

RESEARCH ARTICLE

Novel soil quality indicators for the evaluation of agricultural management practices: a biological perspective

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Abstract Developments in soil biology and in methods to characterize soil organic carbon can potentially deliver novel soil quality indicators that can help identify management practices able to sustain soil productivity and environmental resilience. This work aimed at synthesizing results regarding the suitability of a range of soil biological and biochemical properties as novel soil quality indicators for agricultural management. The soil properties, selected through a published literature review, comprised different labile organic carbon fractions [hydrophilic dissolved organic carbon, dissolved organic carbon, permanganate oxidizable carbon (POXC), hot water extractable carbon and particulate organic matter carbon], soil disease suppressiveness measured using a *Pythium-Lepidium* bioassay, nematode communities characterized by amplicon sequencing and qPCR, and microbial community level physiological profiling measured with MicroResp™. Prior studies tested the sensitivity of each of the novel indicators to tillage and organic matter addition in ten European long-term field experiments (LTEs) and assessed their relationships with pre-existing soil quality indicators of soil functioning. Here, the results of these previous studies are brought together and interpreted relative to each other and to the broader body of literature on soil quality assessment. Reduced tillage increased carbon availability, disease suppressiveness, nematode richness and diversity, the stability and maturity of the food web, and microbial activity and functional diversity. Organic matter addition played a weaker role in enhancing soil quality, possibly due to the range of composition of the organic matter inputs used in the LTEs. POXC was the indicator that discriminated best between soil management practices, followed by nematode indices based on functional characteristics. Structural equation modeling shows that POXC has a central role in nutrient retention/supply, carbon sequestration, biodiversity conservation, erosion

control and disease regulation/suppression. The novel indicators proposed here have great potential to improve existing soil quality assessment schemes. Their feasibility of application is discussed and needs for future research are outlined.

Keywords labile carbon, long-term field experiments, organic matter addition, soil biological indicators, tillage

1 Introduction

Agricultural soils have traditionally been managed mainly for productivity because they underpin our existence through food, feed, fiber and timber production. However, they have the potential to sustain a wide range of functions (or processes, a term used synonymously here) related to environmental resilience^[1–3]. Soil quality is defined as the capacity of the soil to perform multiple functions^[4]. Soil quality comprises two aspects, inherent soil quality as determined by nominally fixed factors, i.e., climate, organisms, topography, parent material and time^[5]; and dynamic soil quality which refers to those aspects of soil quality that change as a result of land use and soil management^[6].

Intensive agricultural management has been highly successful in increasing production but often with detrimental effects on dynamic soil properties. These impacts can in turn disrupt soil processes, soil multifunctionality, and soil-based ecosystem services^[7–10] defined as the benefits for humankind derived from ecosystems^[11]. Ultimately, these negative impacts can render soils less reliant on self-regulating processes^[12]. In this context, the assessment and the monitoring of soil quality as affected by agricultural management is a pre-requisite of the fundamental redesign of agricultural systems^[8,13,14] that aim to maintain or increase both agricultural productivity and environmental resilience through the adoption of alternative soil management practices.

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Soil quality can be assessed by measuring the status or the (rate of) change induced by perturbations of soil chemical, physical and biological properties that together determine the capacity of the soil to perform processes^[12,15]. Mainly chemical and physical soil properties were taken into account in early soil quality assessments^[16]. Bünemann et al.^[15] showed that soil chemical and physical indicators are still the most measured properties in soil quality assessments up to now. Biological properties are under-represented, although the soil biota have a primary role in many soil processes that determine soil quality because they are closely linked with physical and chemical properties^[17,18]. This under-representation is likely due to the fact that soil biology is a complex and recently developed discipline, lacking, in many cases, standardization for sampling and laboratory protocols. In addition, soil biological measurements can vary considerably depending on season, weather and other factors, hampering proper establishment of reference values and the interpretation of these changes in terms of soil functioning^[17]. Establishing a direct link between biological indicators and functions is therefore challenging, also because of the difficulties related with the determination of the active part of populations and communities of organisms^[19].

It is widely recognized, however, that despite these challenges, the composite use of chemical, physical and biological properties is crucial to effectively assess soil quality in its entirety^[18,20,21]. Fortunately, rapid technological and knowledge developments in the field of soil biology and organic matter have the potential to deliver novel soil quality indicators that can help farmers and other land managers to most effectively assess the effects of soil management on soil functioning, especially because biological properties are more easily and quickly influenced than most chemical or physical properties^[14,22,23]. Novel biological soil quality indicators can overcome the limitations of the currently-used indicators by being faster

to assess, more sensitive to management, and/or delivering more information about soil processes^[19]. This can ultimately lead to the evaluation and the adoption of alternative agricultural practices that effectively sustain both agricultural production and environmental resilience^[24–26].

The main objective of previous studies^[27–29] was to investigate the suitability of a range of soil biological and biochemical properties as novel soil quality indicators for agricultural management. In this selection of indicators, based on a thorough review of the literature^[15], different but complementary dimensions of the biological and biochemical soil characteristics linked with multiple soil functions were accounted for (Fig. 1), namely soil labile organic carbon^[27], soil disease suppressiveness^[28], soil free-living nematode community characteristics^[29] and soil microbial functionality^[30]. These properties were considered novel because they are not extensively used in soil quality assessment schemes (in particular in Europe), in contrast to currently measured soil quality indicators which are regularly included in assessment schemes as reported in Bünemann et al.^[15].

Each previous study addressed the main objective by assessing (1) the sensitivity of the pertinent novel indicators to soil management comprising tillage (reduced vs. conventional) and organic matter addition (low vs. high), and (2) their linkages with traditionally measured soil quality indicators [e.g., total organic carbon (TOC), pH, water stable aggregates and microbial biomass]. The novel indicators were screened in ten long-term field experiments (LTEs) across Europe to maximize their potential to be added to, or replace, indicators measured in current soil quality assessment schemes. Here, the main findings from Bongiorno et al.^[27–30] and are analyzed together in a unique way compared to the previous single studies, summarized, contextualized and discussed, in respect of the research objective outlined above. Based on the results of previous studies it was hypothesized that the

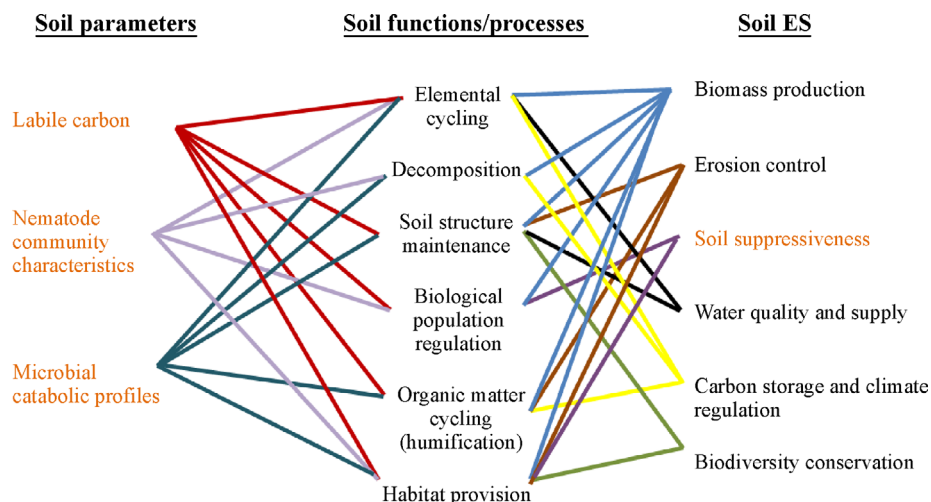


Fig. 1 Linkages between novel soil quality indicators (in orange), processes and ecosystem services (ES). Adapted from Bünemann et al.^[15].

labile carbon fractions were the novel soil quality most sensitive to tillage and organic matter addition among the novel indicators measured. In addition, labile carbon was expected to have a multifunctional role in soil, being positively linked with various soil properties used as proxies for soil-based ecosystem processes and services. Recommendations for future research are subsequently presented, pointing out where scientific research can help the development of soil quality assessments to the benefit of farmers and other land managers.

2 Materials and methods

2.1 Experimental sites and management

Ten European LTEs with a management history between three and 19 years were chosen to investigate the effects of tillage and organic matter addition on a selection of novel soil quality indicators. The ten LTEs were located in different pedoclimatic zones covering six different soil types and consisted of arable (eight LTEs) and permanent (two LTEs) crops (Table S1)^[27–29]. The main treatment factors were classified as tillage: CT, conventional tillage with plowing to 20–35 cm depth (deep soil inversion cultivation) vs. RT, reduced tillage to 10–15 cm depth (very shallow or non-inversion cultivation); and organic matter addition: LOW, no mineral fertilizer and organic matter addition or mineral fertilizer only vs. HIGH, organic matter addition only or organic matter addition with mineral fertilizer^[27–29]. The LTEs had either a complete randomized block design or a split plot design with three or four replicate plots per treatment (Table S1).

2.2 Sampling

Sampling was done in spring 2016 before any major soil management was applied to the fields. Each sample consisted of 20 soil cores randomly collected in the central

area of the plots to avoid border effects. Soil in experiments with tillage included as a management factor (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) were sampled from depths 0–10 and 10–20 cm, except for NL1 which was sampled from depths 0–15 and 15–30 cm (Table S1). Soil in experiments with organic matter addition as the only management factor (CH3, HU1 and PT1) were sampled from depth 0–20 cm. After collection, subsamples were air-dried (40°C) and the remainder stored moist at 3°C. Moist field samples were transported in insulated boxes to Wageningen University and Research (Wageningen, The Netherlands), the Research Institute of Organic Agriculture (FiBL, Frick, Switzerland), the University Miguel Hernandez (Alicante, Spain) and the University of Trier (Trier, Germany), and air-dried samples were sent to the University of Ljubljana (Ljubljana, Slovenia) shortly after collection. Soil samples were sieved at ≤ 5 mm at the sampling location or immediately after reaching the laboratory of destination and, if the soil was moist, stored at 3°C until further processing.

2.3 Standard chemical, physical and biological soil quality indicators

Various standard chemical, physical and biological soil quality indicators were determined in previous studies^[27–30]. All the indicators are shown in Table S2. Here, only soil respiration, water stable aggregates, yield, microbial biomass, and carbon stock were used for statistical analysis (Table 1). These indicators were selected because of their tight link with the following ecosystem services: nutrient retention/supply (soil respiration), erosion control (water stable aggregates), biomass production (yield), biodiversity conservation (microbial biomass) and carbon storage and climate regulation (carbon stock). This selection of standard indicators was used to test and visualize the relationship between labile organic carbon and soil ecosystem services that was initially hypothesized in an *a priori* model (Fig. S1).

Table 1 Overview of methods used to determine chemical, physical, and biological soil quality indicators as measured in the framework of the European project “Interactive Soil Quality Assessment in Europe and China for agricultural Productivity and Environmental Resilience” (iSQAPER) and used for the current study. Adapted from Bongiorno et al.^[28]

Properties	Methodology	Units	Analytical laboratory
Chemical properties			
Total organic carbon	SIST ISO 10694: Soil quality - Determination of organic and total carbon after dry combustion (elementary analysis)	%	University of Ljubljana
Physical properties			
Water stable aggregates	Wet sieving method modified as in Öhlinger & Kandeler ^[31]	mg·kg ⁻¹ soil	FiBL
Biological properties			
Microbial biomass carbon	Fumigation-extraction method ^[32]	mg·kg ⁻¹ soil	University of Trier
Soil respiration	Incubation of soil at 25°C for 72 h in thermostatic bath	μg·h ⁻¹ ·g ⁻¹ soil	University Miguel Hernandez
Yield	Harvesting total biomass and product in 2016	Mg·ha ⁻¹ dry matter	Field assessment

Carbon stock was calculated taking into account the different soil depths in the study as follows.

$$\text{C stock (t} \cdot \text{ha}^{-1}\text{)} = [\text{BD (g} \cdot \text{cm}^{-3}\text{)} \times \text{Soil depth (cm)} \\ \times \text{TOC (g} \cdot \text{kg}^{-1}\text{)}] \times 100$$

where BD is bulk density and soil depth is the soil depth sampled. In the case of the LTEs in which depths 0–10 and 10–20 cm were sampled separately, C stocks in each soil depth sampled were summed to obtain the carbon stock at depth 0–20 cm. In the PT1 experiment fresh yield in $\text{Mg} \cdot \text{ha}^{-1}$ was used because dry yield was not available, and the models used took into account the LTE as a random factor.

2.4 Novel soil quality indicators

The following novel soil quality indicators have been selected and measured in previous studies for their linkages with various soil processes (Fig. 1): (1) labile carbon fractions^[27], (2) soil suppressiveness^[28], (3) free-living soil nematodes^[29], and (4) soil microbial catabolic profiles^[30]. Detailed information on the methodology for assessing the novel soil quality indicators can be found in the references cited above. Brief descriptions of the methodologies with the main principles of the methods are given below.

2.4.1 Labile organic carbon fractions

Various labile carbon fractions were measured as detailed in Bongiorno et al.^[27]. Briefly, the dissolved organic carbon (DOC) was extracted by shaking the soil samples for 1 h with ultrapure water and filtering through a 0.45 μm filter^[33]. Then the hydrophilic part of the DOC (Hy-DOC) was fractionated with a simplified fractionation scheme adapted from van Zomeren and Comans^[34] that used a polymeric adsorbent resin to remove the hydrophobic component of the DOC. The total carbon concentration of DOC and Hy-DOC was determined in a TOC analyzer. DOC and Hy-DOC fractions were further analyzed for specific ultraviolet absorbance (254 nm) to assess their aromaticity^[35]. Hot water extractable carbon (HWEC) was extracted, filtered (0.45 μm) and assayed according to Ghani et al.^[36]. Permanganate oxidizable carbon (POXC) was extracted in KMnO_4 and assayed following Weil et al.^[37]. Particulate organic matter (POM) was characterized with a physical fractionation protocol (wet sieved, 53 μm) as reported by Wyngaard et al.^[38] as modified from Salas et al.^[39]. The particulate organic matter carbon (POMC) was calculated by dividing POM by 1.724, assuming that the percentage of organic carbon in POM was 58%. All soil samples from depths 0–10, 10–20, and 0–20 cm were used for the labile carbon fraction analysis.

2.4.2 Soil suppressiveness

General soil suppressiveness was measured with the established model pathosystem *Pythium ultimum*–*Lepidium sativum* (cress)^[40,41] as detailed in Bongiorno et al.^[28]. Briefly, the capacity of the soil to suppress pathogens was measured by comparing cress fresh weight in soil where *P. ultimum* was added with cress fresh weight in soil where the pathogen was not added.

Soil suppressiveness was calculated as follows.

$$\text{SSni (\%)} = 100 \times (\text{Wni} \times \text{Wn}^{-1})$$

where Wni is shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and Wn is mean shoot weight in natural soil not inoculated with *P. ultimum*. Soil samples from depths 0–10 (0–15 for NL1) and 0–20 cm only were used for this analysis.

2.4.3 Free-living soil nematodes

Free-living soil nematodes were extracted from moist field soils with an Oosterbrink elutriator and cotton wool extraction and subsequently processed following Bongiorno et al.^[29]. Briefly, DNA was extracted from the nematodes according to Vervoort et al.^[42] and used as templates in qPCR to assess total nematode densities^[42,43]. For brevity, qPCR-based quantification of nematode densities is referred to as total nematode abundance. The DNA was also used for 18S rRNA gene amplification and sequencing on the Illumina MiSeq platform^[44] and the data were analyzed by the Genetic Diversity Centre, ETH Zurich. From the sequencing data, indices of richness [sum of operational taxonomic units (OTUs) or genera], diversity (Shannon diversity index), evenness (Sheldon evenness index), percentages and absolute abundances of trophic groups (bacterivorous, fungivorous, herbivorous, predacious and omnivorous nematodes), colonizer-persister (c-p) groups, maturity index (MI), enrichment index (EI), structure index (SI), and channel index (CI) were calculated. In addition, a general beta diversity analysis was conducted on the nematode communities of all the sites. All soil samples from depths 0–10, 10–20, and 0–20 cm were used for the analysis.

2.4.4 Microbial catabolic profile

The MicroRespTM system was used to measure the community level physiological profiling (catabolic profile) of the soil microbial community according to the methodology described in Campbell et al.^[45] and Creamer et al.^[46]. Briefly, the colorimetric gel detection plates were prepared by mixing 150 μL of noble agar and a pH indicator solution containing 12.4 ppm, wt/wt cresol red, 150 $\text{mmol} \cdot \text{L}^{-1}$ KCL and 2.5 $\text{mmol} \cdot \text{L}^{-1}$ NaHCO_3 . Soil sieved at 2 mm was added to the MicroRespTM deep-well

plates using the MicroResp™ filling device and the plates were stored in an incubator for 6 h at 25°C. After incubation, 25 µL of carbon substrate (prepared to deliver 30 mg·g⁻¹ of C to the soil) was dispensed into each well of the deep well plate containing the soil and the plates were left open for 30 min. Seven substrates were added to the soil and subsequently used to produce respiration rates, namely deionized water as control, glucose, alanine, gamma-amino butyric acid, n-acetyl-glucosamine, alpha-ketoglutaric acid and lignin. The initial colorimetric values of the detection plates were read at 570 nm to obtain initial absorbance values (T₀) before the detection plates were sealed and incubated at 25°C for 6 h. Following the incubation, the colorimetric values of the detection plates were read again (T₁) and these final absorbance values were normalized using the T₀ absorbance values. Absorbance data were converted to CO₂ concentration using the calibration curve: CO₂ (%) = 0.02 × A₅₇₀^{-3.11} (R² = 0.93), where CO₂ (%) is the concentration in the headspace after incubation and A₅₇₀ is the normalized absorbance^[47]. The CO₂ (%) concentrations were then converted to respiration rates (µg CO₂-C g⁻¹ dry soil h⁻¹) using the formula provided in the MicroResp™ procedure. Absolute (µg CO₂-C g⁻¹ dry soil h⁻¹) and relative respiration rates (%) were obtained after correcting for the average respiration rate for soil to which deionized water was added. Subsequently, the multiple substrate induced respiration (MSIR; calculated as the sum of the absolute respiration rates of all the substrates per sample) and the Shannon diversity index (H'), used as a measure of microbial functional diversity, were calculated. Only soil samples from depths 0–10 (0–15 for NL1) and 0–20 cm were used for this analysis.

2.5 Statistical analysis

All statistical analyses were conducted using R version 3.6.0^[48]. If tillage was one of the treatments investigated, 0–10 cm depth soil samples were used and, if the only treatment investigated was organic matter addition, 0–20 cm depth samples were used. In total, 101 samples were used. Only these soil depths (not 10–20 cm) were used because all the novel indicators were measured in soil samples coming from these depths. Furthermore, all soil samples from depth 0–20 cm were exposed to conventional tillage and therefore large differences between depths 0–10 and 10–20 cm depths at these sites were not expected because of the mixing of the soil by plowing^[27–29].

Random forest classification^[49] was done to test the importance of the novel soil quality indicators in classifying the different combinations of soil management practices (i.e., CT-Low, CT-High, RT-Low, and RT-High). Random forest classification uses the classification results from many classification trees, where for each tree a single

classification result for each observation is obtained. Each tree is grown with a subset of samples (on average two-thirds of the samples) from the entire data set (bagging or bootstrapped aggregation), and at each node of the tree a random subset of variables (m) of the p independent variables is selected to classify those samples. The class of a sample is determined by the majority of the votes of all the trees in the forest to check the quality of the trees and to assess the importance of the variables in classifying the samples. For the random forest model, the function *randomForest* from the package *randomForest* was used^[50], using 2000 trees and the default value of $m = \sqrt{p}$ (m represents the subset of variables, and p is the total number of variables used for the random forest). In this case, $m = 4$. A large number of trees is needed to obtain a stable estimation of the importance of a variable^[51]. The variable importance measure reported here is the mean decrease in model accuracy (%) on the out-of-bag (OOB) samples (samples that were not part of bootstrapped samples used to create the trees of the forest) when the values of the respective feature are randomly permuted. The OOB estimate of error rate is reported as a measure of the accuracy of the model.

Redundancy analysis (RDA) was used to visualize the profiles of the novel soil quality indicators in soil with either heavy (clay + fine silt < 50%) or light (clay + fine silt > 50%) texture. LTEs CH1, CH2, CH3, SL1 and ES4 were characterized as heavy soils ($n = 42$) and LTEs NL1, NL2, PT1, HU1 and HU4 were characterized as light soils ($n = 59$). The function *rda* in the *vegan* package was used for the RDA^[52] with the novel indicators as dependent variables, the soil management as a constraining variable, and the LTEs as conditional variable. Statistical significance of the RDA was assessed using the *anova* function. The scores of the substrates on the first two axes of the RDA were used to assess the importance of the substrates in differentiating between soil management practices. The soil quality properties were then correlated with the first two RDA axes to check their association with the agricultural management. In the RDA graph the dependent variables with a highly significant correlation ≤ 0.001 with either one of the two RDA axes are reported.

Piecewise structural equation modeling (SEM) was used to evaluate the direct and indirect effects of POXC on various ecosystem services taking into account the dependent structure of the data derived from the same LTE^[53]. An *a priori* model of the relationships between labile carbon and ecosystem processes and services was established, where the hypothesized relationships acted as a framework for the optimization of the piecewise SEM (Fig. S1). The data matrix was fitted using the log-transformed variables but soil suppressiveness was logit-transformed, nematode abundance was square-root transformed, and microbial functional diversity was elevated to the power of two. The evaluation of the AIC was used to

estimate the robustness of the models and to select the appropriate final model^[54]. The Fisher chi-square test (χ^2 ; the model has a good fit when $0 \leq \chi^2/\text{d.f.} \leq 2$ and $p \geq 0.05$) was used to test the overall goodness of fit of the model^[53]. The total standardized effects (reported as path coefficients) of the predictors were calculated and reported on the side of the arrows in the final SEM model representation. R_m^2 (marginal coefficient of determination) and R_c^2 (conditional coefficient of determination) are reported for the response variables, indicating the proportion of the variation explained by the fixed predictor variables and the proportion of the variation explained by the fixed and random predictor variables, respectively. The *lavaan* and *piecewiseSEM* packages were used for the structural equation model^[55,56] and the results were considered statistically significant at $p \leq 0.05$. This SEM model used microbial functional diversity (Shannon diversity index), soil respiration and MSIR as measures of *nutrient cycling*, water stable aggregates as a measure of *erosion control*, C stock as a measure of *carbon storage* and *climate regulation*, soil suppressiveness as a measure of *disease regulation/suppression*, microbial biomass carbon, nematode abundance and nematode richness as measures of *biodiversity conservation*, and yield as a measure of *biomass production*. This set of properties was selected as necessary properties to create the *a priori* SEM model because of their direct relationship with the abovementioned ecosystem services (Fig. S1). POXC was used as labile organic carbon fraction in the model because it was the most sensitive of the labile fractions and correlated best with various soil quality properties^[27–30]. In addition, while comparing SEM models conducted with the other labile carbon fractions, the model with POXC was the best fitting, although the results of the modeling were similar for all the other labile carbon fractions.

3 Results and discussion

3.1 Sensitivity of novel soil quality indicators to tillage and organic matter addition

Reduced tillage and addition of organic matter are widespread agricultural practices used to reduce the impact of soil management on soil properties and processes such as carbon cycling and soil structure formation and maintenance^[8], counteracting multiple soil threats such as soil organic matter depletion, soil erosion and compaction^[57]. Across the ten European LTEs studied, the novel soil quality indicators were sensitive to changes brought about by these agricultural practices despite the large site effects of the LTEs. In particular, compared to the other indicators, POXC, HWEC, and POMC^[27] were sensitive to both tillage and organic matter addition, while soil suppressiveness, free-living soil nematode communities and microbial catabolic profiles were more affected by

tillage than by organic matter addition^[28–30]. Random forest analysis was used to test which of the novel indicators were the most important in discriminating between the different combinations of soil management practices (i.e., CT-Low, CT-High, RT-Low, RT-High). As hypothesized, the labile carbon fractions were the most sensitive according to the mean percentage decrease in accuracy (Fig. 2, OOB estimate of error rate = 52.5%). In particular, POXC was the most important variable in discriminating between the soil management practices.

Previous studies also highlight labile organic carbon, determined by various methodologies, as a highly sensitive fraction of the total soil organic carbon^[36,58–60]. This is likely due to the dependency of labile organic carbon on soil aggregation, aggregate turnover, microbial biomass and residue input^[61]. Soil labile carbon concentrations, therefore, tend to decrease upon agricultural disturbances that lead to aggregate disruption and turnover, release of nutrients from dying microbial cells, and lower residue input. In addition, previous studies found POXC to be one of the most sensitive of a wide range of indicators determined^[58,62–64]. After the labile organic carbon fractions, the soil nematodes, in particular the indices based on functional characteristic of the nematode communities (i.e., SI and EI), were the most sensitive novel soil quality properties in distinguishing the different soil management practices (Fig. 2). This suggests a higher utility of functional information derived from the soil biota compared to taxonomic information in soil quality evaluations.

Tillage exerted a stronger effect on the novel soil indicators than organic matter addition in previous works and this was particularly evident for soil suppressiveness, nematode communities, and microbial catabolic profiles^[28–30]. Conventional tillage destroys soil aggregates, making available resources that boost microbial activity in the short-term^[65]. In addition, conventional tillage entails destruction of the soil as a habitat for organisms where these can be directly killed and exposed to predators by the mechanical action of the plow^[1,66]. In these same studies, reduced tillage practices had a positive effect on various soil processes, increasing the quantity of available carbon for microbial activity^[27] and creating a stable environment which sustained soil suppressiveness^[28], nematode diversity and richness^[29], and microbial decomposition capacity and functional diversity^[30]. Previous studies also show the beneficial effect of reduced tillage on chemical, physical and biological aspects of soil quality compared to conventional tillage^[67–71]. In addition, reduced tillage increased the relative and absolute abundance of herbivorous nematodes^[29] as shown previously by Treonis et al.^[72,73]. This result highlights the possible tradeoffs in ecosystem services (i.e., biomass production and biodiversity conservation) with reduced tillage systems. The effect of tillage was more evident in the upper than in the lower soil depths, confirming the results of previous

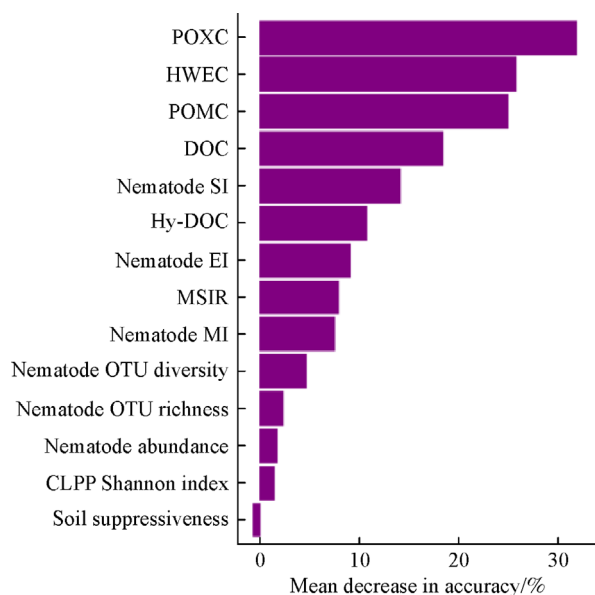


Fig. 2 Importance of novel soil quality indicators in discriminating between different soil management practices (i.e., CT-Low OM, CT-High OM, RT-Low OM and RT-High OM), expressed as variable importance metric (mean percentage decrease in accuracy) from random forest classification analysis. CT, conventional tillage; RT, reduced tillage; OM, organic matter; POXC, permanganate oxidizable carbon; HWEC, hot water oxidizable carbon; POMC, particulate organic matter carbon; DOC, dissolved organic carbon; SI, structure index; Hy-DOC, hydrophilic dissolved organic carbon; EI, enrichment index; MSIR multiple substrate induced respiration; MI, maturity index; OTU, operational taxonomic unit; and CLPP, community level physiological profiling.

studies^[61,74,75]. In addition, reduced tillage often causes stratification of various soil properties, and soil compaction deeper in the soil^[74,76]. These results underline the importance of studying the effect of tillage on soil quality at different soil depths^[77]. The plow layer of the reference system may serve as a minimum sampling depth, but further distinction of depths within the plow layer may increase our understanding of reduced tillage effects.

The weaker effect of organic matter additions than reduced tillage on the novel soil quality indicators may be due to higher variation induced by the heterogeneous nature of the organic matter added in the different LTEs, including biochar, compost, biowaste and farmyard manure (Table S1). The quantity and the composition of the added organic matter and the soil organic matter already present have been shown to be important factors affecting the composition of soil microbial communities, the abundance of components of the communities, and the soil processes they perform such as nutrient cycling, humification, decomposition, and soil suppressiveness^[78–84]. Organic matter addition will preferentially increase microbial biomass and activity if it adds labile and available C and N components to the soil^[81,85].

Based on the results of my group and other studies it may be concluded that farmers and land managers can generally benefit from the adoption of reduced tillage and increased organic matter addition for multiple soil processes such as humification, biological population regulation, nutrient cycling decomposition and habitat provision for biodiversity. However, the implementation of these management measures must be accompanied by awareness of their limitations and with careful evaluation of the site-specific conditions for site-specific management and vice versa^[25,86] in order to optimize the benefits obtained.

3.2 Soil texture

Soil texture can affect the way tillage and organic matter addition impact on soils^[87]. In Bongiorno et al.^[27–30], soil texture was not taken into account because the main aim was to assess the general suitability of novel soil quality indicators across a large number of LTEs. However, soil texture was indirectly considered by including the LTEs as a random factor in the analyses. Here, the effects of tillage and organic matter addition on the novel soil quality indicators were investigated separately in samples of heavy (clay + fine silt > 50%) and light (clay + fine silt < 50%) soils using RDA (Fig. 3).

The treatments of both soil texture classes are located in the same position in Fig. 3(a) and 3(b), with the exception of RT-Low and RT-High, which are swapped in the two figures. In both cases, the most intensive soil management, i.e., CT-Low, was separated from the other management treatments on the first RDA axis, similarly to the RDA of all samples analyzed together in Bongiorno et al.^[30]. This highlights the strong negative effect of the most intensive agricultural practice on soil quality. In the heavy soils (Fig. 3(a)), CT-Low and RT-High resulted in different positions while the two intermediate treatments, i.e., RT-Low and CT-High, clustered closely to each other on RDA axis 1. The labile carbon fractions and the nematode abundance were the ones that discriminated most between the different treatments on RDA axis 1 (Table 2). In the light textured soils (Fig. 3(b)) organic matter addition discriminated between the novel indicators more strongly on RDA axis 2 than in the heavy textured soils. Tillage had a stronger effect when low organic matter was applied, but similarly to heavy soils the discrimination was mainly on RDA axis 1. Light soils are less structured than heavy soils and have limited capacity to protect soil organic matter, and their potential for enhancing soil quality might therefore be higher with direct additions of organic matter than with application of reduced tillage which is more focused on enhancing physical properties^[76,88,89]. However, reduced tillage might be particularly effective when organic matter addition to the soil is low because of a higher potential for improvement. In addition, in the light soils the nematode indicators (MI, EI, OTU richness and

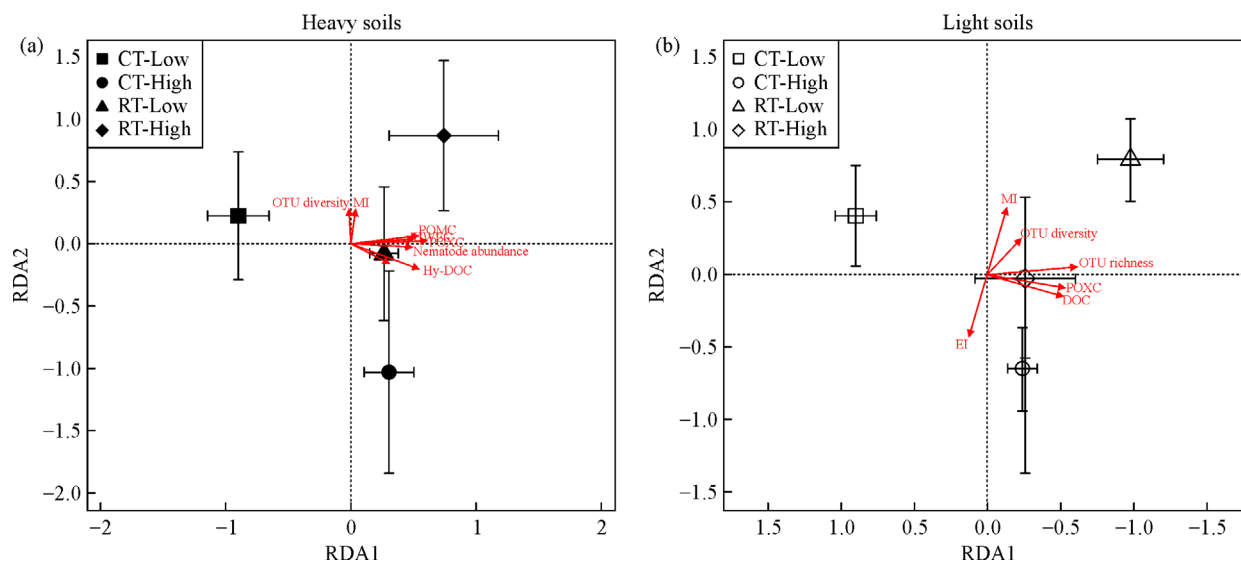


Fig. 3 Influence of soil texture on the novel soil quality indicators, expressed by redundancy analysis (RDA), of novel soil quality indicators assessed in samples with (a) heavy soil texture (clay + fine silt > 50%; $n = 42$) and (b) light soil texture (clay + fine silt < 50%; $n = 59$). The soil quality indicators that had a significant correlation at $p \leq 0.001$ with either RDA axis are reported in red with their vectors. CT-Low, conventional tillage and low organic matter input; CT-High, conventional tillage and high organic matter input; RT-Low, reduced tillage and low organic matter input; RT-High, reduced tillage and high organic matter input; OTU, operational taxonomic unit; POMC, particulate organic matter carbon; HWEC, hot water extractable carbon; POXC, permanganate oxidizable carbon; Hy-DOC, hydrophilic dissolved organic carbon; DOC, dissolved organic carbon; MI, nematode maturity index; and EI, nematode enrichment index.

Table 2 RDA scores, Pearson correlation coefficients (r) and related p -values of the novel soil quality indicators on the first two RDA axes for heavy textured and light textured soils

Indicators	Heavy soils (clay + fine silt > 50%)						Light soils (clay + fine silt < 50%)					
	RDA1			RDA2			RDA1			RDA2		
	Score	r	p	Score	r	p	Score	r	p	Score	r	p
POXC	0.60	0.61	***	0.03	0.03		0.55	0.43	**	-0.10	-0.08	
HWEC	0.49	0.51	**	0.03	-0.02		0.34	0.27	*	-0.02	0.01	
Hy-DOC	0.54	0.61	***	-0.18	-0.11		0.44	0.39	*	-0.17	-0.21	
DOC	0.37	0.44	*	-0.01	-0.02		0.51	0.43	**	-0.11	-0.01	
POMC	0.53	0.55	**	0.05	-0.02		0.42	0.33	*	0.05	-0.09	
Soil suppressiveness	0.12	0.25		0.08	0.02		0.02	0.006		0.18	0.03	
Nematode OTU diversity	-0.02	-0.007		0.27	0.68	***	0.23	0.37	*	0.25	0.57	***
Nematode OTU richness	0.17	0.22		0.11	0.39	*	0.60	0.58	***	0.04	0.26	*
Nematode abundance	0.49	0.62	***	-0.02	-0.38	*	0.18	0.15		-0.13	-0.33	*
MI	0.03	-0.05		0.24	0.55	**	0.13	0.26	*	0.45	0.76	***
SI	0.04	0.002		0.12	0.36	*	0.12	0.16		0.33	0.39	*
EI	-0.12	-0.10		-0.14	-0.35	*	0.09	0.17		-0.41	-0.54	***
MSIR	0.43	0.40	*	0.10	0.02		0.20	0.14		0.009	0.01	
CLPP Shannon index	0.28	0.49	**	-0.15	-0.38	*	0.21	0.24		0.06	0.08	

Note: POXC, permanganate oxidizable carbon; HWEC, hot water oxidizable carbon; Hy-DOC, hydrophilic dissolved organic carbon; DOC, dissolved organic carbon; POMC, particulate organic matter carbon; OTU, operational taxonomic unit; MI, maturity index; SI, structure index; EI, enrichment index; MSIR, multiple substrate induced respiration; and CLPP, community level physiological profiling. * $p \leq 0.05$, ** $p \leq 0.001$, and *** $p \leq 0.0001$.

diversity) were more important in discriminating between the treatments than in the heavy soils, especially on RDA axis 1 (Table 2). Even so, the labile carbon fractions

remained of prime importance, similarly to the heavy soils. It is concluded that soil management had a similar effect on soil quality indicators in heavy and light soils, and that

taking into account soil texture does not fundamentally alter the interpretation of the results presented in Bongiorno et al.^[27–30]. Nevertheless, soil texture seemed to affect some of the nematode indicators, corroborating the results of Quist et al.^[90]. This supports the view that soil texture might influence the impact of soil management on soil quality and warrants more attention in future studies.

3.3 Key role of labile organic carbon

In Bongiorno et al.^[27], labile carbon, in particular POXC, was correlated with various traditionally measured soil quality indicators related to nutrient cycling (total nitrogen, cation exchange capacity, available P and K, Mg and soil respiration), soil structure (water stable aggregates, water holding capacity and bulk density), carbon sequestration (TOC) and habitat provision (microbial biomass carbon, soil respiration). In addition, labile carbon was tightly linked to the other novel indicators assessed, showing its potential as an overarching indicator linking different quality aspects of agricultural soils^[28–30]. Labile organic carbon is also tightly linked to TOC and it constitutes the primary energy source for soil organisms, being the fuel for their activities and processes such as humification and nutrient cycling^[59]. Various studies have found labile carbon to be linked with soil quality properties, and POXC and HWEC were found to be particularly linked with biological properties^[36,91,92]. In addition, POXC has been proposed as an indicator of carbon sequestration in previous studies^[58,93]. Labile organic carbon and its aromaticity have been linked with changes in taxonomic microbial community composition^[78], which are likely to correspond with changes in soil functionality. Therefore, not only TOC, but also the nature of the organic compounds that it comprises can have a strong impact on soil processes, especially in terms of microbe-related processes such as nutrient cycling, decomposition, humification and biological population regulation^[81,84,94]. However, at the moment there is evidence that POXC is not only quantifying the labile part of carbon, but also more processed^[58] and recalcitrant compounds such as lignin^[95,96]. Hence, it is important to stress that in the case of the labile carbon fractions, and in particular POXC, a better understanding of the nature of the compounds will facilitate interpretation and acceptance by farmers and other land managers.

The other novel indicators were generally enhanced by reduced tillage and addition of organic matter and were positively correlated with various traditional soil quality indicators, such as TOC, total nitrogen, microbial biomass and activity^[27–30]. Nevertheless, the novel indicators have the potential to reveal different and unique characteristics of soil quality that might not be derived from traditionally measured indicators (i.e., soil suppressiveness, food-web information and biodiversity, and microbial functional capacity). Therefore, these could be used in

a complementary way in soil quality assessments to gain more information about soil functionality.

3.4 Linking soil quality indicators with ecosystem processes and services

For novel indicators to be adopted in practice, their links with functions and ecosystem services have to be established^[16,97]. For this reason, SEM was used in search for confirmation of hypothesized mechanistic relationships between indicators^[98]. The SEM models in Bongiorno et al.^[28,30] support the hypothesized primary role of labile organic carbon in sustaining soil disease suppressiveness and microbial functional diversity. Here, in addition to these individual models, the hypothesized key role of POXC (the other labile carbon fractions have also been tested; data not shown) in sustaining various soil ecosystem services was tested and visualized in a comprehensive SEM model (Fig. 4).

In agreement with the results presented in previous studies and with the hypothesis specified in the current work^[27–30], POXC was found to have a multifunctional role in agricultural soils. POXC (but also the other labile carbon fractions; results not shown) had positive links with carbon sequestration, nutrient retention/supply, biodiversity conservation and erosion control, the latter partly through the positive effect on microbial biomass carbon, an association that confirms previous studies^[22,99]. In particular, fungal biomass has been positively associated with micro-aggregate stability^[100–102]. Labile organic carbon also had an indirect positive effect on biodiversity conservation through its stimulation of the active part of the microbial community (i.e., soil respiration), and on disease regulation/suppression through its stimulation of the competitive ability of the microbial community (viz., microbial biomass carbon and MSIR) against the pathogen. This latter finding is consistent with Dignam et al.^[103] who found that higher carbon availability selected for a richer and more competitive and suppressive community controlling *Rhizoctonia solani*. Likewise, Bastida et al.^[104] demonstrated the key position of labile carbon measured as DOC in the multifunctionality of soil microbial communities. The negative relationship between C stock and soil disease suppressiveness may be explained by the enhancement or inhibition of soil suppressiveness by organic amendment depending on the quality of the organic carbon added to the soil^[83,84]. The quality of the organic carbon additions was variable in the different LTEs studied (e.g., compost, biochar and biowaste), and the net effect resulted in a negative relationship between C stock and soil suppressiveness. An earlier work indicates that the more labile part of the TOC sustains the capacity of the microbial community to suppress soil pathogens through general disease suppressiveness mechanisms^[28].

Most of the links between labile organic carbon and ecosystem services were positive, underlining the

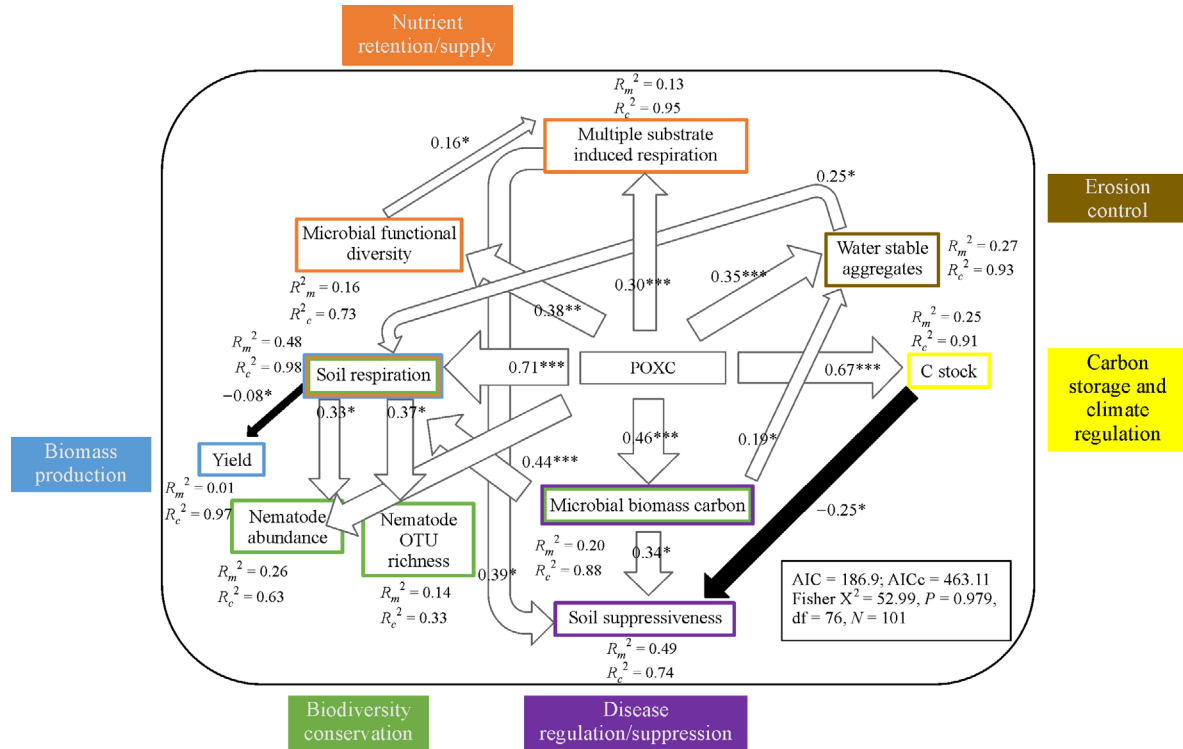


Fig. 4 Structural equation model (SEM) showing the central role of POXC as a predictor of various ecosystem services, which are placed in colored boxes outside the SEM frame. White boxes within the SEM frame represent measured variables and arrows represent the unidirectional relationship between the properties. The color of the border of the boxes specifies the ecosystem service they indicate. White and black arrows indicate positive and negative relationships, respectively. Numbers on the side of the arrows indicate standardized effect size whose strength is proportional to the width of the arrow. Numbers close to the boxes of the response variables are R_m^2 (marginal coefficient of determination) and R_c^2 (conditional coefficient of determination). Akaike information criterion (AIC), corrected Akaike information criterion (AICc), Fisher chi-square (Fisher χ^2), p -value (P) of the test, degrees of freedom (df), and the number of observations (N) are indicated. POXC, permanganate oxidizable carbon; C stock, carbon stock; and OTU, operational taxonomic unit. $^0p \leq 0.1, *p \leq 0.05, **p \leq 0.01$ and $***p \leq 0.001$.

synergies between soil processes. However, even though weak, POXC had an indirect negative effect on biomass production through soil respiration. This result is at odds with those from previous studies where positive relationships between POXC and yield were found [37,64,93,105]. This opposite result is nevertheless important because it suggests tradeoffs between different ecosystem services that have to be accounted for when managing soils. Agricultural practices considered to be sustainable such as organic matter additions, reduced tillage, and cover cropping often coincide with increased values of soil quality indicators that are linked to environmental functions at the expense of productivity [25,74,106–111]. In particular, this is often the case when soil management aims to sustain multiple functions related to productivity but also to environmental resilience [112].

4 Feasibility of the application of the novel soil quality indicators

The advantages and disadvantages of each of the novel soil

quality indicators are presented in Table 3.

In addition, why the use of the novel indicators in soil quality assessment may be challenging was examined. These considerations apply to the novel indicators, but also to more traditionally used soil biological, physical and chemical indicators. The latter, however, have a longer history of consensus building and standardization.

Firstly, standardization of the methodologies including sampling time (i.e., season relative to crop developmental stage and time of soil management application) should be addressed to facilitate comparisons in space and time [99,113,114] and to reach consensus between different laboratories [115]. However, it must be recognized that standardization is not always possible and that sometimes methods tailored for specific conditions are more effective [116].

Secondly, data interpretation depends on data collection and availability. These are necessary for making information about the state and the changes in soils more precise while developing thresholds, scoring curves, reference values, and benchmarks [12,117,118]. The interpretation of the values obtained can also be challenging due to seasonal

Table 3 Advantages and disadvantages of the novel soil quality indicators

Novel indicator	Advantages	Disadvantages
Labile carbon fractions	Sensitive Multifunctional indicators Unified protocols are available	Individual laboratory protocols vary, hampering general standardization and comparability Pre-treatment conditions (sieving and storing) affect all the fractions; also quantity of soil and soil organic carbon affects POXC determination Not clear which part of the total carbon is quantified, complicating the interpretation of the results (POXC might not quantify only the labile part of TOC)
Soil suppressiveness	Highly reproducible, fast and easy assay Close to <i>in situ</i> conditions Sensitive	Other factors, not quantified in our study, affect soil suppressiveness Assessment of <i>potential</i> , which does not take into account the specificity of a particular host-pathogen interaction in the field Bioassays with different pathogen can give different results Bioassay should be combined with <i>in situ</i> characterization of disease severity and/or with a bioassay using the crop and the pathogen that are present in the area and cause disease
Free-living soil nematode communities	Sensitive Molecular techniques gave results in accordance to more established microscopic techniques. Data obtained with molecular methods can be interpreted using knowledge on nematode community composition (i.e., trophic and life strategy groups) Molecular characterization will become ever faster, cheaper and with higher throughput than morphological identification Information on taxonomic as well as functional and ecological aspects based on food preferences and life-history is available	Variable efficiency of the extraction of nematodes and DNA from soil, and high variability in the methodology between laboratories Optimization and standardization of the method is needed: primer selection, database completeness and bioinformatic analysis workflow Number of copies of targeted genes varies with species and life stage, complicating the assessment of relative abundances, and standardization of the sequencing results Assessment of relic DNA
Microbial catabolic profile (MicroResp™)	Easy and practical functional characterization of the soil microbial community which combines functional diversity and degradation rates Sensitive	The method selects only species adapted to rapid growth on simple substrates The choice of the substrates is critical and the current set of substrates has low discriminating capacity The same amount of carbon source is added to the soil, not the same amount of carbon Final values are highly dependent on a laboratory-specific calibration line, making comparison between laboratory results problematic

and spatial variation. This is particularly true for biological indicators, which can respond to a plethora of environmental conditions and whose dynamics can, therefore, be high^[119]. Data collection should consider local variation in biological functioning as affected by factors such as pedoclimatic zone and land use^[88]. Also, data availability might contribute to improved predictive power of models that simulate soil processes^[120] and facilitate the link between soil properties and soil functions such as habitat provision, soil structure formation and maintenance, and nutrient cycling^[121].

Finally, whereas the indicators used in *visual* soil quality assessment have the benefit of being easily understandable, translation of *analytical* soil quality indicators values to meaning for farmers and other land managers often has to be mediated by scientists, extension service advisors and/or computer algorithms. It is essential that these measure-

ments have the potential to be translated into suggestions for the implementation of sustainable agricultural practices^[122,123]. The novel indicators studied here seem to have this potential.

5 General remarks and suggestions for future research

The approach used for the identification and measurement of novel soil quality indicators in Bongiorno et al.^[27–30] required simplification of the management practices in the ten LTEs in broad categories (tillage and organic matter addition). This type of generalization can be important for the development of soil conservation policies^[124]. However, site specificity is important in the assessment of supply and demand of soil functions because these depend

on soil type, land use and specific management^[6,65,86,125]. There is, in fact, a need for more regional and management-specific soil quality assessments^[126] that can eventually generate context-specific solutions in agriculture^[127,128].

In addition, it should be stressed that the technological and knowledge advancement that is creating the possibility to develop novel soil quality indicators should not be blindly followed. Thoughtful evaluation of the relative merits of the potential indicators is needed as in the case of molecular methods^[129,130].

Nevertheless, the novel soil quality indicators presented offer the potential to be added to, or partly replace, indicators measured in existing soil quality assessment schemes because of their sensitivity to management, and linkages with soil processes such as nutrient and carbon cycling, habitat for biodiversity and soil suppressiveness. In-depth time and cost analysis is needed to evaluate whether these aspects make them appropriate elements of soil quality assessment schemes. At present, the addition of POXC as a soil quality indicator seems the most feasible possibility if the challenges outlined above (Section 4) are taken into account, in particular regarding standardization and interpretation.

In this respect, the following research opportunities should be explored.

- Studies focusing on (1) elucidating which part of the TOC is measured as labile carbon (i.e., POXC might not only measure the labile fraction of the TOC), (2) developing a rapid and easy way to assess organic carbon quality, and (3) elucidating the relationship between labile carbon, different organic carbon compounds and functions are needed. Spectroscopic methods seem very promising in this respect, e.g., mid-infrared photoacoustic spectroscopy^[131] and diffuse reflectance Fourier transformed mid-infrared spectroscopy; they could also be used to assess microbiological characteristics of the soil community such as microbial biomass carbon^[84].

- Elucidation of which methodologies may help with the assessment of effectiveness indicators of soil disease suppressiveness is needed. In this regard, sequencing, transcriptomics, quantitative PCR, metabolomics, and proteomics techniques are promising^[132]. However, the link between potential antagonistic activity of the microbial community assessed by molecular methods (e.g., presence of genes coding for antagonistic properties) and the actual soil suppressiveness measured with bioassays as well as the predictive value for field conditions need to be established.

- Validation of the results of food-web indices of free-living nematode communities, calculated with sequencing results, is needed, together with the optimization of databases, pipelines for the method (primer selection, bioinformatics analysis), and standardization of the sequencing results to obtain corrected relative abundances^[133].

- Better interpretation and validation of the MicroResp™ results are needed to ensure that results will be understandable and easily translated to management recommendations.

- There is a need to strengthen the link between taxonomic and functional diversity and soil processes, to make more effective use of soil biota information in soil quality assessment.

- Further studies are also needed for other management practices such as crop rotations, intercropping, cover crops, and more specific organic matter input practices (e.g., farmyard manure, slurry and biochar). In addition, the effects of soil texture should be further considered to give more specific management recommendations.

- There is a need to investigate when and to what extent involvement of different stakeholders (e.g., farmers and other land managers) in the development, validation and use of novel soil quality indicators might help to render research activities in the field of soil quality indicators more effective.

6 Conclusions

Assessing biological soil quality indicators is essential to monitor the status and the changes in soil processes as affected by anthropogenic pressure. In this work the potential of different soil properties, i.e., labile organic carbon, soil disease suppressiveness, free-living nematode community characteristics and microbial catabolic profiles, as novel soil quality indicators in agricultural systems is summarized and discussed. Reduced tillage in particular, and organic matter addition to a lesser extent, affected these different dimensions of soil quality. POXC, and to a lesser degree the other carbon fractions of organic matter, were found to be particularly suitable indicators, as is apparent from the quantitative analysis of their sensitivity to soil management and their direct and indirect contributions in sustaining multiple soil ecosystem services (nutrient retention/supply, erosion control, carbon storage, disease suppression, biodiversity conservation, and biomass production). Although a better understanding of their mechanistic relationships with soil functions, and of which part of the organic matter is quantified by the labile carbon fractions is needed, the novel indicators assessed were linked to functionality. The novel indicators may therefore play a valuable role in translating soil quality assessment into agricultural management options. The functional characterization of soil nematodes was shown to be more sensitive to management than a mere taxonomic characterization, which could point out the important role of information based on biota functioning in soil quality assessment. In addition to being sensitive indicators for long-term agricultural management effects, it is speculated that the novel indicators can serve as sensitive indicators of short-term agricultural management effects also. This

study together with our previous studies can contribute to the further development of soil quality assessment by adding information about the suitability of novel indicators to assess soil quality. Future work should focus on the validation of the indicators studied and to optimize their use in combination with, or substituting for, existing soil quality indicators.

Supplementary materials The online version of this article at <https://doi.org/10.15302/J-FASE-2020323> contains supplementary materials (Tables S1 and S2; Fig. S1).

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