Pure

Scotland's Rural College

Applying soil health indicators to encourage sustainable soil use: the transition from scientific study to practical application

Griffiths, BS; Faber, J; Bloem, J

Published in: Sustainability

DOI: 10.3390/su10093021

First published: 24/08/2018

Document Version Publisher's PDF, also known as Version of record

Link to publication

Citation for pulished version (APA):

Griffiths, BS., Faber, J., & Bloem, J. (2018). Applying soil health indicators to encourage sustainable soil use: the transition from scientific study to practical application. *Sustainability*, *10*(9), [3021]. https://doi.org/10.3390/su10093021

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
 You may freely distribute the URL identifying the publication in the public portal ?

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Article



Applying Soil Health Indicators to Encourage Sustainable Soil Use: The Transition from Scientific Study to Practical Application

Bryan S. Griffiths ^{1,*}, Jack Faber ², and Jaap Bloem ²

- ¹ SRUC, Crop and Soil Systems Research Group, West Mains Road, Edinburgh EH9 3JG, UK
- ² Wageningen Environmental Research, 6700 AA Wageningen, The Netherlands; jack.faber@wur.nl (J.F.); jaap.bloem@wur.nl (J.B.)
- * Correspondence: Bryan.Griffiths@sruc.ac.uk; Tel.: +44-131-535-4311

Received: 30 July 2018; Accepted: 16 August 2018; Published: 24 August 2018



Abstract: The sustainable management of land for agricultural production has at its core a healthy soil, because this reduces the quantity of external inputs, reduces losses of nutrients to the environment, maximises the number of days when the soil can be worked, and has a pore structure that maximises both the retention of water in dry weather and drainage of water in wet weather. Soil health encompasses the physical, chemical, and biological features, but the use of biological indicators is the least well advanced. Sustainability also implies the balanced provision of ecosystem services, which can be more difficult to measure than single indicators. We describe how the key components of the soil food web contribute to a healthy soil and give an overview of the increasing number of scientific studies that have examined the use of biological indicators. A case study is made of the ecosystem service of water infiltration, which is quite an undertaking to measure directly, but which can be inferred from earthworm abundance and biodiversity which is relatively easy to measure. This highlights the difficulty of putting any monitoring scheme into practice and we finish by providing the considerations in starting a new soil health monitoring service in the UK and in maintaining biological monitoring in The Netherlands.

Keywords: ecosystem services; soil food web; earthworms; monitoring; water infiltration

1. Introduction

Human societies are highly dependent upon healthy soils for the delivery of ecosystem goods and services, including provisioning (e.g., food, fibre, timber, fuel), regulation (e.g., climate, disease, natural hazards), waste treatment, nutrient cycling, and cultural services [1]. Many ecosystem services are driven by trophic interactions in the soil food web (i.e., who eats who) [2] and interactions between organisms in the soil food web are the critical determinant of soil function [3]. In this paper we will explain the role of the soil food web for soil health, give examples to demonstrate the linking of soil biology to function (as in Reference [4]) and provide observations on the practical issues of developing and maintaining a monitoring programme for soil health. In particular we discuss the relationship between an easily measured biological indicator (i.e., earthworms) and an ecosystem service that is technically challenging to measure (i.e., water infiltration). We then examine the challenges in rolling out a new soil health monitoring programme for farmers in the Northern UK and those of an established policy-related programme in The Netherlands.

2. Ecosystem Services and the Soil Food Web

In terrestrial ecosystems, higher plants are the major primary producers of biomass. Carbon and energy are released into the soil by root exudates and plant residues. In soil, bacteria and fungi are the primary decomposers of dead organic matter such as plant residues, root exudates, decaying micro-organisms, and animal manure. Other groups of organisms feed on bacteria (bacterial-feeders), fungi (fungal-feeders), plant roots (micro herbivores), or animals (predators and top predators). Some organisms are very selective and feed only on a few other species, whilst others, the omnivores, exploit various food sources. All these trophic interactions and biological activity drive carbon and nutrient cycles, soil structure formation, disease suppression, and ultimately soil ecosystem services [5].

Carbon and nutrient cycling is performed not only by microbes but also by microbivores (i.e., grazers) and predators which decompose microbes and other organisms. In a field study at the Lovinkhoeve experimental farm in The Netherlands, microbes, microbivores, and N mineralisation were monitored in a winter wheat field under conventional and integrated management. The microbial biomass was strongly dominated by bacteria and was not significantly larger in the integrated field than in the conventional field, whereas protozoan and nematode biomasses were 64% and 22% larger, respectively [6]. Average N mineralisation was also 30% greater in the integrated field. The differences were attributed to the approximately 30% larger soil organic matter content of the integrated field which appeared to increase the activity (but not biomass because of increased turnover) of bacteria, and biomasses of protozoa and nematodes [6]. A food web model indicated that an important part of the observed N mineralisation can be explained by the grazing activity of protozoa and nematodes [7]. While this study indicated a direct faunal contribution to N mineralisation of up to 45%, other studies have also indicated indirect contributions of the fauna to C and N mineralisation rates. Rashid et al. [8] used a production-ecological model to show that N mineralisation by earthworms, enchytraeids, fungi, and protozoa together added up to almost all the N mineralisation measured as herbage N uptake. There were significant contributions from fungi (32–41%), protozoa (16–35%), and earthworms (9–30%).

There is a positive role of microbial activity on soil structure and stability, with fungi having three types of effect: physical entanglement, production of extracellular polysaccharides, and production of hydrophobic substances [9]. A meta-analysis [10] shows the positive relationship between the responses of fungi and soil aggregate stability, demonstrating a strong functional link between fungi and soil structure. In addition to fungi, earthworms also contribute to soil structure formation, being major ecosystem engineers: accelerating microbial activity; mixing organic matter and creating macro-pores in the soil. Earthworm abundances and functional group composition were shown to be positively correlated with water infiltration rate, with a consistent trend of increased earthworm and fungal community abundances and complexity following transitions to lower intensity and later successional land uses [10]. Andriuzzi et al. [11] found that anecic earthworms, which create vertical macropores, can counteract the effects of intense rain events on soil and plants. This is important information as some of the strongest ecological and agronomic effects of climate change will occur through pulse events, rather than altered average trends.

Carbon sequestration is an important aspect of sustainable agricultural systems, and to increase soil organic matter (SOM) and sequester carbon, decomposition must be slightly slower than the input of plant material, on a long-term basis. The major factors controlling SOM dynamics are: (1) the quality of the incoming substrates, (2) the role of the soil biota and especially the microorganisms, (3) physical protection such as in aggregation, (4) interaction with the soil matrix, and (5) the chemical nature of the SOM itself [12]. Whether plant inputs are first converted to microbial residues before stabilization influences how SOM responds to land use and climate change. Plant residues that accumulate in soil through physical protection (e.g., inside aggregates) or in zones with low biological activity, are susceptible to destabilization following disturbances such as cultivation or in response to environmental change (e.g., temperature increases). If, however, plant materials are synthesized into microbial proteins, lipids or polysaccharides, the resulting organo-mineral associations may include ligand bonds or other strong interactions that have lower temperature sensitivity and may better

withstand perturbations. Kallenbach et al. [13] demonstrated that microbial processing of simple C substrates such as sugars and the lignin monomer syringol, produced an abundance of stable, chemically diverse SOM dominated by microbial proteins and lipids. The actual substrate chemistry may be less important for SOM accumulation than how it influences fungal abundance and microbial carbon use efficiency in the long term.

The resistance and resilience of soil food webs to climate change is increasingly recognised as an important inherent property [14]. De Vries et al. [15] showed that the fungal-based food web of an extensively managed grassland soil, and the processes of C and N loss it governs, was more resistant, although not resilient, and better able to adapt to drought than the bacterial-based food web of an intensively managed arable soil in the south of England. Across four European countries of contrasting climatic and soil conditions, soil food web properties strongly and consistently predicted processes of C and N cycling across land-use systems and geographic locations, and were a better predictor of these processes than land use [16]. Beyond the well-known role of arbuscular-mycorrhizal fungi (AMF) in improving plant nutrient uptake, AMF can contribute to water use efficiency and resistance against drought and salinization [17–19]. Laboratory studies have also shown that AMF reduce leaching of N and phosphorus (P). These findings show that more extensive management promotes more resistant, and adaptable, fungal-based soil food webs.

The soil food web is also implicated in disease suppression. Soil-borne fungal and bacterial root pathogens can cause serious losses to agricultural crops and are difficult to manage, especially in narrow rotations. Enhancement of disease suppressive properties of soils is of great importance for sustainable agriculture, by limiting the ability of pathogens to establish or to produce disease symptoms. Postma et al. [20] analysed soil samples from 10 organic arable farms for disease suppressiveness and showed significant correlations between suppressiveness and the occurrence of specific beneficial microorganisms, as well as with more general microbial properties. Probably the soil suppressiveness is a combined effect of general and specific disease suppression.

Soil health is fundamentally underwritten by the assemblages that carry out the various key processes. These assemblages are predominantly biological in origin, but actually involve a particular configuration of the biology, physics, and chemistry of the soil constituents. What is quite clear is that any measure of soil health must be multivariate—single properties will not adequately encompass or integrate the features or issues that underwrite soil health [21].

3. Measuring and Monitoring Soil Condition to Preserve Ecosystem Services

Ecosystem services are under threat from biodiversity decline, compaction, contamination, erosion, landslides, organic matter decline, salinization, and sealing, all exacerbated by climate change [22]. Assessments of the condition that soils are in and what policies might be followed for their preservation are very similar across a range of scales. Globally, unprecedented demand is stressing the land and water systems that underpin food production, such that global and national approaches need to be aligned [23]. Indeed, a global soil resilience programme to monitor soil fertility and function and the ecosystem services provided by soils has been suggested [24]. Across Europe the unsustainable use and management of land is leading to increased soil degradation, the control of which requires harmonisation of soil monitoring and data collection programmes [22]. Gregory et al. [25] critically reviewed the effects of soil threats for likely effects on UK soils and yield, noting many reductions in ecosystem services (not just crop yield) as a result and again recommending a monitoring programme. A recent gap analysis at the European level [26] recommended additional soil biological and physical parameters within the LUCAS soils survey and called for further development of national soil monitoring schemes. Given this increasing recognition of the importance of soil, O'Sullivan et al. [27] looked at practicalities of implementing a soil monitoring network for Ireland, suggesting a 16 km² grid with baseline analytical costs of €0.7–3 million for each sampling round. This strong and increasing policy requirement for the effective monitoring of soils at local, regional and national scales has been recognised previously and consistently [28–34].

Most soil processes are mediated by soil biota in direct relationship with the physico-chemical properties of their environment, although there is still a need to better understand how soil biodiversity links with soil functioning [4]. Biological indicators are relevant in supporting policy and decision-making to achieve sustainable soil management [32,35,36], and the inclusion of biological indicators to assess changes in the delivery of ecosystem functions is accepted practice both at national and European scales [5,32,37–39]. Biological indicators have long been developed and applied in specific environmental situations, making the extrapolation of values and applicability under different conditions difficult. Furthermore, despite recent efforts to standardise, a wide range of different methods and procedures are applied, which makes comparison all the more difficult. National [40,41] and European [42]) initiatives have been undertaken to recommend indicators across Europe and elsewhere [32,43–46].

Reviews have compared a large range of biological indicators for scientific and technical relevance to assist policy-makers in land management [32,33,42,46–48], with the consensus being that major efforts remain to be made in order to standardise operational procedures and to validate them for different types of land use [37]. The selection of potential biological indicators is only a step in developing a practical monitoring scheme [49], as there are operational issues to be solved such as: ease of application, robustness, sensitivity, laboratory accuracy, throughput, economic value and descriptiveness. The selection criteria for biological indicators are well described [33,46,47], but consideration also has to be given to the cost-effectiveness of the indicators and the interpretation of the results from the monitoring. Different stakeholders have different information needs, and different indicators have to be developed to answer their specific requirements [46]. All these factors have been recognised as crucial steps in the development of a soil quality assessment procedure [15].

4. Measuring an Indicator Rather than the Actual Ecosystem Service

With global climate changing faster than politicians can discuss, the frequency and intensity of rain storms is increasing, posing threats of flooding and waterlogged soils in agricultural and sedentary areas alike. Land management aiming to enhance the water regulation capacity of soils, and thus climate-proofing agriculture and the urban environment (mitigation and adaptation), is in dire need of cost-effective and policy-relevant indicators to evaluate (e.g., precipitation surplus infiltration capacity and soil porosity). Direct measurement of water infiltration rates in the field (e.g., using a ring infiltrometer, [50]) is a simple method, but is time consuming (it can take several hours to make measurements at a single point) and is logistically challenging as many tens of litres of water are required. So it is not practical as a routine or rapid way to monitor this particular ecosystem service. Earthworms, on the other hand, are relatively easy to measure (approximately 15 min for one point) and it logistically quite feasible to do many tests per day.

Pioneering has been done on establishing and quantifying the relationship between earthworm communities and the water infiltration capacity of the soils they inhabit. Wilfried Ehlers [51] already in 1975 estimated earthworm contribution to soil water infiltration as more than 1 mm/min (via conducting burrows), although the volume of such channels amounted to only 0.2 vol % in untilled grey-brown podzolic soil derived from löss. Tilled soils did not feature effective channels in the plough layer or below, because of lacking connection to the soil surface. Earthworm densities in untilled plots had doubled in four years of no-tillage practice. Bouché [52] in 1977 measured infiltration rates in 17 soils and gave a mean rate of 150 mm h⁻¹ per 100 g m⁻² of earthworms and even 282 mm h⁻¹ per 100 g m⁻² of anecic species. A further study was made in nine sites analysing hydraulic (ctive) burrows and their structural properties. Infiltration rate was correlated to earthworm biomass (r = 0.975), burrow length, surface and volume (r = 0.99), but not with burrow diameter, hydraulic tortuosity or with earthworm number and soil profile depth [53].

Recent studies at 16 arable cropping farms in Limburg in the South of The Netherlands comparing non-inversion tillage to conventional ploughing, have shown that reducing tillage intensity results in: larger earthworm populations, (Figure 1); more functional earthworm diversity (in ecological groups)

(Figure 2); and more earthworm activity in the top soil (Figure 3), with the outcome being a significant increase in infiltration rate (Figure 4) and greater aggregate stability across all aggregate size classes.



Figure 1. Earthworm density (i.e., individual numbers m^{-2}) in conventionally tilled fields (CT) and fields under reduced tillage (RT), averaged over a 3-year period (2009–2011).



Figure 2. Earthworm community composition in ecological groups (anecic deep dwelling species, endogeic soil dwelling species, epigeic top soil, and litter dwelling species) under reduced tillage (various methods of non-inversion tillage were used) and conventional tillage (mouldboard ploughing).



Figure 3. Depth distribution ratio of earthworms under reduced tillage (various methods of non-inversion tillage were used) and conventional tillage (mouldboard ploughing), expressed as the ratio of the numbers of individuals found in the 0–20 cm topsoil over the number of individuals extracted by mustard oil (AITC) extraction from the pit after removal of a $30 \times 30 \times 20$ cm soil block (i.e., topsoil, for hand sorting).



Figure 4. Water infiltration rate (mm/minute) under reduced tillage (RT) and conventional mouldboard ploughing (CT) (data for 2011 only), and an estimate for pore volume capacity (imensionless), calculated as the difference between initial infiltration rate and the rate at constant infiltration in a double ring infiltrometer).

Whilst there was a high degree of variability, there was a strong tendency for earthworms to be present in top soil under reduced tillage against populations residing in subsoil in the case of conventional tillage. In a pairwise comparison of adjacent fields, reduced tillage (RT) showed faster rates of water infiltration and a larger pore volume than the neighbouring conventionally tilled fields. Variability under RT was much higher, probably related to the use of different methods of tillage, and while there was no significant relationship with earthworm densities the trend was clearly positive. Soil porosity and water infiltration may be related to soil aggregation, as larger aggregates can have larger pores between them and can hold moisture for a longer time. We found no differences in soil aggregate size in relation to tillage or associated with earthworms (not shown), but the stability of aggregates was significantly higher under reduced tillage particularly in the 0.5–1.0 mm size class (Figure 5).



Figure 5. Soil aggregate stability (% stable aggregates by weight) in different size classes under reduced tillage (RT) and conventional mouldboard ploughing (CT).

Thus, as the stability of soil aggregates is decisively enhanced by earthworms [54], and earthworms are specifically vulnerable to tillage [55], earthworms would give a general indication of relative changes in soil structure and water regulation, while not being an exact measure of water infiltration, in sufficient detail to justify their inclusion on a soil health monitoring scheme. Guides to earthworm extraction and identification are readily available (i.e., [56]) and methods to calculate soil health indices for earthworms have been published [57].

5. Considerations for the practical Use of Soil Health Indicators.

Soil health is no longer an esoteric niche interest for academics but is increasingly in the public domain, as can be seen from these recent UK news clips:

- "African soil crisis threatens food security" (2014) [58]
- "Careless farming adding to floods" (2014) [59];
- "EU pesticide bans could hit UK crops" (2014) [60];
- "UK faces significant shortage of farmland" (2014) [61];
- "Members of Parliament sound alarm over neglected soils" (2016) [62];
- "Nature loss linked to farming intensity" (2016) [63];
- "Prince of Wales joins soil boosting project" (2016) [64];
- "Scottish research finds soil crucial to climate change fight" (2016) [65];
- "Farm subsidies must be earned" (2017) [66];
- "Bread's environmental costs are counted" (2017) [67];

However, setting up a soil health monitoring scheme has a new set of practical criteria in addition to the scientific validation of potential indicators. Soil ecosystems are complex. Therefore, many different aspects need to be measured [68]. It is important to use a set of various indicators, and not a few a priori selected indicators which are supposed to be the most sensitive. Some indicators are more sensitive to contamination (e.g., bacterial growth rate), others are more sensitive to differences in soil fertility and agricultural management (e.g., N mineralisation) [69].

The practical aspects can be seen from the examples of a policy related programme in The Netherlands and an agricultural service in Scotland.

6. Biological Indicator of Soil Quality (BISQ) in The Netherlands

The Convention on Biological Diversity (CBD) recognized the importance of biodiversity for ecosystem functioning and the provisioning of soil services to mankind [70]. After the ratification of the CBD, the Dutch government produced a National Action Plan in 1995 to meet the CBD obligations. The role of biodiversity in the maintenance of ecological functions (life support functions) in the soil was one of the subjects needing more attention. More data had to be gathered to enable policy-makers to assess the quality and resilience of soil ecosystem services. Therefore, the Biological Indicator system for Soil Quality (BISQ) was designed in 1997 [71]. In the BISQ, the link between biodiversity and soil functioning is represented in a stepwise and deductive way, from the point of view that the soil food web offers the opportunity to link diversity to specific functions: the life support functions. Thus, BISQ aimed at major trophic groups and processes of the soil food web. BISQ was incorporated into an already operational abiotic soil monitoring programme, The Netherlands Soil Monitoring Network (NSMN). The monitoring network is based on more than 300 sites, arranged in various land use/soil type categories (farms, natural areas and urban sites), and represents approximately 75% of the total land surface area of The Netherlands. Sampling for biological soil properties was carried out in parallel with sampling in the NSMN for soil and chemical analysis. Use was made of the same infrastructure and some of the biological analyses (such as for microbes, nematodes and microbial processes) were carried out using the same mixed samples.

For the application of biological indicators, a lot of methodological choices have to be made [69]. Samples can be taken from replicated field plots, or can be composed from larger areas. In The Netherlands, per category of soil type and land-use 10–20 farms (replicates) spread over the country are sampled. Per farm (about 5 to 50 ha) one mixed sample is composed from 320 cores. These mixed samples are used for chemical, microbiological and nematode analyses. Separate soil cores or blocks (six replicates per site) are taken for mites and springtails (microarthropods), enchytraeids and earthworms. Some reference sites consist of smaller contaminated areas or experimental fields. Here replicated field plots (about 10×10 m) are sampled. Sampling depth is best decided by considering soil

horizons and tillage depth. In a ploughed arable field 0–25 cm would be appropriate, in grassland and especially in forest thinner, and more, layers would be better. However, this would result in a variable sampling depth or increase the number of samples by taking more than one layer. Given the large number of samples, analysing more than one depth would cost too much time and money. Sampling 0–25 cm would dilute microbial activity considerably in some grassland and forest soils where life is concentrated closer to the surface. Dilution hampers detection of differences. Therefore, in The Netherlands monitoring network samples are taken from 0–10 cm depth and litter is removed

before sampling. For microbiological parameters early spring or late autumn is the best time to sample, as soil conditions are relatively mild and stable and short-term effects of the crop are avoided. In The Netherlands for practical reasons samples are taken from March to June. The soil must be dry enough to access, and farmers prefer sampling of arable land before soil tillage and sowing new crops. Sampling of about 50 farms takes two to three months. Storage is inevitable when large numbers of samples from many sites have to be handled. Soil fauna samples can be preserved for later analysis, so earthworms are hand sorted within 5 days, and enchytraeids, microarthropods, and nematodes are stored in 70% ethanol after extraction. For microbiological samples an a priori storage temperature of 12 °C was chosen, which is close to the average annual soil temperature. The soil is sieved through a 5 mm mesh, as practically it is very difficult to pass field moist, heavy clay soil through a 2 mm sieve. Sieving is useful to reduce variation in process rate measurements such as respiration and mineralisation, and to facilitate mixing and sending identical sub-samples to different laboratories. Sampling and sieving are however major disturbances, which also reduce soil structure and generally increase microbial activity. Therefore, results of the first week of 6-week soil incubations are not used for calculation of process rates (potential C and N mineralisation).

To reduce variation caused by variable weather conditions, samples are pre-incubated for four weeks at constant temperature (12 °C) and moisture content (50–60% of water holding capacity) before microbiological analyses are performed. Since each soil- and land-use type in the monitoring network is analysed once in six years, effects of for instance a dry summer should be minimized.

This pre-incubation applies to the analyses of bacterial and fungal biomass (direct microscopic measurements), bacterial growth rate (3H-thymidine and 14C-leucine incorporation into DNA and proteins) and community level physiological profiles using BiologTM ECOplates. Soil samples used for measuring potential C and N mineralisation by 6-week incubation at 20 °C, and for measuring potentially mineralisable N by 1 week of anaerobic incubation at 40 °C, are not pre-incubated because incubations are already included in the methods.

From 2004, part of the budget was allocated to study effects of agricultural management and nature restoration in existing long term field experiments. Samples from such experiments are not pre-incubated. One reason is the increasing interest in fungal/bacterial ratios and the observation that fungal hyphal length showed rapid decreases when soil was incubated, especially thinner and non-septate hyphae (presumably mycorrhiza) in soils with low fertilization De Vries et al. [72].

After 17 years within the practical and budgetary limitations, BISQ is still considered as state of the art in soil monitoring Rutgers et al. [71]. Stability and continuity is the basis for building a long term (and therefore valuable) monitoring system. This requires a stable set of methods and indicators. Repeated seasonal measurements and inclusion of protists is still desirable but still not feasible for financial and technical reasons. Molecular DNA and RNA techniques are still developing rapidly, but the information is in line with classic taxonomic methods and does not necessarily offer new or better opportunities for assessment of soil ecosystem services. Instead phospholipid fatty acid (PLFA) analysis is regarded as a more applicable measure of microbial community structure. Besides some major groups of bacteria, it includes a biomarker for saprotrophic fungi. In addition, arbuscular mycorrhizal fungi (AMF) can be included by measuring also neutral lipid fatty acids (NLFA). Based on multi criteria analysis Rutgers et al. [71] proposed a minimum data set for monitoring soil ecosystem services:

- Soil biological indicators: earthworms, enchytraeids, nematodes, microarthropods, fungi, bacteria, N mineralisation, C mineralisation, and root mass (grassland only).
- Abiotic soil indicators: soil type and texture, penetration resistance, bulk density, organic matter parameters including labile fractions, pH, nutrients.
- System indicators: land use, vegetation, agricultural management (crop, rotation, tillage, fertilization, crop protection (pesticides), traffic) and groundwater level.
- If costs are a major aspect for the soil monitoring, these can only be reduced by reducing the number of indicators.

7. A Soil Health Test as a Practical Tool for Scottish Growers

Soil health is of practical interest to farmers as the increasingly wet winters in the UK have emphasised the importance of good soil structure to reduce flooding and trafficability issues in soil, and the increasing use of precision farming is also highlighting good and bad areas of their fields. So it was timely to start a soil health testing programme.

Soil health is all about balancing the integrated physical, chemical and biological components of the soil system Stockdale and Watson [34]. Many farmers already have their soils routinely tested for nutrients (P, K, Mg, Ca, Na) and pH, and are used to seeing and interpreting a nutrient report. These analyses are carried out by a commercial analytical laboratory. Thus, there was a base of farmer experience, a network of advisors and analytical facilities on which to base the new soil health testing programme. The primary considerations were cost, interpretability and understandability. Any test had to be affordable and to be seen as value for money. This was related to ease of sampling and analytical practicalities. Thus, while microbial biomass is relatively highly regarded as a key property of soils [33], the scientific standard method of chloroform-fumigation-extraction [73] is not a practical option for commercial laboratories because of health and safety regulations surrounding the use of chloroform. The alternative substrate-induced respiration [74] protocol required either a gas chromatograph for measuring CO₂, which our laboratory did not have, or a titration approach that would have been too time consuming (and so expensive). A measure of potentially mineralisable nitrogen was taken as a practical alternative, as it was measureable using existing equipment and expertise and correlated well with microbial biomass measured by fumigation extraction [75].

Another consideration was the understandability of the test, with growers really taking to the test if the measures were ones that they could relate to. Earthworms being a case in point given their almost iconic status in representing healthy soil. For the interpretation of the test results, we required the indicator to have threshold values which would trigger some management options for the farmer. Probably the best known example is soil pH, which has well-known optima for crop production. This then led to a traffic-light system that could be adapted to the existing nutrient management report (Table 1). **Table 1.** Example of nutrient management report using a traffic-light system as the output from a proposed soil health test in Scotland. The traffic light colours give a quick overview regarding the risk of reduced crop production and/or environmental damage, so "Green" (low risk, continue to monitor), "Amber" (moderate risk, need to investigate further), "Red" (high risk, need to investigate urgently). This example is for an arable farm on a sandy loam soil and would come with management advice on how to improve soil health.

Measure	Overview	Score	Target range
Potentially Mineralisable N		$28.7 { m mg kg^{-1}}$	$>21 \text{ mg kg}^{-1}$
Organic Matter (LOI)		5.96 %	>9.5 %
pH		6.1	6.5–7.5
Extractable Phosphorus		4.39 mg L^{-1}	$4.5-13.5 \text{ mg L}^{-1}$
Extractable Potassium		$87.9 m mg L^{-1}$	$>76 \text{ mg L}^{-1}$
Extractable Magnesium		$154 { m mg} { m L}^{-1}$	$61–1000 \text{ mg } \text{L}^{-1}$
Extractable Calcium		$1500 { m mg} { m L}^{-1}$	$>3000 \text{ mg L}^{-1}$
Extractable Sodium		11.2 mg L^{-1}	$>50 \text{ mg L}^{-1}$
Visual Evaluation of Soil Structure		2.75	<2.4
Earthworm count		6.25 per 20cm ⁻²	>8

Standardisation is the one common message across all the reports on soil monitoring (see introduction) but in this case that fact that all analyses would be done by the same lab ensured standardisation, but we also made sure to use common protocols where available (i.e., nutrients, pH and potentially mineralisable nitrogen). Finally, we recognised the huge potential for data-mining in future, the accumulation of soil measurements across the country and over many years would provide potentially important information only if the accompanying metadata (GPS location, cropping history, etc.) was collected with every sample. This can be seen from the Australian model (http://www.soilquality.org.au/) where growers can benchmark their soils against regional comparators.

Using approaches as described here, biodiversity and functioning of soil ecosystems can be monitored. In pollution-gradients it is possible to use a local unpolluted control [9]. However, in many cases such a reference is not available. Generally, the value of an indicator is affected not only by stress factors, but also by soil type, land use and vegetation. Therefore, reference values for specific soil types have to be deduced from many observations (e.g., 20 replicates per type). The choice of a desired reference is a political rather than a scientific issue, and depends on the aims of land use. A biologically active and fertile soil is needed in (organic) farming, but a high mineralisation of nutrients from organic matter may hamper conversion of agricultural land to a species rich natural vegetation. Soils showing very low or very high indicator values may be suspect and need further examination. Sufficient data and experience are needed to make judgements of desirable reference values. Monitoring changes of indicators over time can reduce the importance of (subjective) reference values. Such changes may be easier to interpret than momentary values [68]. Spatially extensive and long-term monitoring may be not ideal, but it is probably the most realistic approach to obtain objective information on differences between, temporal changes within, and human impact on ecosystems.

Author Contributions: Conceptualization, B.S.G., J.F., and J.B.; Writing-Original Draft Preparation, B.S.G., J.F. and J.B.; Writing-Review & Editing, B.S.G., J.F. and J.B.

Acknowledgments: This work was in part supported by: the Scottish Government Rural and Environment, Science and Analytical Services Division; Agriculture and Horticulture Development Board/British Beet Research Organisation (Soil Biology and Soil Health Partnership, AHDB: 91140002); and by the Ministry of Agriculture, Nature and Food Quality of The Netherlands. Annemariet van der Hout is gratefully acknowledged for performing field measurements, and Nico van den Brink for data analyses.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Millennium Ecosystem Assessment. *Ecosystems and Human Well-Being: Synthesis;* Island Press: Washington, DC, USA, 2005.
- Barnes, A.D.; Jochum, M.; Lefcheck, J.S.; Eisenhauer, N.; Scherber, C.; O'Connor, M.I.; de Ruiter, P.; Brose, U. Energy Flux: The Link between Multitrophic Biodiversity and Ecosystem Functioning. *Trends Ecol. Evolut.* 2018, 33, 186–197. [CrossRef] [PubMed]
- 3. De Vries, F.T.; Wallenstein, M.D. Below-ground connections underlying above-ground food production: A framework for optimising ecological connections in the rhizosphere. *J. Ecol.* **2017**, *105*, 913–920. [CrossRef]
- 4. Young, I.M.; Bengough, A.G. The search for the meaning of life in soil: An opinion. *Eur. J. Soil Sci.* **2018**, *69*, 31–38. [CrossRef]
- 5. Bünemann, E.K.; Bongiorno, G.; Bai, Z.G.; Creamer, R.E.; De Deyn, G.; de Goede, R.; Fleskens, L.; Geissen, V.; Kuyper, T.W.; Mader, P.; et al. Soil quality—A critical review. *Soil Biol. Biochem.* **2018**, *120*, 105–125. [CrossRef]
- 6. Bloem, J.; Lebbink, G.; Zwart, K.B.; Bouwman, L.A.; Burgers, S.L.G.E.; de Vos, J.A.; de Ruiter, P.C. Dynamics of microorganisms, microbivores and nitrogen mineralisation in winter wheat fields under conventional and integrated management. *Agric. Ecosyst. Environ.* **1994**, *51*, 129–143. [CrossRef]
- De Ruiter, P.C.; Moore, J.C.; Zwart, K.B.; Bouwman, L.A.; Hassink, J.; Bloem, J.; de Vos, J.A.; Marinissen, J.C.Y.; Didden, W.A.M.; Lebrink, G.; et al. Simulation of nitrogen minerlaization in the below-ground food webs of two winter wheat fields. *J. Appl. Ecol.* **1993**, *30*, 95–106. [CrossRef]
- Rashid, M.I.; de Goede, R.G.M.; Brussaard, L.; Bloem, J.; Lantinga, E.A. Production-ecological modelling explains the difference between potential soil N mineralisation and actual herbage N uptake. *Appl. Soil Ecol.* 2014, 84, 83–92. [CrossRef]
- 9. Cosentino, D.; Chenu, C.; Le Bissonnais, Y. Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. *Soil Biol. Biochem.* **2006**, *38*, 2053–2062. [CrossRef]
- Spurgeon, D.J.; Keith, A.M.; Schmidt, O.; Lammertsma, D.R.; Faber, J.H. Land-use and land-management change: Relationships with earthworm and fungi communities and soil structural properties. *BMC Ecol.* 2013, 13, 46. [CrossRef] [PubMed]
- 11. Andriuzzi, W.S.; Pulleman, M.M.; Schmidt, O.; Faber, J.F.; Brussaard, L. Anecic earthworms (*Lumbricus terrestris*) alleviate negative effects of extreme rainfall events on soil and plants in field mesocosms. *Plant Soil* **2015**, *397*, 103–113. [CrossRef]
- Paul, E.A.; Kravchenko, A.; Grandy, A.; Morris, S. Soil organic matter dynamics: Controls and management for sustainable ecosystem functioning. In *The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability*; Hamilton, S.K., Doll, J.E., Robertson, G.P., Eds.; Oxford University Press: New York, NY, USA, 2015; pp. 104–134.
- 13. Kallenbach, C.M.; Frey, S.D.; Grandy, A.S. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nat. Commun.* **2016**, *7*, 13630. [CrossRef] [PubMed]
- 14. Griffiths, B.S.; Philippot, L. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiol. Rev.* **2013**, *37*, 112–129. [CrossRef] [PubMed]
- De Vries, F.T.; Liiri, M.E.; Bjørnlund, L.; Bowker, M.A.; Christensen, S.; Setälä, H.M.; Bardgett, R.D. Land use alters the resistance and resilience of soil food webs to drought. *Nat. Clim. Chang.* 2012, *2*, 276–280. [CrossRef]
- 16. De Vries, F.T.; Thébault, E.; Liiri, M.; Birkhofer, K.; Tsiafouli, M.A.; Bjørnlund, L.; Jørgensen, H.B.; Brady, M.V.; Christensen, S.; De Ruiter, P.C.; et al. Soil food web properties explain ecosystem services across European land use systems. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 14296–14301. [CrossRef] [PubMed]
- 17. Johansson, J.F.; Paul, L.R.; Finlay, R.D. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.* **2004**, *48*, 1–13. [CrossRef] [PubMed]
- Nasim, G. The role of arbuscular mycorrhizae in inducing resistance to drought and salinity stress in crops. In *Plant Adaptation and Phytoremediation*; Ashraf, M., Ozturk, M., Ahmad, M.S.A., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp. 119–141.
- 19. Santander, C.; Aroca, R.; Ruiz-Lozano, J.M.; Olave, J.; Cartes, P.; Borie, F.; Cornejo, P. Arbuscular mycorrhiza effects on plant performance under osmotic stress. *Mycorrhiza* **2017**, *27*, 639–657. [CrossRef] [PubMed]
- Postma, J.; Schilder, M.T.; Bloem, J.; van Leeuwen-Haagsma, W.K. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. *Soil Biol. Biochem.* 2008, 40, 2394–2406. [CrossRef]

- 21. Kibblewhite, M.G.; Ritz, K.; Swift, M.J. Soil health in agricultural systems. *Philos. Trans. R. Soc. B* 2008, 363, 685–701. [CrossRef] [PubMed]
- 22. Jones, A.; Panagos, P.; Barcelo, S.; Bouraoui, F.; Bosco, C.; Dewitte, O.; Gardi, C.; Erhard, M.; Hervás, J.; Hiederer, R.; et al. *The State of Soil in Europe. A Contribution of the JRC to the European Environment Agency's Environment State and Outlook Report–SOER 2010*; Publications Office of the European Union: Luxembourg, 2012.
- 23. FAO. *The State of the World's Land and Water Resources for Food and Agriculture (SOLAW)–Managing Systems at Risk;* Food and Agriculture Organization of the United Nations: Rome, Italy, 2011.
- Smith, P.; House, J.I.; Bustamante, M.; Sobocká, J.; Harper, R.; Pan, G.; West, P.C.; Clark, J.M.; Adhya, T.; Rumpel, C.; et al. Global pressures on soils from land use and management. *Glob. Chang. Biol.* 2015, 22, 1008–1028. [CrossRef] [PubMed]
- Gregory, A.S.; Ritz, K.; McGrath, S.P.; Quinton, J.N.; Goulding, K.W.T.; Jones, R.J.A.; Harris, J.A.; Bol, R.; Wallace, P.; Pilgrim, E.S.; et al. A review of the impacts of degradation threats on soil properties in the UK. *Soil Use Manag.* 2015, *31*, 1–15. [CrossRef] [PubMed]
- Van Leeuwen, J.P.; Saby, N.P.A.; Jones, A.; Louwagie, G.; Micheli, E.; Rutgers, M.; Schulte, R.P.O.; Spiegel, H.; Toth, G.; Creamer, R.E. Gap assessment in current soil monitoring networks across Europe for measuring soil functions. *Environ. Res. Lett.* 2017, *12*, 124007. [CrossRef]
- 27. O'Sullivan, L.; Bampa, F.; Knights, K.; Creamer, R.E. Soil protection for a sustainable future: Options for a soil monitoring network in Ireland. *Soil Use Manag.* **2017**, *33*, 346–363. [CrossRef]
- Cluzeau, D.; Guernion, M.; Chaussod, R.; Martin-Laurent, F.; Villenave, C.; Cortet, J.; Ruiz-Camacho, N.; Pernin, C.; Mateille, T.; Philippot, L.; et al. Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types. *Eur. J. Soil Biol.* 2012, *49*, 63–72. [CrossRef]
- 29. European Union (EU). *Communication from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions;* Thematic Strategy for Soil Protection Plus Summary of the Impact Assessment; COM 231 (2006) Final; European Union: Brussels, Belgium, 2006.
- 30. European Union (EU). *Proposal for a Directive of the European Parliament and of the Council Establishing a Framework for the Protection of Soil and Amending Directive 2004/35/EC;* COM 232 (2006) Final; European Union: Brussels, Belgium, 2006; 30p.
- Griffiths, B.S.; Römbke, J.; Schmelz, R.M.; Scheffczyk, A.; Faber, J.; Bloem, J.; Pérès, G.; Cluzeau, D.; Chabbi, A.; Suhadolc, M.; et al. Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. *Ecol. Ind.* 2016, 69, 213–223. [CrossRef]
- Pulleman, M.; Creamer, R.; Hamer, U.; Helder, J.; Pelosi, C.; Pérès, G.; Rutgers, M. Soil biodiversity, biological indicators and soil ecosystem services—An overview of European approaches. *Curr. Opin. Environ. Sustain.* 2012, 4, 529–538. [CrossRef]
- Ritz, K.; Black, H.I.J.; Campbell, C.D.; Harris, J.A.; Wood, C. Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development. *Ecol. Ind.* 2009, 9, 1212–1221. [CrossRef]
- 34. Stockdale, E.A.; Watson, C.A. *Managing Soil Biota to Deliver Ecosystem Services*; Natural England Commissioned Report NECR100; Natural England: York, UK, 2012.
- 35. Francaviglia, R. Agricultural Soil Erosion and Soil Biodiversity: Developing Indicators for Policy Analyses; OECD: Paris, France, 2008.
- Havileck, E. Soil biodiversity and bioindication: From complex thinking to simple acting. *Eur. J. Soil Biol.* 2012, 49, 80–84.
- Faber, J.H.; Creamer, R.E.; Mulder, C.; Römbke, J.; Rutgers, M.; Sousa, J.P.; Stone, D.; Griffiths, B.S. The practicalities and pitfalls of establishing a policy-relevant and cost-effective soil biological monitoring scheme. *Integr. Environ. Assess. Manag.* 2013, *9*, 276–284. [CrossRef] [PubMed]
- Feld, C.K.; Martins da Silva, P.; Sousa, J.P.; De Bello, F.; Bugter, R.; Grandin, U.; Hering, D.; Lavorel, S.; Mountford, O.; Pardo, I.; et al. Indicators of biodiversity and ecosystem services: A synthesis across ecosystems and spatial scales. *Oikos* 2009, *118*, 1862–1871. [CrossRef]
- Lemanceau, P.; Maron, P.-A.; Mazurier, S.; Mougel, C.; Pivato, B.; Plassart, P.; Ranjard, L.; Revellin, C.; Tardy, V.; Wipf, D. Understanding and managing soil biodiversity: A major challenge in agroecology. *Agron. Sustain. Dev.* 2015, 35, 67–81. [CrossRef]

- 40. Gardi, C.; Montanarella, L.; Arrouays, D.; Bispo, A.; Lemanceau, P.; Jolivet, C.; Mulder, C.; Ranjard, L.; Römbke, J.; Rutgers, M.; et al. Soil biodiversity monitoring in Europe: Ongoing activities and challenges. *Eur. J. Soil Sci.* **2009**, *60*, 807–819. [CrossRef]
- 41. Rutgers, M.; Schouten, A.J.; Bloem, J.; van Eekeren, N.; de Goede, R.G.M.; Jagers op Akkerhuis, G.A.J.M.; van der Wal, A.; Mulder, C.; Brussaard, L.; Breure, A.M. Biological measurements in a nationwide soil monitoring network. *Eur. J. Soil Sci.* **2009**, *60*, 820–832. [CrossRef]
- Bispo, A.; Cluzeau, D.; Creamer, R.; Dombos, M.; Graefe, U.; Krogh, P.H.; Sousa, J.P.; Pérès, G.; Rutgers, M.; Winding, A.; et al. Indicators for monitoring soil biodiversity. *Integr. Environ. Assess. Manag.* 2009, *5*, 717–719. [CrossRef] [PubMed]
- 43. Black, H.I.J.; Parekh, N.R.; Chaplow, J.S.; Monson, F.; Watkins, J.; Creamer, R.; Potter, E.D.; Poskitt, J.M.; Rowland, P.; Ainsworth, G.; et al. Assessing soil biodiversity across Great Britain: National trends in the occurrence of heterotrophic bacteria and invertebrates in soil. *J. Environ. Manag.* **2003**, *67*, 255–266. [CrossRef]
- Ditzler, C.A.; Tugel, A.J. Soil quality field tools: Experiences of USDA-NRCS soil quality institute. *Agron. J.* 2002, 94, 33–38. [CrossRef]
- 45. Fusaro, S.; Squartini, A.; Paoletti, M.G. Functional biodiversity, environmental sustainability and crop nutritional properties: A case study of horticultural crops in north-eastern Italy. *Appl. Soil Ecol.* **2018**, *123*, 699–708. [CrossRef]
- 46. Turbé, A.; De Toni, A.; Benito, P.; Lavelle, P.; Lavelle, P.; Ruiz, N.; Van der Putten, W.H.; Labouze, E.; Mudgal, S. *Soil Biodiversity: Functions, Threats and Tools for Policy Makers. Bio Intelligence Service, IRD, and NIOO*; Report for European Commission (DG Environment); European Communities: Paris, France, 2010.
- 47. Aalders, I.; Hough, R.L.; Towers, W.; Black, H.I.J.; Ball, B.C.; Griffiths, B.S.; Hopkins, D.W.; Lilly, A.; McKenzie, B.M.; Rees, R.M.; et al. Considerations for Scottish soil monitoring in the European context. *Eur. J. Soil Sci.* **2009**, *60*, 833–843. [CrossRef]
- 48. Paz-Ferreiro, J.; Fu, S. Biological indices for soil quality evaluation: Perspectives and limitations. *Land Degrad. Dev.* **2016**, *27*, 14–25. [CrossRef]
- 49. Doran, J.W.; Zeiss, M.R. Soil health and sustainability: Managing the biotic component of soil quality. *Appl. Soil Ecol.* **2000**, *15*, 3–11. [CrossRef]
- 50. Reynolds, W.D. Saturated Hydraulic Properties: Ring Infiltrometer. In *Soil Sampling and Methods of Analysis,* 2nd ed.; Carter, M.R., Gregorich, E.G., Eds.; CRC Press: Boca Raton, FL, USA, 2008; pp. 1043–1056.
- Ehlers, W. Observations on earthworm channels and infiltration on tilled and untilled loess soil. *Soil Sci.* 1975, 119, 242–249. [CrossRef]
- 52. Bouché, M.B. Stratégies lombriciennes. In *Soil Organisms as Components of Ecosystems*; Lohm, U., Persson, T., Eds.; Ecology Bulletin; NFR: Stockholm, Sweden, 1977; pp. 122–132.
- 53. Bouché, M.B.; Al-Addan, F. Earthworms, water infiltration and soil stability: Some new assessments. *Soil Biol. Biochem.* **1997**, *29*, 441–452. [CrossRef]
- 54. Six, J.; Bossuyt, H.; Degryze, S.; Denef, K. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Till. Res.* **2004**, *79*, 7–31. [CrossRef]
- 55. Wardle, D.A. Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. In *Advances in Ecological Research;* Begon, M., Fitter, A.H., Eds.; Academic Press: Cambridge, MA, USA, 1995; Volume 26, pp. 105–185.
- 56. Guide to British earthworms. Available online: https://www.opalexplorenature.org/?q=Earthwormguide (accessed on 23 August 2018).
- 57. Paoletti, M.G.; Sommaggio, D.; Fusaro, S. An earthworm soil quality index proposal (QBS-e) applied to agroecosystems. *Biol. Ambient.* **2013**, *27*, 25–43.
- 58. African Soil Crisis Threatens Food Security. Available online: https://www.bbc.co.uk/news/scienceenvironment-30277514 (accessed on 23 August 2018).
- 59. Careless Farming Adding to Floods. Available online: http://www.bbc.co.uk/news/science-environment-26466653 (accessed on 23 August 2018).
- 60. EU Pesticide BANS Could Hit UK Crops. Available online: http://www.bbc.co.uk/news/uk-29699449 (accessed on 23 August 2018).
- 61. Uk Faces Significant Shortage of Farmland. Available online: https://www.bbc.co.uk/news/scienceenvironment-28003435 (accessed on 23 August 2018).

- 62. Members of Parliament Sound Alarm over Neglected Soils. Available online: http://www.bbc.co.uk/news/ science-environment-36428361 (accessed on 23 August 2018).
- 63. Nature Loss Linked to Farming Intensity. Available online: http://www.bbc.co.uk/news/scienceenvironment-37298485 (accessed on 23 August 2018).
- 64. Prince of Wales Joins Soil Boosting Project. Available online: https://www.bbc.co.uk/news/scienceenvironment-37766919 (accessed on 23 August 2018).
- 65. Scottish Research Finds Soil Crucial to Climate Change Fight. Available online: https://www.scotsman.com/news/education/scottish-research-finds-soil-crucial-to-climate-change-fight-1-4093629#ixzz45DwfHpQY (accessed on 23 August 2018).
- 66. Farm Subsidies Must Be Earned. Available online: http://www.bbc.co.uk/news/science-environment-40673559 (accessed on 23 August 2018).
- 67. Bread's Environmental Costs Are Counted. Available online: http://www.bbc.co.uk/news/scienceenvironment-39106180 (accessed on 23 August 2018).
- 68. Lancaster, J. The ridiculous notion of assessing ecological health and identifying the useful concepts underneath. *Hum. Ecol. Risk Assess.* **2000**, *6*, 213–222. [CrossRef]
- 69. Bloem, J.; Schouten, A.J.; Sørensen, S.J.; Rutgers, M.; van der Werf, A.; Breure, A.M. Monitoring and evaluating soil quality. In *Microbiological Methods for Assessing Soil Quality*; Bloem, J., Hopkins, D.W., Benedetti, A., Eds.; CABI: Wallingford, UK, 2006; pp. 23–49.
- 70. UNCED. United Nations Conference in Environment and Development; Agenda 21; UNCED: Rio de Janeiro, Brazil, 1992.
- 71. Rutgers, M.; Schouten, T.; Bloem, J.; Buis, E.; Dimmers, W.; van Eekeren, N.; de Goede, R.G.M.; Jagers op Akkerhuis, G.A.J.M.; Keidel, H.; Korthals, G.; et al. *Een Indicatorsysteem voor Ecosysteemdiensten van de Bodem: Life Support Functions Revisited*; RIVM Rapport 2014-0145; RIVM: Bilthoven, The Netherlands, 2014; 129p, Available online: http://edepot.wur.nl/345145 (accessed on 23 August 2018).
- 72. De Vries, F.T.; Bååth, E.; Kuyper, T.W.; Bloem, J. High turnover of fungal hyphae in incubation experiments. *FEMS Microbiol. Ecol.* **2009**, *67*, 389–396. [CrossRef] [PubMed]
- 73. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
- 74. Anderson, J.P.E.; Domsch, K.H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **1978**, *10*, 215–221. [CrossRef]
- 75. Schipper, L.A.; Sparling, G.P. Performance of soil condition indicators across taxonomic groups and land uses. *Soil Sci. Soc. Am. J.* **2000**, *64*, 300–311. [CrossRef]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).