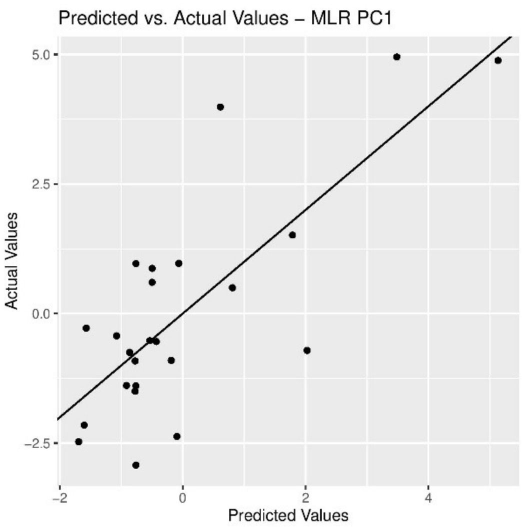


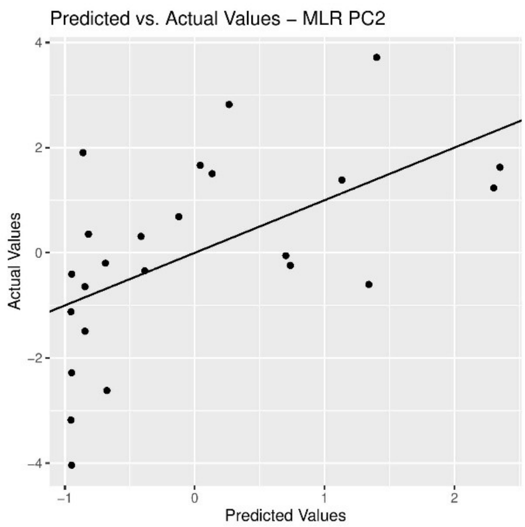




Appendix 4

Predicted vs. Actual value plots

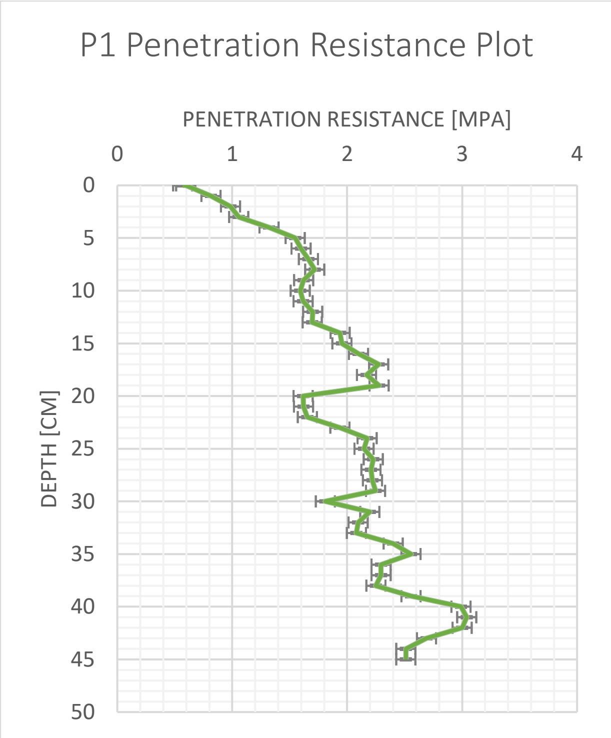


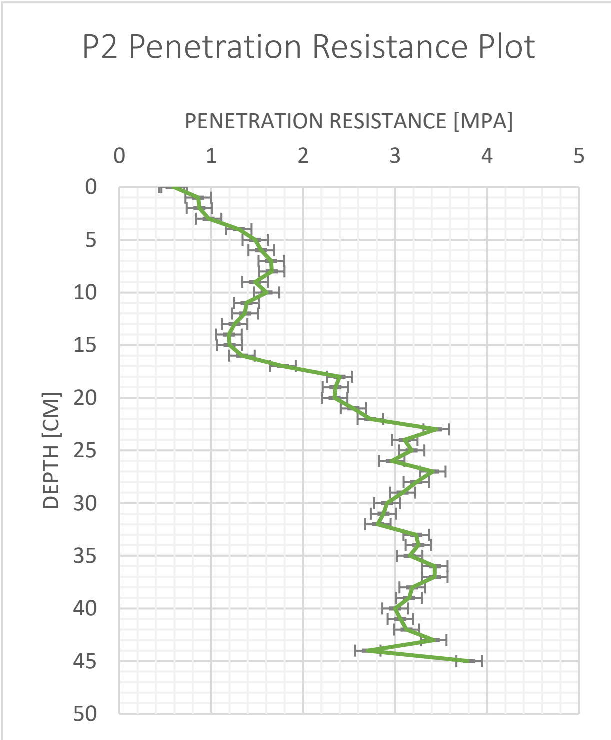


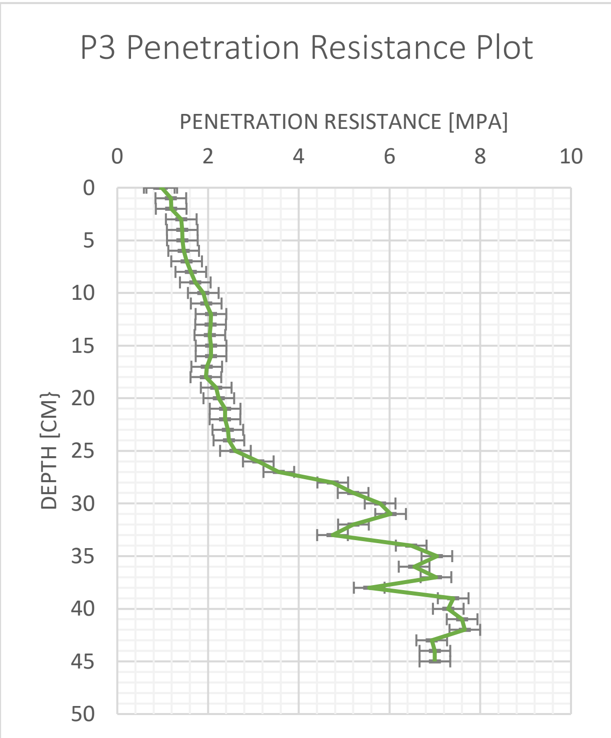
Appendix 5

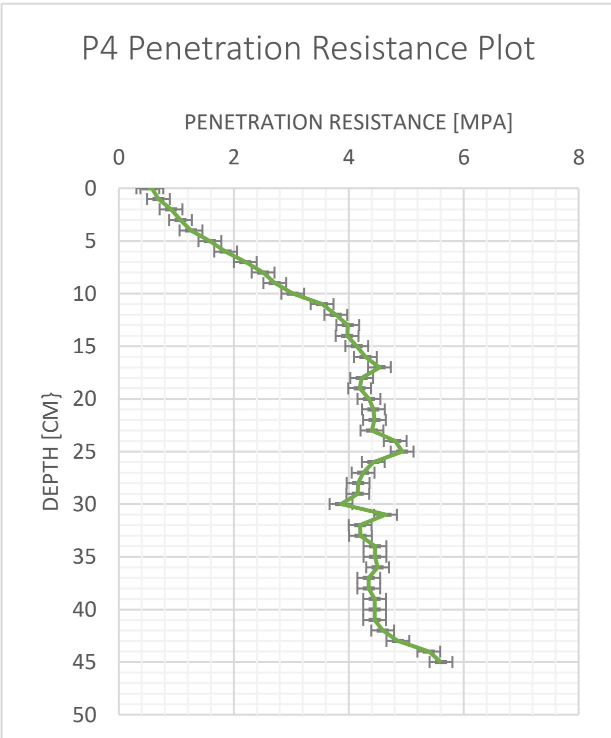
Soil penetration resistance

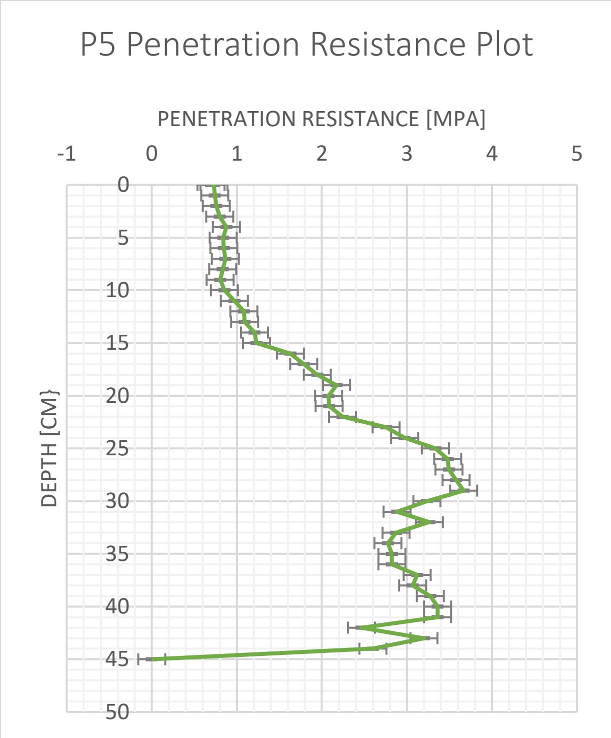
(No data for P13)

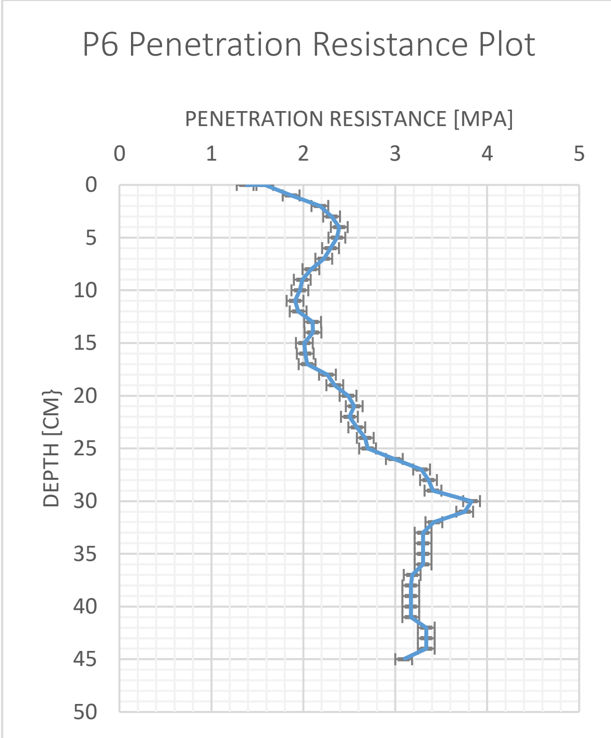


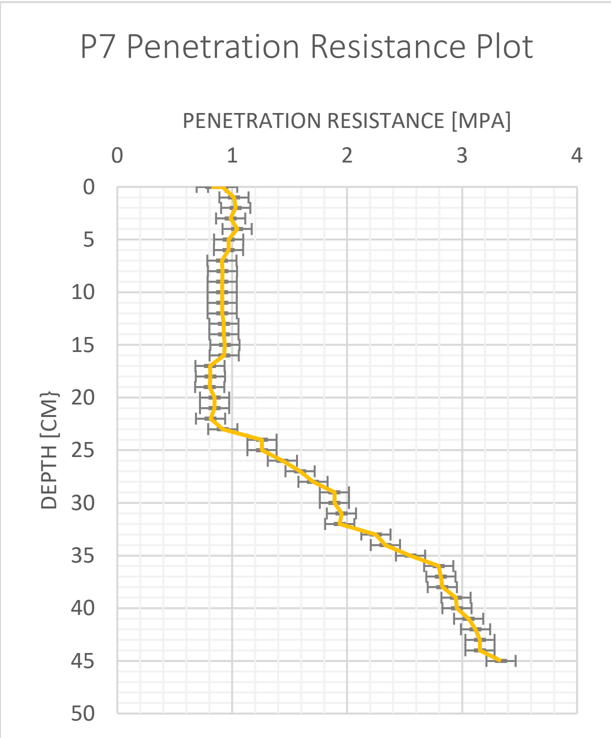


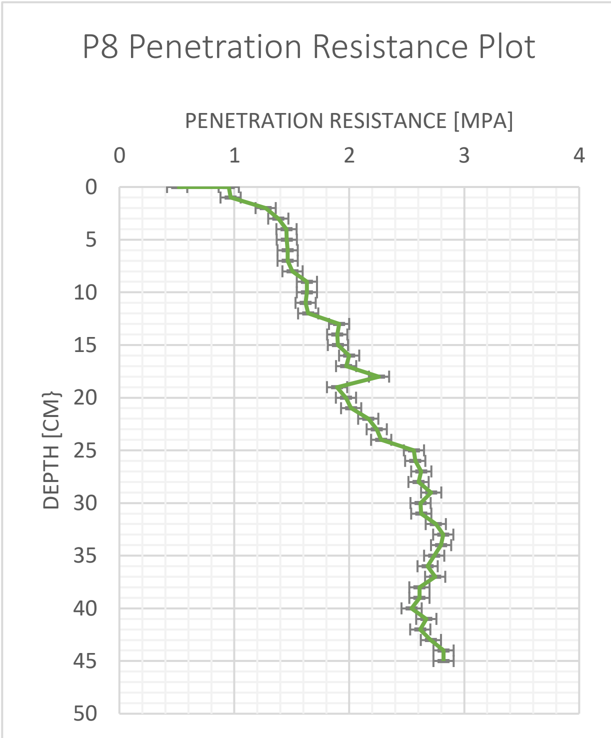


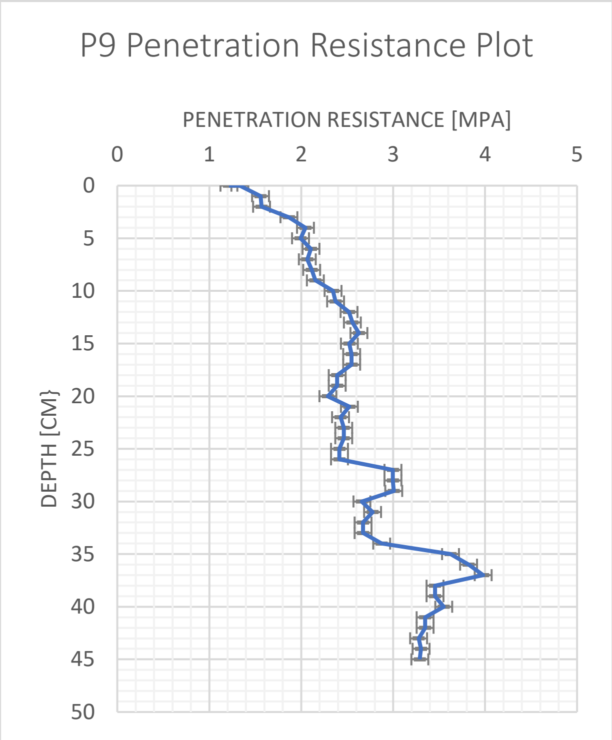


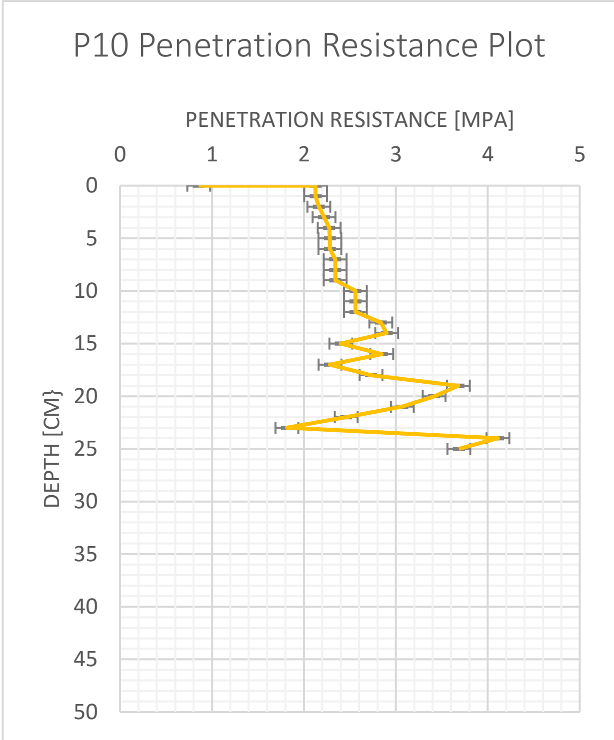

P5 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
-1 0 1 2 3 4 5
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

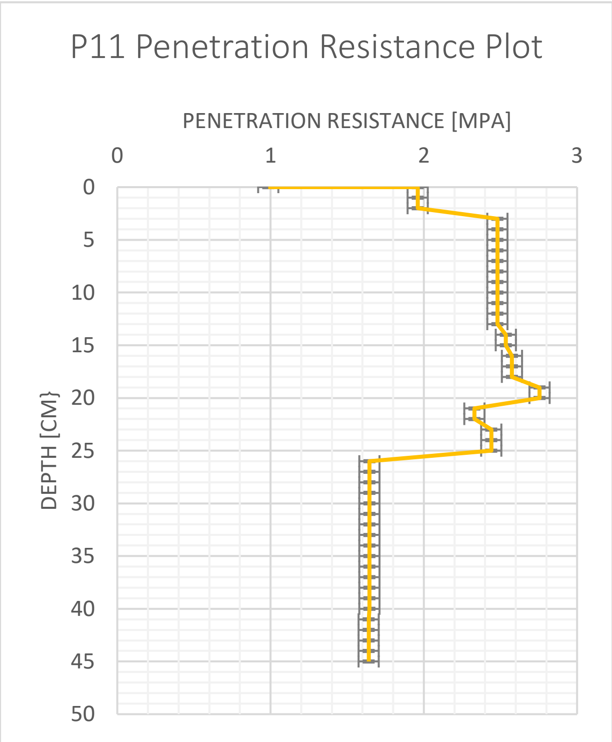

P6 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3 4 5
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

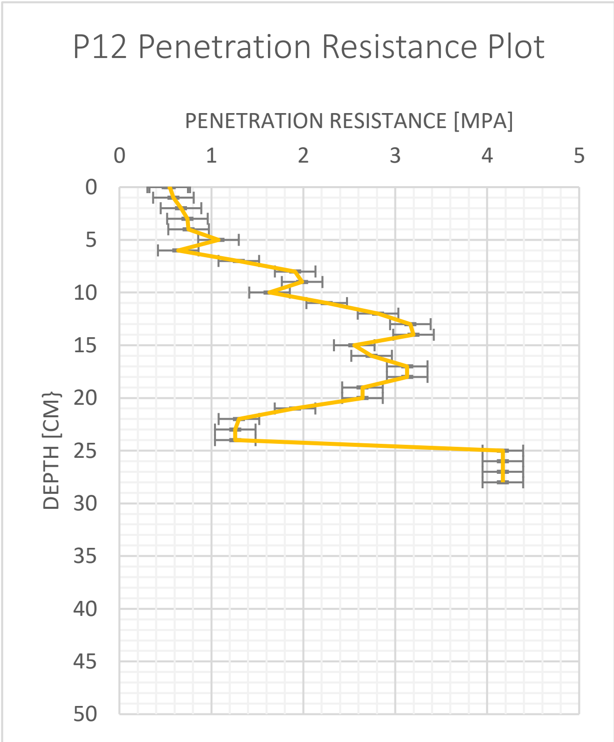

P7 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3 4
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

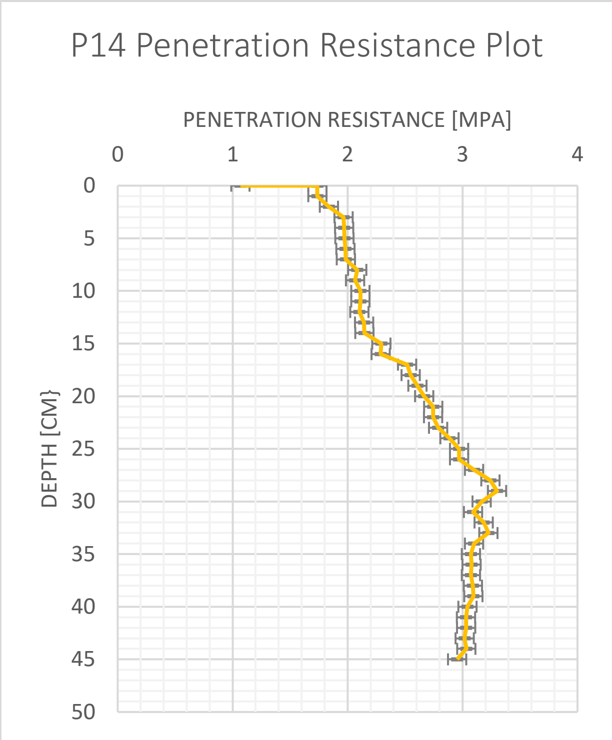

P8 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3 4
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

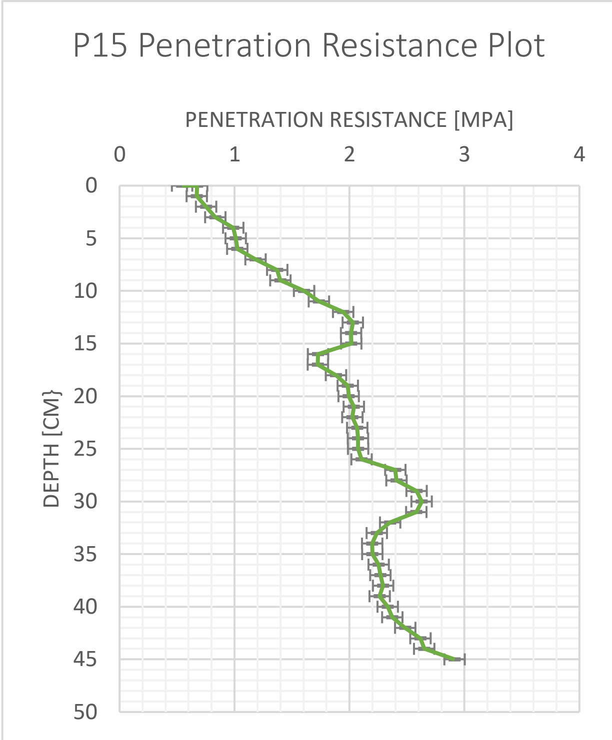

P9 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3 4 5
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

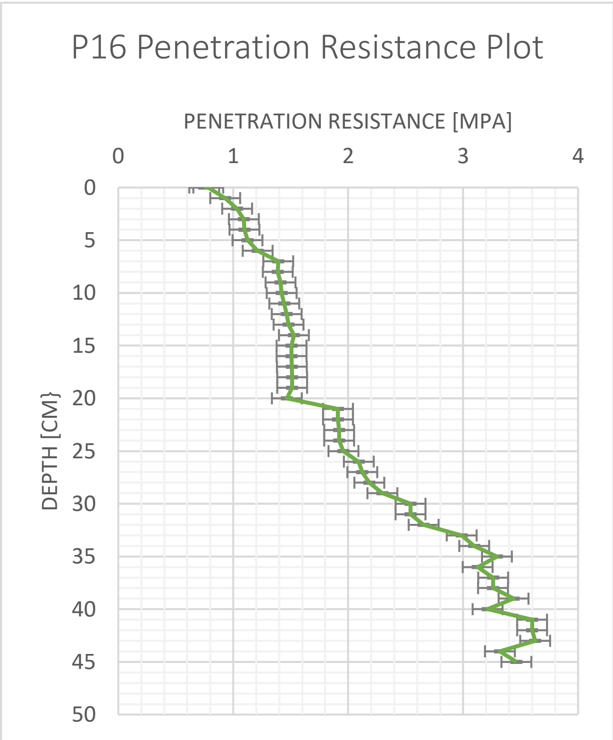

P10 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3 4 5
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

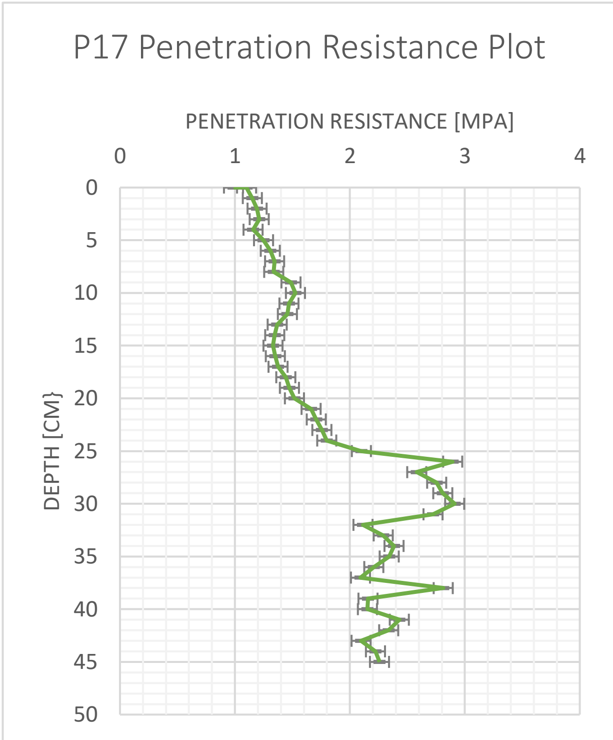

P11 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

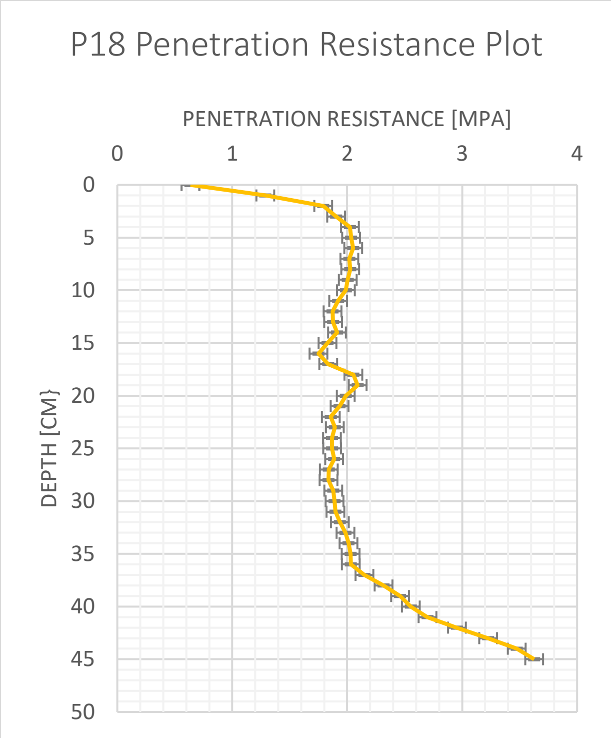

P12 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0 1 2 3 4 5
DEPTH [CM]
0 5 10 15 20 25 30 35 40 45 50

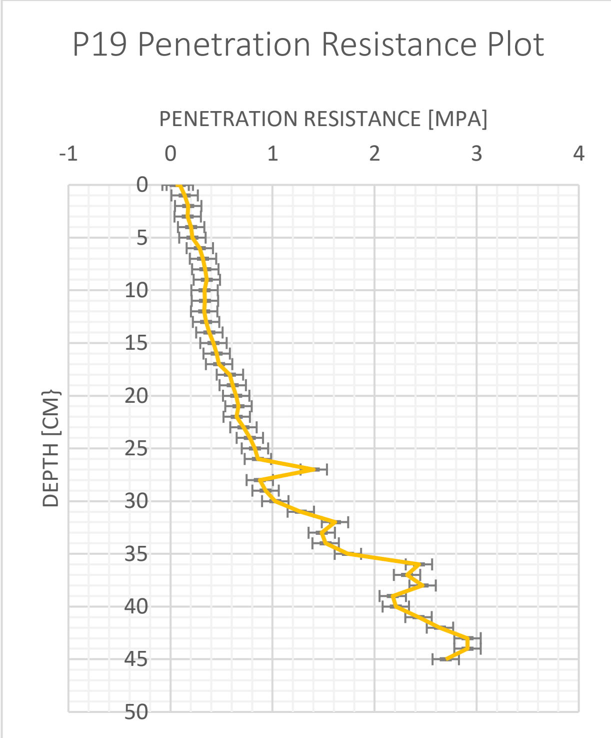

P14 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50

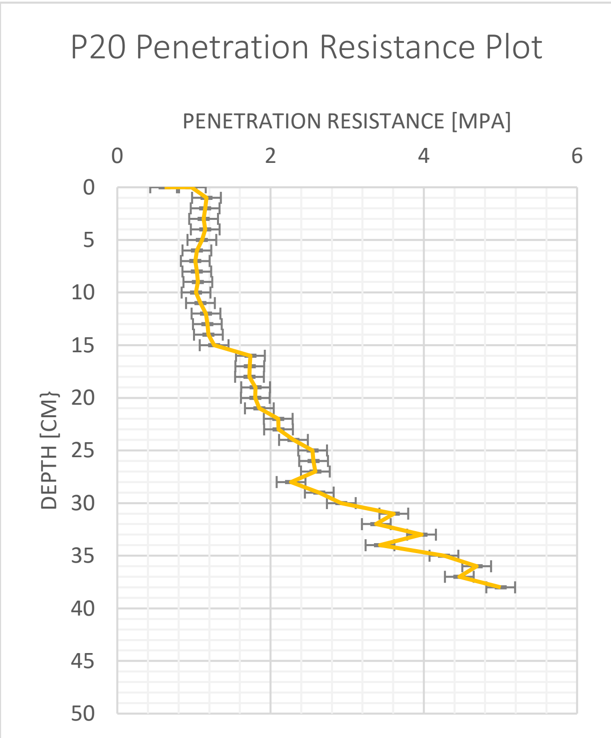

P15 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50

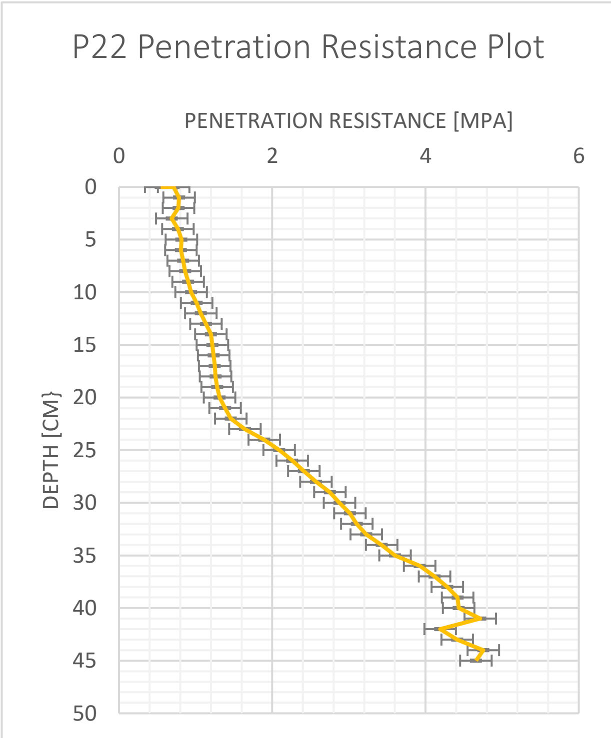

P16 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50

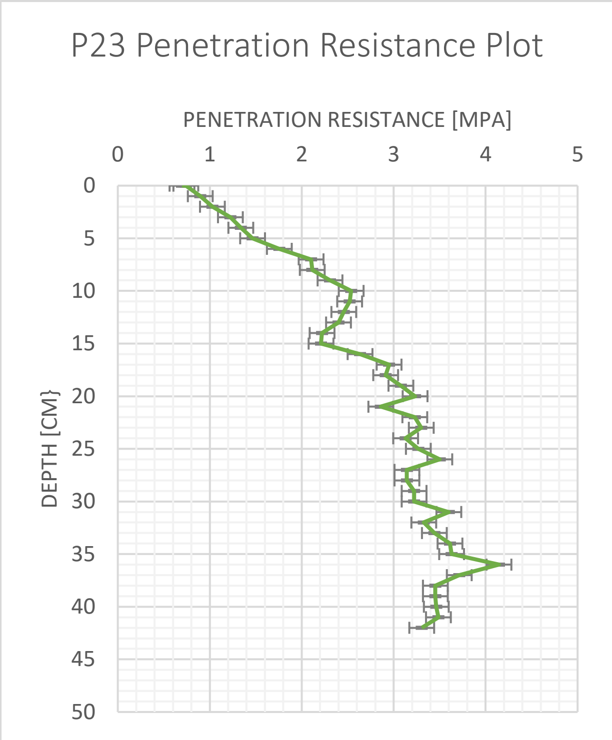

P17 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50

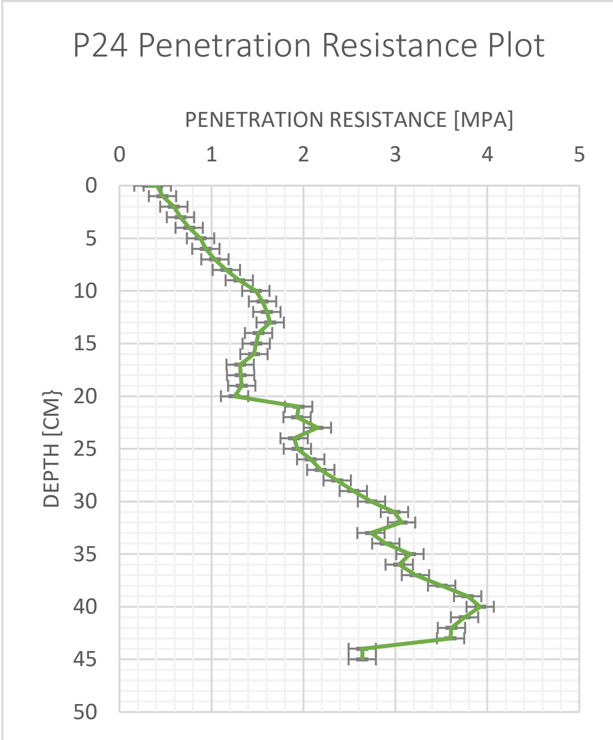

P18 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50

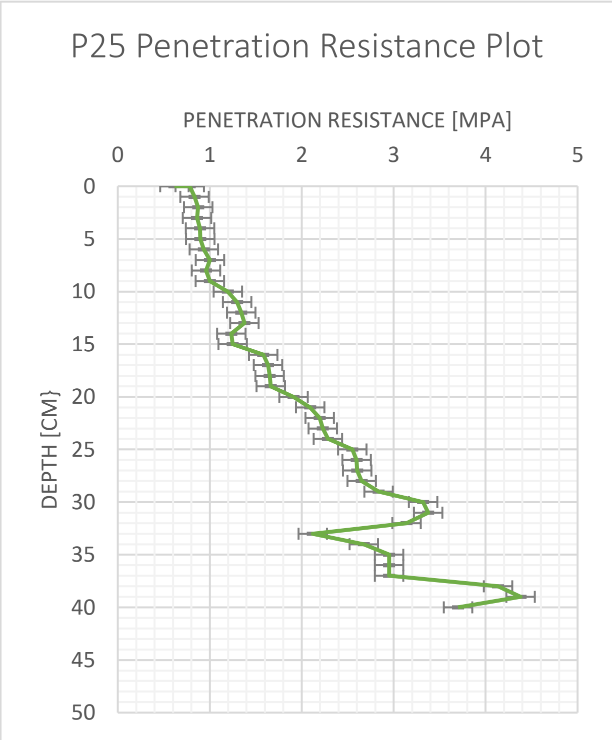

P19 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
-1
0
1
2
3
4
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50


P20 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
2
4
6
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50


P22 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
2
4
6
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50


P23 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
5
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50


P24 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
5
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50


P25 Penetration Resistance Plot
PENETRATION RESISTANCE [MPA]
0
1
2
3
4
5
DEPTH [CM]
0
5
10
15
20
25
30
35
40
45
50