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Drenning, P., Volchko, Y., Enell, A. et al (2024). A method for evaluating the effects of gentle remediation options (GRO) on soil health:

Demonstration at a DDX-contaminated tree nursery in Sweden. *Science of the Total Environment*, 948. <http://dx.doi.org/10.1016/j.scitotenv.2024.174869>

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# A method for evaluating the effects of gentle remediation options (GRO) on soil health: Demonstration at a DDX-contaminated tree nursery in Sweden

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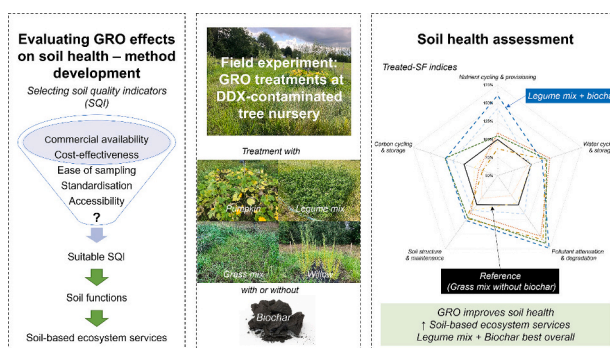
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## HIGHLIGHTS

- A method to evaluate the effects of GRO on soil health is proposed.
- Effects on soil quality indicators linked to soil functions and ecosystem services
- The method is applied to evaluate GRO treatments in a field experiment in Sweden.
- GRO have positive effects on soil health to improve delivery of ecosystem services.
- The positive effects are attributable to biochar amendment and leguminous plants.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Frederic Coulon

### Keywords:

Soil health  
Gentle remediation options (GRO)  
Soil functions  
Ecosystem services  
Multifunctionality  
Contaminated land

## ABSTRACT

Healthy soils provide valuable ecosystem services (ES), but soil contamination can inhibit essential soil functions (SF) and pose risks to human health and the environment. A key advantage of using gentle remediation options (GRO) is the potential for multifunctionality: to both manage risks and improve soil functionality. In this study, an accessible, scientific method for soil health assessment directed towards practitioners and decision-makers in contaminated land management was developed and demonstrated for a field experiment at a DDX-contaminated tree nursery site in Sweden to evaluate the relative effects of GRO on soil health (i.e., the ‘current capacity’ to provide ES). For the set of relevant soil quality indicators (SQI) selected using a simplified logical sieve, GRO treatment was observed to have highly significant effects on many SQI according to statistical analysis due to the strong influence of biochar amendment on the sandy soil and positive effects of nitrogen-fixing leguminous plants. The SQI were grouped within five SF and the relative effects on soil health were evaluated compared to a reference state (experimental control) by calculating quantitative treated-SF indices. Multiple GRO treatments are shown to have statistically significant positive effects on many SF, including *pollutant attenuation and degradation*, *water cycling and storage*, *nutrient cycling and provisioning*, and *soil structure and maintenance*. The SF were in turn linked to soil-based ES to calculate treated-ES indices and an overall soil health index (SHI), which

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<https://doi.org/10.1016/j.scitotenv.2024.174869>

Received 12 March 2024; Received in revised form 16 July 2024; Accepted 16 July 2024

Available online 20 July 2024

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can provide simplified yet valuable information to decision-makers regarding the effectiveness of GRO. The experimental GRO treatment of the legume mix with biochar amendment and grass mix with biochar amendment are shown to result in statistically significant improvements to soil health, with overall SHI values of 141 % and 128 %, respectively, compared to the reference state of the grass mix without biochar (set to 100 %).

## 1. Introduction

Well-functioning, healthy soils are vital to human well-being by supporting not only crop production for food security but also providing other essential ecosystem services (ES), defined as the direct and indirect contributions of an ecosystem to human well-being (TEEB, 2010), such as water purification and regulation, carbon sequestration, nutrient cycling, habitat for biodiversity and many others (Adhikari and Hartemink, 2016; El Mujtar et al., 2019; Greiner et al., 2017; Jónsson and Davíðsdóttir, 2016; Smith et al., 2015). In the recent *Proposal for a Directive on Soil Monitoring and Resilience*, healthy soils have been defined as soils that are in good chemical, biological and physical conditions and are able to continuously provide as many ecosystem services as possible (EC, 2023); however, approximately 60–70 % of the soils in the European Union are considered unhealthy due to degradation (Veerman et al., 2020). Soil contamination (used here as synonymous to pollution) is one of the main causes of soil degradation that can both pose serious risks to human health and negatively affect soil biota in performing their functions, which can inhibit the soil's capacity to provide ES (FAO et al., 2020; FAO and UNEP, 2021; Orgiazzi et al., 2016; Turbé et al., 2010). There are ca. 2.8 million potentially contaminated sites in Europe alone (Pérez and Eugenio, 2018), and remediating these sites to improve the health of soils is an important objective in the proposed *Soil Monitoring Law* (EC, 2023). In general, soil contamination with inorganic (e.g., metals) and organic contaminants (e.g., pesticides, POPs) is often a result of human activity that commonly degrades urban, industrial, and mining soils but can also negatively affect agricultural soils (EC, 2023; FAO and UNEP, 2021).

In Sweden, for instance, historical use of dichlorodiphenyltrichloroethane (DDT) as an insecticide at tree nursery sites has resulted in a large-scale contamination problem with >700 such sites requiring remediation due to elevated concentrations of DDT and its metabolites (DDX) persisting in the soil over decades (SGI, 2017). In addition to the potential risks to human health and the environment, studies have shown that long-term DDX contamination can also have significant negative impacts on soil health by reducing e.g., fungal counts, microbial biomass carbon, respiration and enzyme activity, which may not recover even after a long period of field ageing (Edvantoro et al., 2003; Megharaj et al., 2000, 1999). Many of these tree nursery sites are large, diffusely contaminated areas to relatively shallow depths and have good quality natural soils of high value to preserve and reuse for a biologically productive end use. For such soils it is important to consider that the remediation techniques employed can have variable impacts on soil functions (O'Brien et al., 2017; Volchko et al., 2013) and can even be completely destructive to the soil ecosystem in the case of conventional excavation-based methods (Breure et al., 2018). The links between soil biodiversity and soil functioning should be maintained or restored during the remediation process where possible, appropriate and cost-effective (EC, 2023; Gómez-sagasti et al., 2012). For, the ultimate objective of remediation should be not only to manage risks but also restore soil health (Epelde et al., 2008; FAO et al., 2020; Gómez-sagasti et al., 2012). In this regard, gentle remediation options (GRO) are highly relevant as a subset of nature-based solutions utilizing plants, bacteria, fungi, and/or soil amendments that can potentially be used to both effectively manage risks from contamination while at the same time improving (or at least not reducing) soil functionality over time (Cundy et al., 2016). Indeed, several studies have used a combination of physical, chemical and biological indicators to evaluate the effects of GRO on soil health, including from phytoremediation (Burges et al., 2017, 2016;

Epelde et al., 2014b; Mench et al., 2022) or various soil amendments such as lime (Mijangos et al., 2010) and biochar (Bera et al., 2016), which can show broad improvements within only a few growth seasons. Despite the progress and many proposed methodologies in recent years (Andrews et al., 2004; Creamer et al., 2022; Epelde et al., 2014a; Moebius-Clune et al., 2016; Rutgers et al., 2012; Volchko et al., 2014a), there is not yet a consensus on terminology nor methodological approach. While *soil health* and *soil quality* tend to be used interchangeably (Bünemann et al., 2018), soil health is used in this study in accordance with the terminology and conceptual framework established in the recent EJP SOIL SIREN project; where soil health refers to a soil's actual or current 'capacity' or 'status' to perform its functions and deliver ecosystem goods or services given the soil's condition at the local field level under current management practices or degradation levels (Faber et al., 2022). Assessing soil health entails investigating the soil's capacity to provide multiple ecosystem services (i.e., multifunctionality), and how it would change under different management practices. Evaluation is based on the quantification and measurement of soil quality indicators (SQI), or 'descriptors' (EC, 2023), related to soil properties and processes together making up important soil functions (Bünemann et al., 2018; EEA, 2022; Faber et al., 2022; Lehmann et al., 2020; Van der Meulen and Maring, 2018). *Soil function* (SF) is defined here in line with Bünemann et al. (2018) as (*bundles of*) *soil processes that underpin the delivery of ecosystem services*, e.g., the bundle of biogeochemical processes regulating nutrient availability and retention together contribute to the soil function 'Nutrient cycling and provision' which underpins the ES 'Biomass production'. Grouping individual, correlated SQI into higher-level categories such as SF and ES can facilitate interpretation of laboratory data for soil health assessments, improve communication with stakeholders as well as provide long-term monitoring programs with the ability to adapt through time against changes in techniques, methods, interests, etc. (Burges et al., 2018; Epelde et al., 2014a, 2014b; Faber et al., 2013; Garbisu et al., 2011; Gómez-sagasti et al., 2012). In Sweden, practitioners have reported that many aspects of soil health assessment, including measuring biological indicators, still belong primarily to the scientific realm and are not practically applicable (Faber et al., 2022). Indeed, developing practical assessment methods and facilitating communication about soil health with stakeholders are important objectives in line with the upcoming *Soil Monitoring Law* (EC, 2023).

There are however still uncertainties and knowledge gaps regarding the assessment of soil health, particularly in the context of contaminated sites. The multifunctionality of soils and their contribution to providing multiple ES is still not fully accounted for in many ES assessments or ontologies such as the Common International Classification of Ecosystem Services, CICES (Faber et al., 2022; Haines-Young and Potschin, 2018; Paul et al., 2021). There is not yet a prevailing method for the systematic selection of indicators and their connection to SF and ES to evaluate the effects of remediation techniques such as GRO on soil health. In those studies evaluating GRO, the indicators for various soil properties are rarely connected to specific soil functions or relate to the potential increased delivery of ecosystem services as a result of GRO application. Also, many methods measure inherent soil properties relating to the capability of a soil to provide ES (an 'absolute' assessment) and may not be able to capture the dynamic responses and short-term changes in soil health resulting from the effects of soil management (Obriot et al., 2016), e.g., using different soil remediation techniques. To bridge these gaps, this study builds on these approaches to further develop an accessible, scientific method directed towards practitioners

and decision-makers to integrate current knowledge on soil health assessment into contaminated land management for assessing and interpreting the relative impacts of GRO on soil health for improving ES delivery.

The overall aim of this study is twofold: i) to develop a systematic method to evaluating the effects of GRO on soil health by assessing the relative change in the soil's 'current capacity' to perform its functions and supply ES, and ii) to demonstrate the application of the developed method for a field experiment at a DDX-contaminated tree nursery site to determine whether the tested GRO improve soil health at this site. The specific objectives are to: a) select a battery of SQI for evaluating GRO's effects on soil health by using a simplified 'logical sieve'; b) perform statistical analysis of the effects of eight different GRO treatments on selected SQI to determine statistical significance; c) link the selected SQI to specific SF and ES; d) evaluate the effects of GRO on soil health in terms of relative improvements, or reductions, in the soil's capacity to perform its functions and provide ES by comparing to a reference state and calculating quantitative treated-SF indices, treated-ES indices and an overall treated-soil health index. It should however be noted that risk management and the fate of DDT and its metabolites (DDX) is not the focus of this paper.

## 2. Materials and methods

Fig. 1 gives an overview of the steps taken to develop a method for evaluating the effects of GRO on soil health.

### 2.1. Field experiment

#### 2.1.1. Site description

The field experiment site, Kolleberga, is located at a former tree nursery in Southern Sweden (Ljungbyhed), with a previous cultivation of pine and spruce plants on 23 ha to serve the forest industry. Since its initial usage in 1950s, technical DDT was used to control different types of pests, both by dipping the plants in barrels of dissolved DDT as well as spraying across the field by hand and with tractors. Despite the Swedish ban on DDT in 1969, DDT and its metabolites (including both *p,p'* and *o,p'* isomers) dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), hereafter collectively referred to as ΣDDX, are still detected in the agricultural fields. Soil concentrations of ΣDDX at Kolleberga have been found to be in the range between 5 and 15 mg/kg<sub>dw</sub> to a depth of approximately 0.35 m below ground level due to repeated ploughing and mixing of the soil in the fields (Nilsson, 2019). These concentrations exceed the Swedish generic soil guideline value of acceptable levels for a less sensitive land use of 1 mg/kg<sub>dw</sub> for ΣDDX.

The agricultural fields are no longer used for productive forestry but are managed by sowing a mixture of grasses, periodically cutting and ploughing the grass back into the soil. The parent soil is a glacio-fluvial sediment of loamy sand consistency with 87 % fine-medium sand, 4 % silt, 7 % clay, and 2 % gravel and larger stones, with a bulk density of approximately 1500 kg/m<sup>3</sup>, and is well-drained with moderate levels of organic carbon and neutral pH (Table S1). Depth to the groundwater table is ca. 4–5 m.

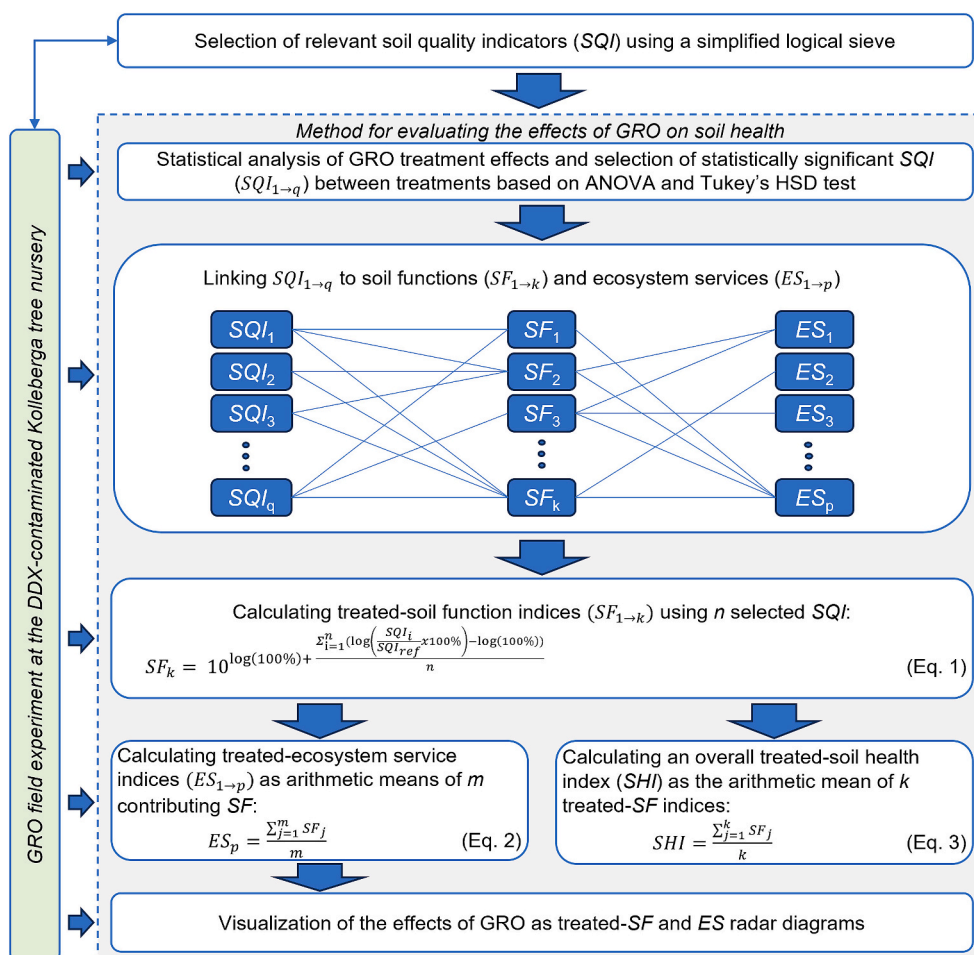


Fig. 1. Schematic illustration of the methodology followed for evaluating the effects of GRO on soil health at the Kolleberga tree nursery site.

### 2.1.2. Experimental set-up

The pilot-scale field experiment was established by excavating soil from the contaminated field at Kolleberga to a depth of 35 cm, homogenizing, and divided into two piles of equal volume. Biochar (produced by pyrolysis of wood chips and bark using a floating bed reactor at 750 °C for 20 min) was added as a soil amendment to one of the piles at a ratio of 3 % (w/w) and thoroughly mixed. The experimental plots were set up according to a randomized block design with the treatments in triplicate in 2 × 2 m plots with a depth of 35 cm. The soil was distributed randomly to the plots divided into three blocks where half contained the biochar-amended soil and half the unamended soil (24 plots in total). Four different plant species (or mixes of species), as determined through reviewing comparable studies to manage DDX-contaminated land, were planted in the biochar amended (even treatment (T) numbers) and unamended soil plots for eight treatments in total: T1 & T2 pumpkin (*Cucurbita pepo* ssp. *pepo* cv. Howden), T3 & T4 grasses (mixture of *Festuca rubra*, *Festuca pratensis*, *Phleum pratense*, *Poa pratensis*, and *Lolium perenne*), and T5 & T6 legumes (mix of clover/alfalfa; *Trifolium repens*/*Medicago sativa*), and T7 & T8 willow (*Salix viminalis* cv. Emma and Ester ratio of 50/50 %). The plant species were intended for three different GRO strategies identified as potentially feasible for the site (Drenning et al., 2022): phytoextraction using pumpkin (Denyes et al., 2016; Lunney et al., 2010; Paul et al., 2015; White, 2002; White et al., 2005); phytostabilisation using willow and grasses separately (Lunney et al., 2004; Mitton et al., 2012); and phyto/rhizodegradation using legumes (Gregory et al., 2015; Purnomo et al., 2011; Wu et al., 2008). The purpose of adding biochar to the soil was primarily to aid/improve the stabilization of DDX (i.e., to decrease the soil porewater concentration and bioavailability of the DDX), which has been successful in other applications (Denyes et al., 2016; Enell et al., 2020; Wang et al., 2018). Amendment with biochar can also improve the physical, chemical, and biological properties of soil (Gul et al., 2015; Kookana et al., 2011; Lehmann et al., 2011; Rijk et al., 2024), potentially having positive effects on phytoremediation as well as improving soil health, so it is evaluated in combination with all plants. The plants were watered regularly using an automated irrigation system, with each plot receiving the same amount of water, and the plants were chemically fertilized (NPK 11–5–18) in the beginning of each growth season then minimally to pumpkin and willow according to need. The grass mixture without biochar (T3) serves as an experimental control (reference state) that resembles Kolleberga's present vegetation (according to the prevailing management plan for the site), while controlling for irrigation effects. See the SM for more details on the biochar (Table S2), experimental establishment (Fig. S1) and design (Fig. S2).

### 2.1.3. Soil sampling and analysis

At the start of the experiment, before any planting, the field soil and experimental plots (Fig. S1) were sampled and analysed to determine the initial soil parameters (Table S1). Soil samples were collected by digging multiple test pits in the plots to a depth of 25 cm, and then collecting soil using a hand shovel across the soil profile, and thoroughly mix the soil in a bucket to homogenize. After each subsequent growth season, sampling points within the plots were randomized to select sampling locations, the plant biomass harvested by cutting the stems close to the ground surface level, and soil samples were collected using a small (Ø 2 cm) core sampler and extracting 20 soil cores at four random locations in each plot, to a depth of 20–25 cm, which were then homogenized, collected in diffusion-tight plastic bags, labelled for each individual plot and stored in cooling boxes before sending to the labs. The samples were sieved through a Ø 2 mm sieve either by the responsible laboratory (first year) or directly in the field (second year).

Accredited commercial and university labs were contracted to perform soil analysis for the field experiment and standardized methods followed whenever possible (overview of the methods is provided in Table S3). Total ΣDDX concentrations in the soil were measured using GC–MS according to (Rashid et al., 2010) and moisture content by

thermogravimetry according to SS-EN 12880:2000. The following soil physicochemical parameters were measured: determination of pH in water (ISO 10390:2021); extraction and determination of Ca, K, Mg, and P content in soil using the AL-method (SS 028310:1993); determination of soil particle size distribution using the sieving and sedimentation method (ISO 11277); determination of total nitrogen (N-tot) after dry combustion (ISO 13878); determination of total organic carbon (TOC) after dry combustion (ISO 10694); determination of plant-available nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) content by flow analysis and spectrometric detection (ISO 13395); determination of maximum water holding capacity, WHC<sub>max</sub> (ISO 11268-2:2012, Annex C). Microbial biomass was determined as microbial biomass carbon (MBC) according to the fumigation-extraction method (ISO 14240-2:1997). Microbial basal respiration (BR) was determined as O<sub>2</sub> consumption over five days by the use of the OxiTop® method (WTW GmbH; Platen and Wirtz, 1999), according to (ISO 16072:2002). A conversion factor of 0.9 was assumed for the respiratory quotient to convert O<sub>2</sub> consumption to release of CO<sub>2</sub> (Ben-Noah and Friedman, 2018). Potential nitrification was determined according to (ISO 15685:2012). The earthworm bioassay was conducted with the test species *Eisenia fetida* according to ISO 11268-1 (acute toxicity) and – 2 (reproduction). Five of the living adult earthworms from each sample were randomly selected and removed on day 28, weighed individually, held on filter paper for 24 h to purge guts, weighed after purging, and then placed in glass vials and frozen to use for measuring the DDX uptake into their biomass. The ΣDDX concentration in earthworms was analysed by modifying the method described in Henriksson et al. (2017) (complete method described in the SM). The bait lamina (filled strips purchased from TerraProtecta GmbH) field assessment was carried out according to ISO 18311:2016.

## 2.2. Quantification and evaluation of the effects of GRO on soil health

### 2.2.1. Selection of SQI via logical sieve

A gross list of SQI was compiled through a non-exhaustive, narrative literature review, including findings from other major reviews and reports compiling the most commonly used indicators for soil monitoring programs (e.g., Bünemann et al., 2018; Faber et al., 2013; Griffiths et al., 2016; Ritz et al., 2009; Stone et al., 2016; Turbé et al., 2010). Many indicators have been considered for soil monitoring programs to assess soil quality, which may not be suitable for this application. Indeed, only dynamic parameters were considered for this assessment since inherent parameters (e.g., particle size distribution) are typically not responsive to GRO treatment. Emphasis was placed on studies in the context of contaminated sites that have used indicators for both ecological risk assessment and evaluating the performance of phytoremediation and/or the addition of soil amendments (e.g., (Borges et al., 2016, 2017; Epelde et al., 2008, 2009; Gómez-sagasti et al., 2012; Gutiérrez et al., 2015; Kumpiene et al., 2009; Niemeyer et al., 2012; Quintela-Sabarís et al., 2017). Standardized methods such as ISO 19204:2017 for performing ecological risk assessment using the Triad approach (ISO, 2017) and proposed minimum datasets (MDS) or test battery for evaluating GRO (GREENLAND, 2014) or soil functions in remediation projects (Volchko et al., 2014b, 2019, 2020) were used as a starting point. To filter the gross list of potential SQI, a simplified logical sieve, modified from the method proposed in Ritz et al. (2009), was used whereby indicators were filtered according to a list of five pertinent criteria to determine suitability for use in this context: i) accessibility for non-experts, ii) commercial availability, iii = cost-effectiveness, iv) ease of field sampling, and v) standardization (see Table S5 in SM). Scores were assigned to the SQI for each criterion using a simple binary system based on literature review, best professional judgement in collaboration with experts, and the results of previous logical sieve studies (Faber et al., 2013; Griffiths et al., 2016; Ritz et al., 2009; Stone et al., 2016), with a score of 1 if it fulfilled the criterion or a 0 if it did not. As this study is an applied research with no closely connected research laboratory, and an important aim has been to ensure replicability by

practitioners, the criterion of *availability in commercial laboratories* and *cost-effectiveness* were particularly emphasized and became the main determinants of which SQI were suitable. Following the logical sieve, a test battery of highest scoring indicators was then selected for use and complemented with other non-standard measurements for this particular case.

2.2.2. Statistical data analysis

Analysis of variance (ANOVA) was used to statistically analyse the data and determine if the treatment effects of GRO were statistically significant ( $p < 0.05$ ) for individual SQI, where only those that were significantly different (i.e., sensitive to changes in management) were included in calculating the treated index value for each soil function (SF). A correlation matrix was used to determine if there were significant correlations (Pearson's  $r > 0.8$ ) between measured indicators to remove any redundancies (Obriot et al., 2016; Rinot et al., 2019). Using data from the second year of the experiment (Y2), the differences between GRO treatments' effects on the SQI and interaction effects were assessed by means of two-way ANOVA, with Plants (P) and Amendment (A, with or without biochar) as fixed factors and random effects of blocks in a split-plot model design. GRO treatment effects were also statistically analysed to determine if there were statistically significant differences between the calculated index value for each SF according to a two-way ANOVA with Plants (P) and Amendment (A) as fixed factors and including random effects of blocks. For each ANOVA analysis, homoscedasticity was evaluated by visually observing a plot of the variance of error residuals and Levene's test ( $p < 0.05$  means non-homogeneous variance between groups), and normality was evaluated by observing Q-Q plots and the Shapiro test ( $p < 0.05$  means data not normally distributed). If homogeneity and normality assumptions were not met, a non-parametric Kruskal-Wallis, one-way ANOVA was performed instead for Plants and Amendment individually. When significant results were obtained using ANOVA ( $p < 0.05$ ), Tukey's HSD test was used to make multiple pairwise comparisons and differences between groups deemed significant if  $p < 0.05$ . All statistical tests were performed using R statistical software v. 4.2.1 (R Core Team, 2022). The linear mixed-effects models were created using the lme4 package (Bates et al., 2015), statistical tests and ANOVA in linear mixed-effects models using the lmerTest package (Kuznetsova et al., 2017), the car package (Fox and Weisberg, 2019), pairwise comparison and Tukey's HSD test using the emmeans package (Lenth, 2022), and compact letter display using the multcomp package (Hothorn et al., 2008) and multcompView (Graves et al., 2019). The correlation matrix was performed using jamovi and R statistical code (Jamovi, 2022; R Core Team, 2022).

2.2.3. Connecting indicators to soil functions and soil-based ecosystem services

A non-exhaustive narrative literature review was carried out to compile frequently proposed SF, soil-based ES, sub-functions, and processes. Redundancies, repetition, and overlap between definitions, processes, and terminology were aggregated and an overarching term and definition proposed if there is no clear consensus. This synthetic list of SF and soil-based ES was also tentatively matched with classes of the Common International Classification of Ecosystem Services CICES v5.1 (Haines-Young and Potschin, 2018) where there existed an equivalent service and gaps highlighted where there was not, as proposed by Paul et al. (2021). Using the EJP SIREN conceptual framework (Faber et al., 2022) as the theoretical basis, the SQI used in this study were grouped within the higher-level categories of SF based on correlations/associations between indicators and specific soil functions/processes. Based on prevailing literature, the SF were then linked to the specific soil-based ES to which the particular function contributes, which could be to one or several different services. The resulting hierarchical connections between the selected SQI, SF and soil-based ES are used in the proposed soil health assessment.

Table 1 presents a conceptualization of the hierarchical connections

Table 1

Linkages between selected SQI, SF and ES. NCP: nutrient cycling and provision; WCS: water cycling and storage; PAD: pollutant attenuation and degradation; SSM: soil structure maintenance; CCS: carbon cycling and storage.

	Soil functions				
	NCP	WCS	PAD	SSM	CCS
<b>Soil quality indicators</b>					
Basal respiration (BR)	x		x		x
Microbial biomass carbon (MBC)	x		x	x	
Potential nitrification/ammonification (PotNit)	x				
Earthworm biomass growth (EW <sub>Growth</sub> )		x		x	x
Earthworm DDT uptake (EW <sub>DDX</sub> )			x		
Bait lamina (BaitLam)	x				x
Total organic carbon (TOC)		x	x	x	x
Water holding capacity (WHC <sub>max</sub> )		x			
Total organic nitrogen (N-tot)	x				
Plant-available nitrate- and nitrite-nitrogen (NO <sub>2</sub> <sup>-</sup> + NO <sub>3</sub> <sup>-</sup> -N)	x				
Available nutrients (P-AL, K-AL, etc.)	x				
<b>Ecosystem services</b>					
Soil decontamination & bioremediation (SDB)			x		
Water purification, supply & regulation (WPSR)	x	x	x	x	
Climate regulation & carbon sequestration (CRCS)	x				x
Erosion control (EC)				x	
Biomass production (BMP)	x	x	x	x	x

by which the selected SQI used to evaluate effects of GRO were grouped within the higher-level categories of soil functions (SF), which in turn underpin the delivery of soil-based ecosystem services (ES). The selected indicators were linked to five aggregated, well-established SF with a variety of constituent sub-functions and processes, included in this assessment: nutrient cycling and provisioning (NCP), water cycling and storage (WCS), pollutant attenuation and degradation (PAD), soil structure maintenance (SSM), and carbon cycling and storage (CCS) (Table S15). By compiling their principal sub-functions and processes, the aggregated SF were in turn connected to one or more relevant soil-based ES whose delivery they underpin (Table S16).

2.2.4. Calculation of soil health indices

The treated-soil function indices to evaluate the effects of GRO on soil health were calculated using the index proposed by Epelde et al. (2014b), which was modified from the 'treated soil quality index' from Mijangos et al. (2010) and further developed in Burges et al. (2017, 2016). This index was used to demonstrate the positive or negative impacts of a remediation treatment on soil health through grouping the measured SQI within a set of ecosystem attributes of ecological relevance (Epelde et al., 2014b; Garbisu et al., 2011; Mijangos et al., 2010). The novelty of the method suggested here is attributable to first linking the SQI with intermediate SF and then to each specific soil-based ES, instead of connecting directly to ecosystem services (ES), as done in e.g., Burges et al. (2017, 2016).

First, the effects of GRO on each SF were calculated as the log-transformed difference in each SQI for a specific treatment compared to a reference state with same soil type (here T3) and an index value was calculated for each individual SF, based on the following equation (Epelde et al., 2014b):

$$SF_{1 \rightarrow k} = 10^{\log(100\%) + \frac{\sum_{i=1}^n \left( \log \left( \frac{SQI_i}{SQI_{ref}} \times 100\% \right) - \log(100\%) \right)}{n}} \tag{1}$$

where,  $SQI_{ref}$  corresponds to the mean value of the reference (ref) state for each indicator;  $SQI_i$  is the measured value for each treatment and replicate;  $n$  is the total number of SQI grouped within each SF used to

calculate the index ( $SF_{1 \rightarrow k}$ ); the mean values of the reference state are set to 100 % to calculate the factorial deviation (see the SM for more details and an example calculation).

Eq. (1) assumes that a higher value is better for each indicator in comparison to the reference state. Where a lower value is a better indication of soil health (i.e., earthworm DDX uptake for Pollutant attenuation and degradation), the factorial deviation for the specific indicator was reversed and included in the summation. Biotic and abiotic SQI were grouped within each SF according to Table 1 and Fig. S3.

Further, the relative change in soil health as the ‘current capacity’ of the soil to perform its functions were then used to derive trends regarding the eventual delivery of specific ES for human benefit. The arithmetic mean of the set of contributing SF for each soil-based ES ( $ES_{1 \rightarrow p}$ ) was calculated using Eq. (2) as treated-ES indices to give an indication of the expected change (positive, negative or no effect) compared to the reference state:

$$ES_p = \frac{\sum_{j=1}^m SF_j}{m} \quad (2)$$

An overall treated-soil health index (SHI), which provides an integrated score of the effects of each GRO treatment on soil health compared to the reference state, was calculated as the arithmetic mean of all the treated-SF indices using Eq. (3):

$$SHI = \frac{\sum_{j=1}^k SF_j}{k} \quad (3)$$

The current study evaluates GRO treatment effects on specific SQI from the first two years of the experiment (Y1 & Y2). However, the SHI is calculated just using data from the Y2, since the dataset for Y1 was incomplete for the treatment soils.

A sensitivity analysis of the effects of GRO on ES was also carried out by using both the arithmetic mean and geometric mean, which prevents extreme values from overly influencing the resulting mean, and comparing the results to determine if there were changes.

### 3. Results

#### 3.1. Selection of soil quality indicators

The results of the simplified logical sieve to filter a shortlist of possible SQI for evaluating GRO are presented in Table 2. A test battery of the highest scoring indicators was selected based on the results from this process and used to evaluate the effects of GRO treatment on soil health. Many SQI resulted in a total score of ‘5’ as they were recommended by many different references, commercially available, cost-effective, and accessible for non-experts. However, commercial availability was a significant bottleneck as it was difficult to find commercial labs to source many of the potential SQI, and some indicators that were possible to source commercially were prohibitively expensive (e.g., dehydrogenase activity). It is worth pointing out, however, that a score of 0 does not indicate that they are impossible to source everywhere, but rather were not possible to find when carrying out the logical sieve.

#### 3.2. GRO treatment effects on SQI

Statistical analysis of the data to determine the SQI where GRO treatment had statistically significant effects (according to ANOVA and Tukey’s HSD test), and removing indicators with strong correlation, resulted in 6 physical and chemical indicators (Fig. 2, Table S10) and 6 biological indicators (Fig. 3, Table S11) for calculation of the treated-SF indices (see the SM for more details on GRO treatment effects).

Regarding the effects of biochar amendment (A), highly significant ( $p < 0.001$ ) positive effects were observed on physical and chemical SQI such as TOC,  $WHC_{max}$ , N-tot, and available nutrients (P-AL, K-AL) while

significant ( $p < 0.05$ ) negative effects were observed on plant-available nitrite- and nitrate-nitrogen ( $NO_2^- + NO_3^-$ -N) according to two-way ANOVA (Fig. 2, Table S10). Several studies report an increase in water retention, porosity, aeration and aggregate stability with the addition of biochar (Blanco-Canqui, 2021; Gul et al., 2015; Hou, 2021; Hou et al., 2023; Zhang et al., 2021) and effects may be particularly pronounced in sandy soils (Li et al., 2021; Razzaghi et al., 2020). The fertility of the soil in terms of nutrient and carbon content is significantly improved by the addition of biochar. Biochar is observed to reduce  $NO_2^- + NO_3^-$ -N content in soil, which is in line with other studies on effects of biochar produced in high temperature pyrolysis (Brtnicky et al., 2021; El-Naggar et al., 2019; Kookana et al., 2011; Lehmann et al., 2011; Zhang et al., 2021; Zhao et al., 2023).

Regarding biological indicators (Fig. 3, Table S11), the effects of biochar amendment are more mixed with both significant positive and negative effects. Biochar was observed to significantly ( $p < 0.05$ ) increase the potential nitrification rate (PotNit), and basal respiration (BR). Contrary to expectations, however, biochar amendment was observed to have no significant effect on microbial biomass carbon (MBC), which contrasts with many other biochar studies where a decrease (or no change) in BR and an increase in MBC has been broadly observed across different contexts and types of biochar (Domene et al., 2014; Lehmann et al., 2011; Liu et al., 2020a; Zhang et al., 2014; Zheng et al., 2016; Zhou et al., 2017).

Regarding soil fauna, biochar amendment was observed to have a highly significant ( $p < 0.001$ ) effect on  $EW_{Growth}$ , with a decrease of 6–27 % compared to unamended soils, and significant ( $p < 0.05$ ) effects on the feeding activity of soil meso- and macrofauna as measured using bait lamina (BaitLam) which were observed to be broadly negative with a decrease of 5–71 % compared to treatments without biochar. However, biochar was also observed to have a highly significant ( $p < 0.001$ ) effect on reducing the uptake of DDX in earthworm fatty tissue ( $EW_{DDX}$ ), which could reduce the toxic pressure on soil organisms. In general, these results seem to agree with the scientific consensus as many studies have shown that biochar (across different pyrolysis temperatures and application rates) can negatively impact soil fauna (e.g., earthworms, Collembola, enchytraeids, nematodes), which can include reduced feeding activity as measured with bait lamina (Marks et al., 2016; Prodana et al., 2019, 2021); demonstrating avoidance behavior to certain types of biochar (Domene et al., 2015; Prodana et al., 2019; Tammeorg et al., 2014); reduced abundance, density, growth or reproduction of soil fauna (particularly earthworms) in biochar amended soils (Briones et al., 2020; Brtnicky et al., 2021; Conti et al., 2018; Liu et al., 2020b; Zhao et al., 2023); and toxic effects to soil fauna from biochar, particularly at high pyrolysis temperature and application rates (Brtnicky et al., 2021; Gruss et al., 2019; Zhao et al., 2023). However, other studies have shown contradictory results with no clear negative effects from biochar on soil fauna (Bamminger et al., 2014; Brtnicky et al., 2021; Domene et al., 2014, 2015; Gruss et al., 2019; Honvault et al., 2023; Jeffery et al., 2022; Verheijen et al., 2009).

In terms of plant effects (P), nitrogen-fixing legumes (i.e., clover and alfalfa – T5 & T6) are observed to have a highly significant ( $p < 0.001$ ) positive effect on both N-tot ( $p < 0.01$ ) and  $NO_2^-$ ,  $NO_3^-$ -N ( $p < 0.001$ ) concentrations in the soil, increased by 473 % (T5) and, respectively, 195 % (T6) compared to T3 (ref. state), which can counteract the reduced N availability from biochar amendment (Fig. 2, Table S10). The legume mix was also observed to have a highly significant ( $p < 0.001$ ) effect on both microbial activity (increasing BR and PotNit) and abundance (increasing MBC) compared to the T3 reference state. Pumpkin treatments (T1, T2) also show significantly increased PotNit, but this is likely a result of fertilization rather than direct plant effects. Nutrient availability also increases in pumpkin and willow treatments but the effect from fertilization likely impacts these results. Plant effects on BaitLam were highly significant ( $p < 0.001$ ) where the legume mix was generally higher and pumpkin much lower than the reference state. (Fig. 3, Table S11). Importantly, none of the plants are shown to reduce

**Table 2**

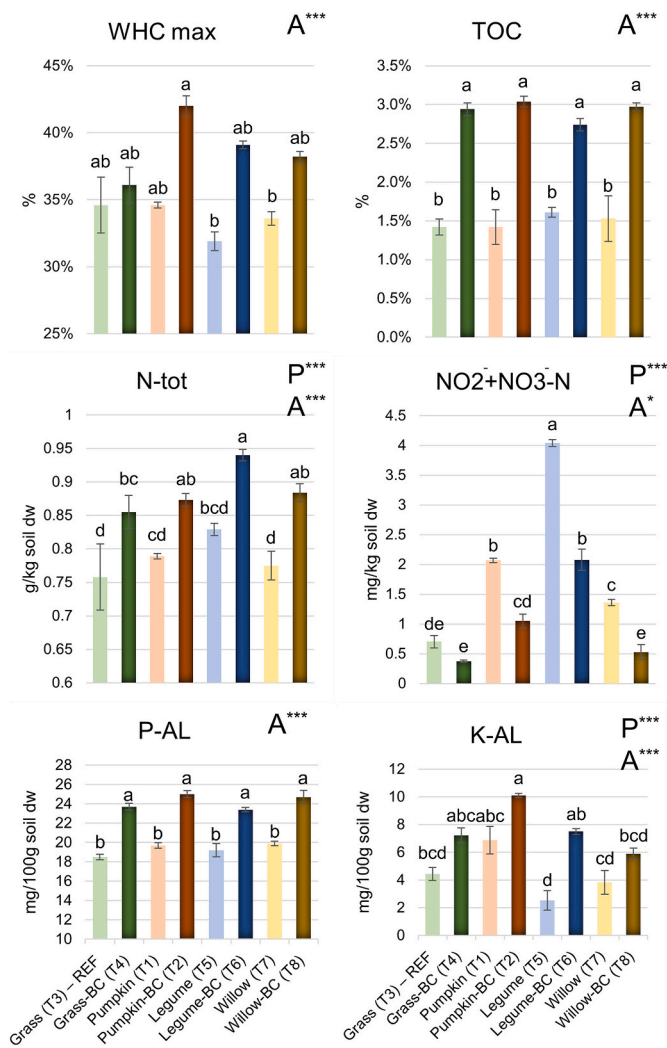
Results of the simplified logical sieve. Indicators presented in descending order according to total score. A '?' indicates reliable information could not be found.

Recommending references	Indicator & method	Filtering criteria					Total
		Accessibility	Commercial availability <sup>a</sup>	Cost-effectiveness <sup>b</sup>	Ease of sampling	Method standardization	
(Volchko et al., 2014a, 2014b, 2019)	Particle size distribution - %L,Si,Sa,Gr (ISO 11277)	1	1	1	1	1	5
(Volchko et al., 2014a, 2014b, 2019)	pH (ISO 10390:2005)	1	1	1	1	1	5
(Volchko et al., 2014a, 2014b, 2019)	Available nutrients (incl. P-AL, K-AL, etc.) (SS 028310:1993)	1	1	1	1	1	5
(Volchko et al., 2014a, 2014b, 2019)	Total N, Kjeldahl method (ISO 13878)	1	1	1	1	1	5
(Volchko et al., 2014a, 2014b, 2019)	TOC (ISO 10694:1995)	1	1	1	1	1	5
(Andrews et al., 2004; Faber et al., 2013; GREENLAND, 2014; Gugino et al., 2009; ISO, 2017; Moebius-Clune et al., 2016; Volchko et al., 2014a, 2014b, 2019)	Potentially mineralizable N (ISO 14238, Gugino et al., 2009) (incl. Other N measurements of nitrification and ammonification potentials)	1	1	1	1	1	5
(Epelde et al., 2014b; Faber et al., 2013; Garbisu et al., 2011; GREENLAND, 2014; Gutiérrez et al., 2015; ISO, 2017; Stone et al., 2016)	Soil microbial biomass carbon (ISO 14240-1&2:2011)	1	1	1	1	1	5
(Andrews et al., 2004; Burges et al., 2016; Epelde et al., 2008, 2009, 2010, 2014b; Faber et al., 2013; Garbisu et al., 2011; GREENLAND, 2014; Gutiérrez et al., 2015; ISO, 2017; Kumpiene et al., 2009; Ritz et al., 2009; Stone et al., 2016)	Soil microbial basal respiration (ISO 16072:2002; Moebius-Clune et al., 2016)	1	1	1	1	1	5
Faber et al., 2013, Epelde et al., 2008, 2009, 2010, 2014a, 2014b, Gutiérrez et al., 2015, Burges et al., 2020	Substrate-induced respiration (ISO 17155:2012; OECD 217)	1	1	1	1	1	5
(Gutiérrez et al., 2015; ISO, 2017; Tiberg et al., 2019)	Earthworm bioassay: behavior, mortality, reproduction, biomass growth (ISO 11268-2:2012)	1	1	0	1	1	4
(Faber et al., 2013; Griffiths et al., 2016; ISO, 2017; Kibblewhite et al., 2008a)	Bait lamina test (ISO 18311:2016)	1	1	1	0	1	4
(Burges et al., 2016; Epelde et al., 2008, 2010; GREENLAND, 2014)	Assessment of dehydrogenases activity (ISO 23753-1&2:2019)	1	1	0	1	1	4
(Alkorta et al., 2003; Burges et al., 2016; Epelde et al., 2008, 2009, 2010; Faber et al., 2013; Garbisu et al., 2011; GREENLAND, 2014; Griffiths et al., 2016; Gutiérrez et al., 2015; Kumpiene et al., 2009; Ritz et al., 2009)	Assessment of biochemical processes through multiple soil enzyme activities and multi-enzyme profiling (ISO 22939:2019)	1	0	0	1	1	2
(Epelde et al., 2010; Griffiths et al., 2016; ISO, 2017; Stone et al., 2016; Tiberg et al., 2019)	Functional gene abundance using qPCR (targeting antibiotic producers, nitrifiers, denitrifiers) (ISO 17601:2016; ISO 11063:2020)	0	0	0	1	1	2
(Andrews et al., 2004; Enell et al., 2016; Faber et al., 2013; Griffiths et al., 2016; ISO, 2017; Kibblewhite et al., 2008a; Ritz et al., 2009; Stone et al., 2016)	Nematode morphological identification (taxa) and abundance of individual functional groups (ISO 23611-4:2019)	0	0	?	0	1	1
(Creamer et al., 2016; Griffiths et al., 2016)	Multiple substrate-induced respiration ( <i>MicroResp</i> )	0	0	?	1	0	1
(Faber et al., 2013; Griffiths et al., 2016; Kibblewhite et al., 2008a; Ritz et al., 2009; Stone et al., 2016)	Earthworm morphological identification (taxa) and abundance of individual functional groups (ISO 23611-1:2018)	0	0	?	0	1	1
(Faber et al., 2013; Griffiths et al., 2016; Kibblewhite et al., 2008a; Ritz et al., 2009; Stone et al., 2016)	Collembola morphological identification (taxa) and abundance of individual functional groups (ISO 23611-2:2006)	0	0	?	0	1	1
(Faber et al., 2013; Griffiths et al., 2016; Kibblewhite et al., 2008a; Ritz et al., 2009; Stone et al., 2016)	Soil microbial community structure and biomass from PLFA (ISO/TC 29843-2:2011)	0	0	0	?	1	1
(Epelde et al., 2014b; Faber et al., 2013; Garbisu et al., 2011; Griffiths et al., 2016; Gutiérrez et al., 2015; ISO, 2017; Ritz et al., 2009; Stone et al., 2016)	Structural diversity through molecular measurements of the microbial community (fingerprinting), using e.g., TRFLP, PCR-DGGE, ARISA (ISO 11063:2012)	0	0	0	0	1	1
(Epelde et al., 2009, 2019; Faber et al., 2013; Gutiérrez et al., 2015; Rutgers et al., 2016)	Bacterial functional diversity /community-level physiological profiling (using e.g., Biolog® Ecoplates)	0	0	0	0	0	0

<sup>a</sup> A score of '0' does not indicate that the analysis is commercially unavailable everywhere, rather that the authors were unable to find a commercial laboratory to perform this analysis during the start-up phase of this project.

<sup>b</sup> If specific information could not be found, the results of the logical sieve by Griffiths et al. (2016) was used to give a relative indication.





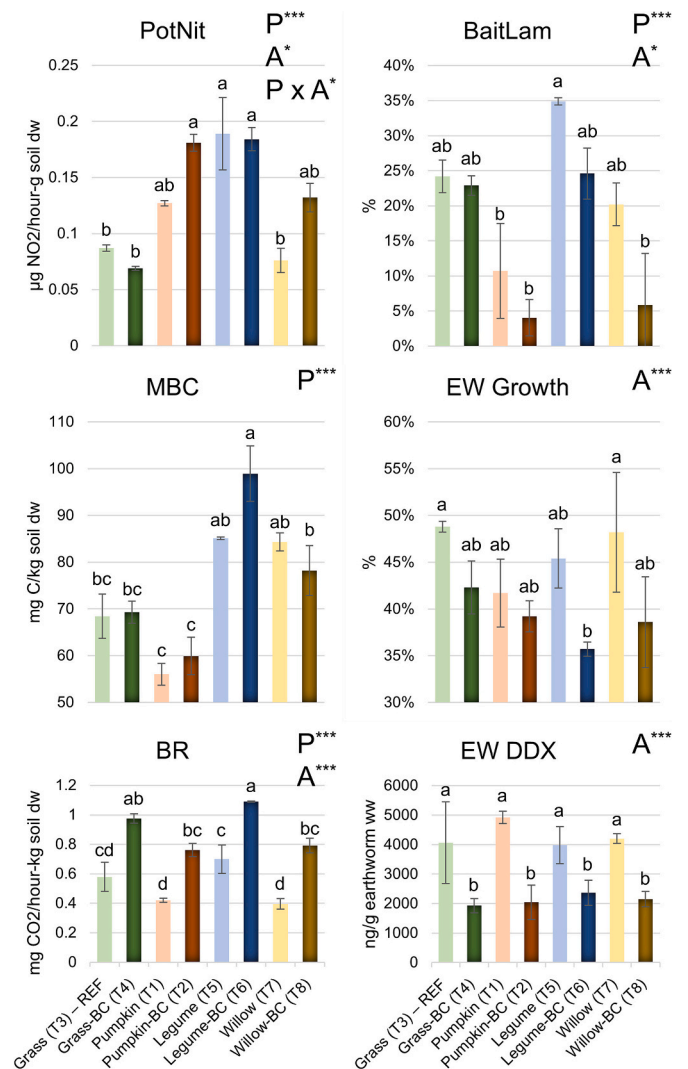
**Fig. 2.** GRO treatment effects on selected physical and chemical soil quality indicators, data from the second year of the experiment (Y2). Data are mean values ( $n = 3$ ), error bars are standard error. The bars with darker shading indicate treatments with biochar soil amendment. Probability values from two-way ANOVA showing effects of plants (P) and biochar amendment (A) shown in the top right corner for each SQI:  $p < 0.001$  (\*\*\*),  $p < 0.01$  (\*\*),  $p < 0.05$  (\*). Pairwise comparisons between groups were calculated using the Tukey HSD test and results shown using compact letter display: if two or more means share the same grouping letter, then they are not shown to be significantly different. TOC: total organic carbon (%<sub>carbon</sub>); WHC<sub>max</sub>: maximum water holding capacity (%<sub>soil dw</sub>); N-tot: total organic nitrogen (g/kg<sub>soil dw</sub>); NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup>-N: sum of nitrite and nitrate-nitrogen (mg/kg<sub>soil dw</sub>); P-AL: available phosphorous (mg/100 g<sub>soil dw</sub>); K-AL: available potassium (mg/100 g<sub>soil dw</sub>). REF: reference state, experimental control.

EW<sub>DDX</sub> which is an important indicator of mitigating the toxic pressure of DDX.

### 3.3. Treated-soil function and ecosystem services indices, and overall soil health index

The effects of GRO on soil health in Kollleberga compared to reference state (grass without biochar, T3) were determined by calculating multiple treated-SF indices (Eq. (1)), presented in Table 3 (including results of ANOVA and Tukey's HSD test) and visualised in Fig. 4A. The overall treated-soil health index (SHI) for each treatment (Eq. (3)) is also shown in Table 3 and the treated ES indices are visualised in Fig. 4B.

Broadly, biochar amendment has a significant positive effect on the resulting index values for NCP ( $p < 0.01$ ) and highly significant positive



**Fig. 3.** GRO treatment effects on biological soil quality indicators, data from the second year of the experiment (Y2). Data are mean values ( $n = 3$ ), error bars are standard error. The bars with darker shading indicate treatments with biochar soil amendment. Probability values from two-way ANOVA showing effects of plants (P) and biochar amendment (A) shown in the top right corner for each SQI:  $p < 0.001$  (\*\*\*),  $p < 0.01$  (\*\*),  $p < 0.05$  (\*). Pairwise comparisons between groups were calculated using the Tukey HSD test and results shown using compact letter display: if two or more means share the same grouping letter, then they are not shown to be significantly different. BR: basal respiration (mg CO<sub>2</sub> / hour-kg<sub>soil dw</sub>); MBC: microbial biomass carbon (mg C / kg<sub>soil dw</sub>); PotNit: potential nitrification rate (μg NO<sub>2</sub><sup>-</sup>/hour-g<sub>soil dw</sub>); BaitLam: bait lamina (puncture count per strip); EW<sub>Growth</sub>: earthworm growth (% increase in body weight between day 0 and day 28 of bioassay); EW<sub>DDX</sub>: earthworm DDX uptake into tissue (ng/g<sub>earthworm ww</sub>). REF: reference state, experimental control.

effects on WCS, PAD, SSM and the overall SHI ( $p < 0.001$ ). The effects of biochar on CCS show no clear statistical differences between groups with and without biochar. Plants are shown to have a significant effect on SSM ( $p < 0.05$ ) and highly significant effects on NCP, CCS and the overall SHI ( $p < 0.001$ ), with most of the positive differences associated with the legume mix (T5, T6) while grass (T3, T4) and willow (T7, T8) were more neutral, and pumpkin (T1, T2) was consistently the most negative. Many of the GRO treatments showed significant improvements in multiple treated-SF indices compared to the reference state, T3 (Fig. 4A; Table 3): NCP – legumes both with (T6) and without (T5) biochar; WCS – pumpkin with biochar (T2) and grass with biochar (T4); PAD – all treatments with biochar (T2, T4, T6, T8); SSM – legumes with

**Table 3**

Treated-soil function indices – using grass control as reference state (mean value set to 100 %). Data are mean values (n = 3) ± standard deviation; Probability values from two-way ANOVA shown below:  $p < 0.001$  (\*\*\*) ,  $p < 0.01$  (\*\*),  $p < 0.05$  (\*), n.s. = not significant. If significant differences were shown in ANOVA, pairwise comparisons between groups were calculated using the Tukey HSD test and results shown using compact letter display: if two or more means share the same grouping letter, then they are not shown to be significantly different. Significant differences from the reference (T3 – REF) are shown in bold. NCP: nutrient cycling and provision; WCS: water cycling and storage; PAD: pollutant attenuation and degradation; SSM: soil structure maintenance; CCS: carbon cycling and storage; SHI: overall treated-soil health index.

Treatment	NCP	WCS	PAD	SSM	CCS	SHI
Pumpkin (T1)	108 % ± 10 <sup>b</sup>	95 % ± 3 <sup>d</sup>	84 % ± 9 <sup>b</sup>	89 % ± 4 <sup>c</sup>	72 % ± 9 <sup>b</sup>	89 % ± 7 <sup>d</sup>
Pumpkin-BC (T2)	109 % ± 4 <sup>bc</sup>	<b>128 %</b> ± 6 <sup>a</sup>	<b>153</b> % ± 23 <sup>a</sup>	115 % ± 5 <sup>ab</sup>	78 % ± 3 <sup>b</sup>	116 % ± 7 <sup>bc</sup>
Grass (T3) – REF	100 % ± 3 <sup>b</sup>	100 % ± 5 <sup>bd</sup>	100 % ± 3 <sup>b</sup>	100 % ± 3 <sup>bc</sup>	100 % ± 2 <sup>ab</sup>	100 % ± 1 <sup>cd</sup>
Grass-BC (T4)	105 % ± 2 <sup>bc</sup>	<b>123 %</b> ± 1 <sup>ac</sup>	<b>166</b> % ± 7 <sup>a</sup>	122 % ± 2 <sup>ab</sup>	<b>128</b> % ± 13 <sup>a</sup>	<b>128</b> % ± 1 <sup>ab</sup>
Legume (T5)	<b>143</b> % ± 1 <sup>ac</sup>	99 % ± 3 <sup>bd</sup>	115 % ± 6 <sup>b</sup>	109 % ± 4 <sup>abc</sup>	116 % ± 2 <sup>ab</sup>	116 % ± 1 <sup>bc</sup>
Legume-BC (T6)	<b>161</b> % ± 2 <sup>a</sup>	116 % ± 15 <sup>abcd</sup>	<b>174</b> % ± 12 <sup>a</sup>	<b>126</b> % ± 18 <sup>a</sup>	<b>127</b> % ± 10 <sup>a</sup>	<b>141</b> % ± 10 <sup>a</sup>
Willow (T7)	98 % ± 6 <sup>b</sup>	101 % ± 3 <sup>bcd</sup>	97 % ± 6 <sup>b</sup>	109 % ± 1 <sup>abc</sup>	84 % ± 13 <sup>ab</sup>	98 % ± 3 <sup>cd</sup>
Willow-BC (T8)	87 % ± 22 <sup>b</sup>	121 % ± 5 <sup>abc</sup>	<b>159</b> % ± 14 <sup>a</sup>	<b>123</b> % ± 5 <sup>a</sup>	72 % ± 32 <sup>b</sup>	112 % ± 12 <sup>bc</sup>
ANOVA	Plant (P)					
	Amendment (A)	n.s.	n.s.	*	***	***
	P × A	n.s.	n.s.	n.s.	n.s.	n.s.

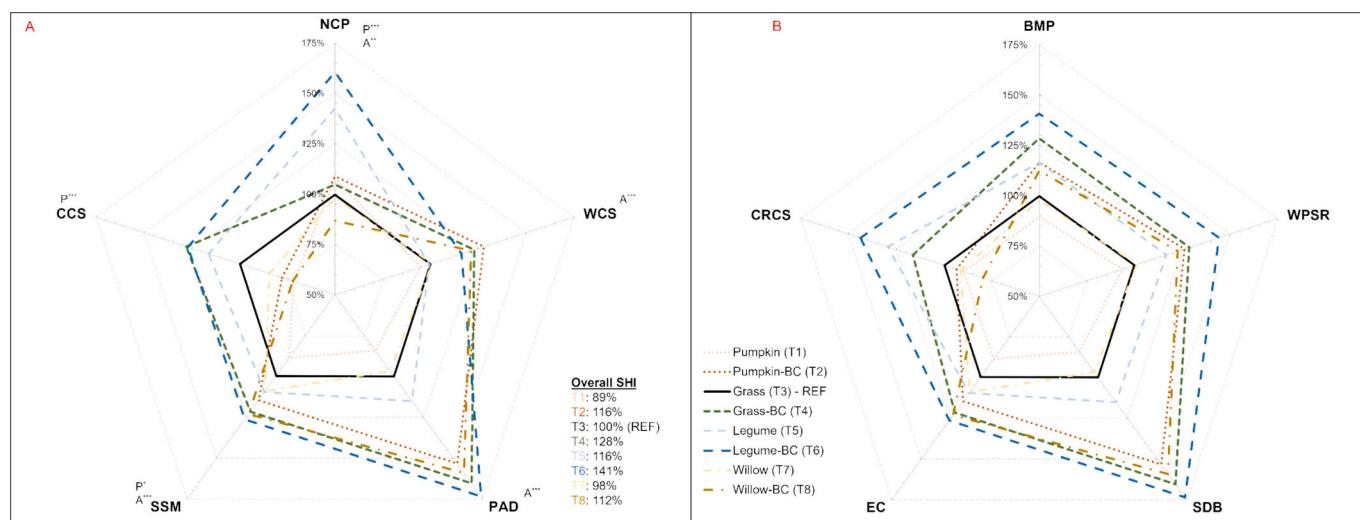
biochar (T6) and willow with biochar (T8); CCS – grass with biochar (T4) and legumes with biochar (T6). Overall, as an aggregated SHI, the treatments that are statistically different compared to the reference state (T3) are grass with biochar (T4) and legumes with biochar (T6). Of these, T6 has the higher comparative index value and this significant difference in soil health is due to the positive effects of both legumes and biochar on multiple SQI, which in turn results in an overall improvement in multiple SF.

Correspondingly, the treated-ES indices show that GRO treatment can have positive effects on multiple soil-based ES compared to the grass experimental control (T3) (Fig. 4B). Importantly for the context of a contaminated site such as Kollberga, the relative improvement is especially strong for soil decontamination & bioremediation (SDB) due to the positive effects of biochar to reduce DDX bioavailability (higher TOC, lowered EW<sub>DDX</sub>) as well as improving overall microbial activity (BR) from biochar amendment and the legume mix. Large relative improvements can also be seen for water purification, supply & regulation (WPSR) and erosion control (EC) for all treatments with biochar due to significant improvements in SQI relating to NCP, SSM and WCS, e.g., large increase in organic carbon and available nutrients. However, the results are more mixed for climate regulation & carbon sequestration (CRCS), despite the large increase in TOC for increased carbon storage, and can be linked to variable effects of biochar amendment on soil fauna relating to carbon turnover. Biochar did not lead to significant improvements in the overall production function, biomass production (BMP), either. A sensitivity analysis to calculating either the arithmetic or geometric mean showed only minor differences in the treated-SF and ES indices that did not substantially change the results.

**4. Discussion**

**4.1. The effects of GRO on soil health**

Overall, the results of the treated-SF and ES indices indicate where there could be potential synergies in GRO treatment to improve the multifunctionality of the soil to provide multiple ES. The overall SHI may not provide specific information regarding the improvement of specific SF or ES, but it can provide a simple indication of the aggregated effects of the GRO treatment for communicating with stakeholders. A main, frequently-cited advantage of GRO is the potential for



**Fig. 4.** Radar diagrams showing the effects of GRO using: A) treated-soil function indices (NCP: nutrient cycling and provision; WCS: water cycling and storage; PAD: pollutant attenuation and degradation; SSM: soil structure maintenance; CCS: carbon cycling and storage) – with probability values from two-way ANOVA showing effects of plants (P) and biochar amendment (A) for each SF:  $p < 0.001$  (\*\*\*) ,  $p < 0.01$  (\*\*),  $p < 0.05$  (\*); and B) treated-ecosystem service indices (BMP: biomass production; WPSR: water purification, supply and regulation; SDB: soil decontamination and bioremediation; EC: erosion control; CRCS: climate regulation and carbon sequestration). The legend is the same for both radar diagrams: the solid black line is the reference state (T3) that has been set to 100 % and thicker lines indicate treatments where biochar was used as a soil amendment.

multifunctionality: to potentially both manage risks and improve (or at least reduce) soil functionality to provide ES (Borges et al., 2018; Cundy et al., 2016; Drenning et al., 2022; Song et al., 2019). Analysis of the specific treatment effects of GRO indicate that the strongest effects on SQI were due primarily to the biochar amendment and the legume mix. While there can be site-specific differences and uncertainty in GRO's effectiveness to reduce total contaminant concentrations, many studies have corroborated these results that GRO can indeed have positive effects on soil health as measured using a variety of SQI, e.g., (Borges et al., 2016, 2018, 2020; Gómez-Sagasti et al., 2021; Lacalle et al., 2018; Mench et al., 2018; Quintela-Sabarís et al., 2017; Touceda-González et al., 2017). The results of the treated-SF and ES indices and overall SHI presented in this study also align with several studies that have utilised a soil health/quality index to demonstrate the positive effects of biochar (Bera et al., 2016; Carnier et al., 2023), phytoremediation (Barrutia et al., 2011; Borges et al., 2016, 2017; Mench et al., 2022), and a combination of these methods (Yadav et al., 2023).

Regarding biochar specifically, there is a large body of literature suggesting that biochar can have highly positive impacts on soil health and improve SF to provide multiple ES such as climate regulation (including reducing N<sub>2</sub>O emission, N leaching and runoff) and carbon sequestration through carbon storage with a long residence time (potentially hundreds to thousands of years (Gul et al., 2015; Kuzyakov et al., 2009)), improving biodiversity and habitat, soil fertility, biomass production, and others (Blanco-Canqui, 2021; Bolan et al., 2021; He et al., 2021; Hou, 2021). Less fertile, degraded and contaminated sandy soils of marginal quality may especially benefit from biochar amendment (Bekchanova et al., 2021; Tang et al., 2013). This seems to be true for the loamy sandy soil at Kollberga, where the biochar amendment is shown to stimulate the soil microbes and increase overall activity, which may be due to additional nutrient availability and retention, a small pool of labile C as well as improved soil pH, porosity, aeration and water retention that provides a favourable soil environment for microbes (El-Naggar et al., 2019; Lehmann et al., 2011). Biochars derived from wood feedstock (such as used in this study) have been noted to promote less microbial abundance (lower MBC) than other types of biochar and slow pyrolyzed biochar produced at high temperatures (>600 °C) can have less pronounced or even negative effects on soil aggregation, microbial biomass and enzyme activities in coarse textured soils (Gul et al., 2015). In general, however, the effects from biochar vary and the type of biochar (temperature, ash content, particle size, feedstock, etc.) and application rate strongly influence its effects on soil fauna and their ability to perform their essential functions (Brtnicky et al., 2021; Lehmann et al., 2011; Marks et al., 2016; Prodana et al., 2019; Verheijen et al., 2009). Biochar has also been shown to be an environmentally sustainable alternative remediation technique to conventional methods (Papageorgiou et al., 2021). There are, however, contradictory or mixed results regarding biochar's improvement on ES in some studies and results may depend on the type and application of biochar as well as soil conditions. For example, while biochar is shown here to generally improve *erosion control* (EC), erosion rates can potentially increase from sandy soils especially when biochar is applied to the soil surface (Brtnicky et al., 2021). Similarly, the effects of biochar on *biomass production* (BMP) also varies between studies, which can be a result of the immobilisation of plant-available forms of nitrogen (Rijk et al., 2024), but is generally considered to improve overall crop production in most cases (Brtnicky et al., 2021). It is also important to account for other potential drawbacks to biochar amendment such as nutrient immobilisation, reduced efficacy of agrochemicals and ecological risks from biochar amendment due to potential toxic effects on different groups of soil organisms such as earthworms (Brtnicky et al., 2021; Zhao et al., 2023). An important positive effect from biochar, particularly in the context of contaminated sites, is that it has a highly significant ( $p < 0.001$ ) effect on reducing the uptake of DDX in earthworm fatty tissue (EW<sub>DDX</sub>). These data indicate that biochar could ameliorate the potential toxic pressure from DDX on soil organisms such as earthworms by

reducing bioavailability, in line with (Denyes et al., 2016; Wang et al., 2018).

Trade-offs between ES are also possible (Blanco-Canqui, 2021), such as between maximizing BMP or CCS, which may not be evident in these results. Also, there are uncertainties in the results as suggested by the standard deviations for both individual SQI (Table S10 and S11) and resulting SF indices (Table 3), which can be large and indicate high variability in the data resulting from differential treatment effects, soil heterogeneity, or other sampling effects. The demand or prioritization of SF and ES may differ depending on the type of soil and land use as well as stakeholder preferences, which are not considered here. For Kollberga, all SF and ES are currently weighted equally but given that it is agricultural land, and the planned future land use is as a tree nursery, the site owner's primary interest is likely to ensure that the soil is fit for *biomass production* (BMP) while also managing the DDX contamination. BMP is here linked to all SF and an overall improvement, shown particularly for NCP, WCS and SSM, is beneficial and the PAD function is also significantly improved indicating that the toxic pressure from DDX is also mitigated.

#### 4.2. Application and limitations of the proposed method for evaluation of GRO effects on soil health

Despite the soil contamination, contaminated land can still have good soil quality and retain some capacity to perform its functions, provide valuable ES, and be worth preserving as a resource (e.g., fertile agricultural land), given that the contamination can be effectively managed (Séré et al., 2024; Volchko et al., 2013). There is therefore a need to integrate additional soil parameters and a broader soil health assessment as part of contaminated land management to improve decision-making and prioritize areas for preservation or development according to their capability and best condition (Blanchart et al., 2018; Volchko et al., 2014a, 2019). The soil system is highly complex and unlikely to be directly measurable, so for practical purposes, proxies for complex soil properties or processes representing multiple functional groups must be sought (Baveye et al., 2016; Kibblewhite et al., 2008b; Séré et al., 2024). While such a 'reductive approach' has been criticized, an indicator-based approach is an accessible and practical means of assessing effects of a soil management strategy, e.g., remediation technique, on soil health. As argued by Smith et al. (2015), there are still important knowledge gaps and more fundamental research is needed, but there is enough knowledge to start moving in the right direction and implement best practices to both improve the delivery of and raise awareness about the valuable ES underpinned by soils and the natural capital they provide. By using SQI as proxies to measure the (change in) state of a soil property or process linked to soil functions, which, as bundles of processes, can be connected to soil-based ecosystem services an indication of the delivery of ecosystem services can be provided (Baveye et al., 2016; Kibblewhite et al., 2008b; Vogel et al., 2018). The connections between SQI, SF and ES conceptualised in this study were based on the prevailing scientific literature, e.g., (El Mujtar et al., 2019; Faber et al., 2022; Kibblewhite et al., 2008b), but in some cases the linkages may not be clear or sufficiently well-supported. Further, the SQI, SF, and soil-based ES included are not exhaustive or encompassing the full range of soil processes and (sub)functions as described in e.g., (Cremer et al., 2022).

A limited set of criteria were applied in the logical sieve for identifying SQI and many potentially relevant indicators were excluded due to e.g., difficulty sourcing from a commercial lab, too expensive, novel or requiring specific expertise for interpretation. Many novel and non-standardized analysis methods that could provide valuable information about soil processes and functions were not possible to source from commercial labs or difficult to interpret (Zwetsloot et al., 2022). Indeed, commercial availability and cost-effectiveness were major bottlenecks for many potential SQI so that frequently recommended indicators that may be sensitive to changes in soil management such as soil enzyme

activities or functional biodiversity and gene abundances were not included. There may thus be limitations in the sensitivity and sophistication of the selected SQI. For instance, ecotoxicological indicators may not be direct measurement of processes or functions but they are useful indicators of the state of target organisms and potential inhibition in degraded soils for performing their functions (Faber et al., 2021), and *Eisenia fetida* is not the most relevant species for soil functionality as a compost earthworm, but is commonly used in ecotoxicological bioassays even though its tolerance to contaminants may be greater than the more functionally relevant endogeic/anecic earthworms (Beesley et al., 2011; Duque et al., 2023). There is also a clear lack of indicators for the 'biological regulators' functional group (e.g., nematodes) and indicators relating to structural and/or functional soil biodiversity, which are still generally lacking in soil health assessment schemes due to various practical limitations (Lehmann et al., 2020; Zwetsloot et al., 2022). Considering the potential detrimental impacts of biochar on soil fauna, certain indicators included in this study such as bait lamina, soil microbial activity and fauna reproduction tests are recommended (Domene et al., 2015; Prodana et al., 2019, 2021), but some potentially important indicators relating to e.g., soil aggregate and organic carbon stability are missing. Further, the fumigation-extraction method for measuring microbial biomass can result in a significant underestimation of microbial biomass carbon by e.g., as much as 70 % with biochar applied at 30 ton/ha due to that biochar can strongly sorb lysed cells and DOC (Jin, 2010; Lehmann et al., 2011), and a correction factor is recommended by some authors to account for this re-adsorption effect (Domene et al., 2014; Jin, 2010; Liang et al., 2010; Marks et al., 2016).

With respect to the selected SQI used to derive the treated-SF indices, ANOVA and correlation analysis was used to derive a minimum dataset (MDS) to select the SQI resulting from the logical sieve that are most responsive to management and used to evaluate GRO more generally (Rinot et al., 2019). However, selecting only the statistically significant indicators can introduce bias into the assessment and should ideally be performed for a larger set of indicators and samples to determine the most sensitive indicators for use in a generalizable method. An MDS is needed though for practical purposes as increasing the number of indicators can increase collinearity, complexity and costs (Bünemann et al., 2018). In this study, strong correlations were observed between P-AL, Mg-AL and Ca-AL as well as between  $EW_{DDX}$  and POM ( $r > 0.8$ , Table S6) that allowed reducing the number of SQI to calculate the index values, but this may differ in other types of soil. In future studies, POM could be used instead of  $EW_{DDX}$  as a proxy measurement of bioavailability (Table S10) and for evaluating the effects of GRO since it is more cost-effective, available, and technically easier to perform (Enell and Holmström, 2020; Wang et al., 2018).

Regarding the selection of SF and ES, different ontologies may include other SF or have a different perspective on which sub-functions and processes are relevant. For example, an important sixth soil function *biodiversity and habitat* was identified in the literature review but not included in this assessment since the SQI used here were not considered to be relevant to this function. Similarly, there are numerous ontologies for ES, and specifically soil-based ES, which have different names or additional ES that could be included in an ES assessment (e.g., local climate regulation, noise abatement, recreational and aesthetic cultural services) but were either not easily accommodated into this soil health assessment or outside the scope of this study. Soil-based ES are primarily regulating services (Turbé et al., 2010), though the *SSM* function can still be considered relevant for the highly vegetation-dependent *ES-erosion control* and the provisioning *ES-biomass production* is linked to all the included SF as they pertain to the capacity of a soil to produce plant food, fibre and fuel for human use.

An important note is that the treated-SF and ES indices as well as the overall SHI are not absolute assessments, but rather assessments of the relative positive or negative effects as a result of a GRO intervention. An important shortcoming is that only one season of data assessed here. However, this method could also be applied to track the changes/

improvement in soil health over a longer time period, which is especially pertinent for GRO which can act slowly to gradually change soil biogeochemical properties. Also, since the assessment is relative, the selection of reference state greatly influences the results so it must be carefully considered. In this study, instead of comparing to an untreated field control, the experimental control reference state (T3) was used for comparison, which is the same parent soil homogenized in the beginning of the experiment, thus eliminating potential confounding effects from irrigation or differing soil types that greatly could affect SF. Multiple references might be preferable to give an indication of the range of improvement that could be expected as a 'positive' and 'negative' control or contaminated and non-contaminated reference state, respectively, as well as accounting for potential heterogeneity (e.g., soil type, contamination levels) that is common at most contaminated sites but not at Kollberga which is relatively homogenous. In general, a soil health index can provide useful information on a large scale but regional comparisons or comparing between different soil types is not appropriate (Lehmann et al., 2020). An alternative approach to assess the actual current condition or health of the soil would entail using scoring functions (i.e., 'more is better', 'less is better', or 'optimum') to define ranges for when the value of a SQI is 'good' for the loamy sandy soil at Kollberga and derive weighted, normalized indicator scores with which to calculate soil health index values (Andrews et al., 2004; Lehmann et al., 2020; Obriot et al., 2016; Rinot et al., 2019; Thoumazeau et al., 2019). For example, soil pH was excluded from the index calculation despite a significant effect of biochar on pH since it aligns better with an 'optimum' scoring function and it was determined that it was already within the optimum range for good soil quality. Such scoring functions and thresholds for when a SQI is in the 'good' range would be highly useful for a risk-based approach to soil health (EEA, 2022). The method developed in this study may be viewed as a prototype and indeed only be suitable for this particular type of soil and site conditions, i.e., DDX-contaminated tree nursery sites. However, the method itself is generalizable and can be replicated and improved so that it can be used across different soil types and contexts to evaluate the effects of GRO on soil health. The focus in this study is on contaminated soil and the effects of GRO, but as knowledge develops and new indicators become available, the method proposed here can be improved and expanded so that it is suitable/generalizable for assessing changes in soil health for wide range of soil types, causes of degradation, and soil management practices (e.g., remediation techniques).

## 5. Conclusion

In this study, an accessible, scientific method for soil health assessment directed towards practitioners and decision-makers in contaminated land management was developed and demonstrated for a field experiment at a DDX-contaminated tree nursery in Sweden to evaluate the relative effects of GRO on soil health (i.e., the 'current capacity' to provide ES). Relevant SQI were selected using a simplified logical sieve of five criteria where *commercial availability* and *cost-effectiveness* were significant bottleneck that limited which indicators were able to be used. Statistical analysis of the effects of GRO treatment on the selected SQI show that biochar amendment has highly significant effects on many SQI that are linked to important SF, including increasing TOC, soil fertility and microbial activity, as well as decreasing the uptake of DDX into earthworms which is especially important for the *pollutant attenuation and degradation* function for the contaminated soil. However, there are potential downsides to biochar including that it can immobilize plant-available forms of N and has mixed, potentially negative, effects on larger soil fauna such as earthworms that could inhibit e.g., soil bioturbation and carbon turnover. The effects of plants on soil functioning varied with the species but, in general, the mix of leguminous clover and alfalfa was shown to have the most positive effects on individual SQI, aggregated SF and resulting ES, and can counteract some of the potential negative effects of biochar. Results indicate that GRO can

generally improve soil health and multifunctionality at the DDX-contaminated former tree nursery site Kollberga in Sweden. The experimental GRO treatment of the legume mix with biochar amendment (T6) and grass mix with biochar amendment (T4) are shown to result in statistically significant improvements to soil health, with overall SHI values of 141 % (T6) and 128 % (T4), respectively, compared to the grass mix without biochar (T3) reference soil (set to 100 %). The treated-SF and ES indices and the overall SHI provide simplified yet valuable information to decision-makers regarding the effects of GRO and can highlight potential trade-offs and synergies in ES delivery. This was demonstrated through the positive effects on the treated-SF indices and consequently the soil's capacity to provide ES.

## Funding

This work was supported by Formas (2021-01428), the Swedish Geotechnical Institute's research programme Tuffo (1.1-2014-0303), COWIfonden (C-147.01), the Swedish Geological Survey (3411-821/2021).

## CRediT authorship contribution statement

**Paul Drenning:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Yevheniya Volchko:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Anja Enell:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Dan Berggren Kleja:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Maria Larsson:** Writing – review & editing, Investigation. **Jenny Norman:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors would like to thank Anna Sorelius, Mattias Axelsson and Ludvig Landén at NSR AB and Hanna Wåhlén and Kristin Forsberg at Geological Survey of Sweden (SGU) for helping to realise the field experiment. The funders of this study are gratefully acknowledged. Thank you to the anonymous reviewers whose feedback has greatly improved this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.174869>.

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