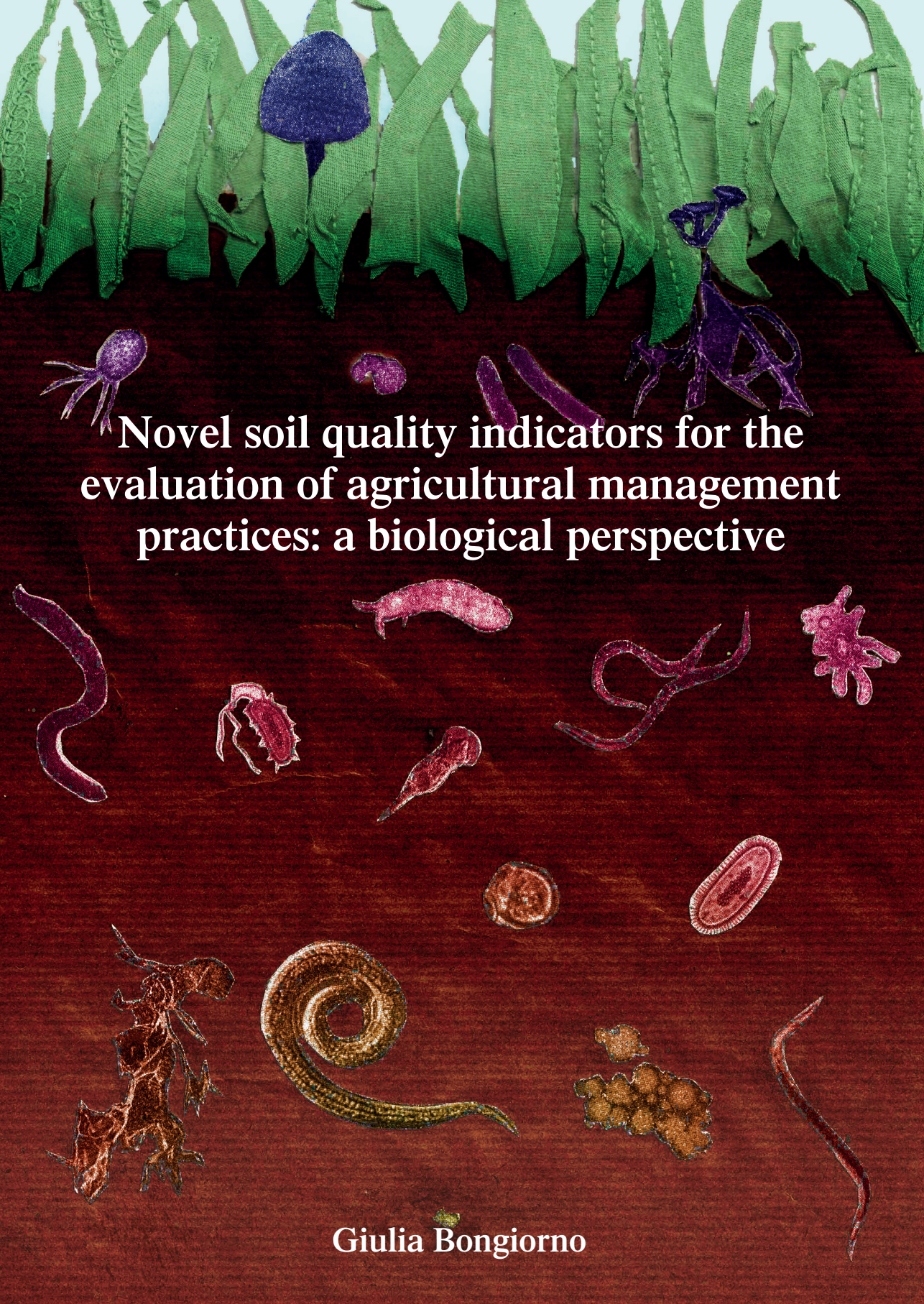

Novel soil quality indicators for the evaluation of agricultural management practices: a biological perspective

Giulia Bongiorno

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

Giulia Bongiorno

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

Giulia Bongiorno

Thesis

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Developments in soil biology and methods to characterize soil organic carbon have the potential to deliver novel soil quality indicators that can help to identify soil management practices that sustain soil productivity and environmental resilience. This thesis aimed at investigating the suitability of a range of soil biological and biochemical parameters as novel soil quality indicators for agricultural management. The soil parameters, selected through a literature review, comprised different labile organic carbon fractions (hydrophilic dissolved organic carbon (Hy-DOC), dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), hot water extractable carbon (HWEC) and particulate organic matter carbon (POMC), ordered here from the smallest to the largest proportion of the total organic carbon), soil disease suppressiveness measured with a *Pythium-Cress* bioassay, nematode communities characterized with amplicon sequencing and qPCR, and microbial community level physiological profiling (CLPP) measured with MicroResp™. We tested the sensitivity of the novel indicators to tillage and organic matter addition in 10 European long-term field experiments, and assessed their relationship with already existing soil quality indicators linked to soil functioning. Lastly, the results of these experimental chapters are interpreted relative to each other and to the broader body of literature on soil quality assessments. Moreover, pros and cons of the novel indicators are discussed, and possibilities and needs for future research are outlined. 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 had a weaker role in sustaining soil quality, possibly due to the different compositions of the organic matter inputs in the long-term field experiments that were sampled. Random forest analysis showed that POXC was the indicator that discriminates soil management most, and structural equation modelling showed its central role in nutrient cycling, 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, but their usefulness is still to be validated and optimized.

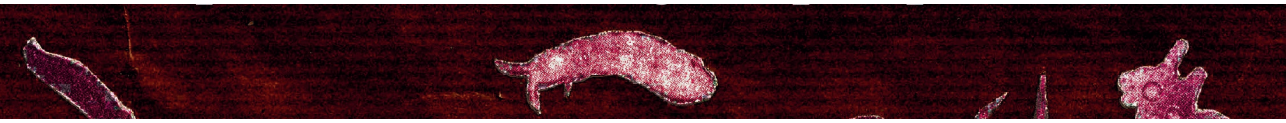
Abstract

Table of Contents

This thesis is dedicated to my grandparents Carlo, Maria, Rosanna and Ugo, and to my nephews India and Lucio. The old and the new generation that are always with me and fill me with joy.


CHAPTER 1
1

General introduction



Giulia Bongiorno

1.1 Soil multifunctionality and the concept of soil quality

Agricultural soils have traditionally been linked mainly to productivity, because they underlie our existence through food, feed, fibre and timber production. However, they have the potential to sustain a wide range of functions (or processes, here used synonymously) related to environmental resilience such as *water cycling, soil aggregation, humification and decomposition, pest and disease population regulation, habitat provision,* and *nutrient cycling* (Kibblewhite et al., 2008b; Dominati et al., 2010; Brussaard, 2012a).

This characteristic of soils to provide multiple functions is referred to as soil **multifunctionality**. Soil multifunctionality is increasingly recognised not only as a potential capacity, but also as a desirable and essential characteristic of 'sustainable' soils (Adhikari and Hartemink, 2016; Jones et al., 2017), i.e. soils that meet our own needs without compromising the ability of future generations to meet theirs (Brundtland et al., 1987).

Soil functions are instrumental for the provision of the so-called soil-based **ecosystem services (ES)**, which are defined as the benefits for humankind derived from ecosystems (Costanza et al., 1997a; Baveye et al., 2016a). Soil-based ecosystem services in agricultural settings are, for example: *biomass production, biodiversity conservation, erosion control, pest and disease control, water quality and supply* and *climate regulation* (Chapter 2; Bünemann et al., 2018). Ultimately, soil-based ecosystem services can help in reaching the United Nations (UN) sustainable development goals (SDGs), international targets related with environmental and societal sustainability (Adhikari and Hartemink, 2016; Keesstra et al., 2016).

Often trade-offs and synergies between processes and between ecosystem services delivered by soils occur (Howe et al., 2014; Stavi et al., 2016; Sandén et al., 2018). For example, O'Sullivan et al. (2015) underlined the trade-off between primary productivity and carbon storage in Irish grassland and Mkhabela et al. (2008) reported lower soil nitrate content and leaching to groundwater, but increased NH_3 and N_2O emissions, in no-tillage compared to conventional tillage.

The capacity of the soil to perform multiple functions is defined as **soil quality** (Doran and Parkin, 1994b). The concept and its development are critically reviewed in Chapter 2 of this thesis (Bünemann et al., 2018). **Soil quality** includes two aspects: **inherent** and **dynamic soil quality**. Inherent soil quality is determined by 'fixed' factors, i.e. climate, organisms, topography, parent material and time (Jenny, 1994); dynamic soil quality refers to those aspects of soil quality that change as a result of land use and soil management (Schulte et al., 2014). Dynamic soil quality is the most relevant aspect of soil quality for humans, since it is the one that is more modifiable through management choices. The concept of soil quality is still largely debated, and this mainly derives from the fact that

soil quality is often considered more in general terms, and not defined in terms of soil functions which are desired from a specific soil (Baveye et al., 2016a). The importance of soil quality is also highlighted by the broader importance of the concept of One Health, where the connection between soil and human health is made explicit (Zornoza et al., 2015; Lal et al., 2017; Schwilch et al., 2018; van Bruggen et al., 2019).

1.2 Soil quality and agricultural management

Since productivity is the main aim of agricultural systems, agricultural practices largely focus on increasing yields. Such practices, especially in more industrialized part of the world, include the use of large amounts of agro-chemicals (e.g. mineral fertilizers and pesticides), monocultures and heavy soil disturbance caused by ploughing (Amundson et al., 2015). These practices were highly successful in increasing production, being economically attractive, but often at the expenses of environmental quality, increasing pollution, decreasing biodiversity and other resources such as water and fossil fuels (Stoate et al., 2001; O'Sullivan et al., 2015; Keesstra et al., 2016). These negative effects of soil management on environmental quality can disrupt soil processes and multifunctionality, in particular functions related to environmental resilience (Vitousek et al., 1997), finally rendering soils less reliant on self-regulating processes (Brussaard et al., 2007; **Figure 1.1**). For example, more and more studies demonstrate that land use intensification has a detrimental effect on biota (Postma-Blaauw et al., 2012; Banerjee et al., 2019), reducing species diversity (Stoate et al., 2001; Tsiafouli et al., 2015), negatively impacting multiple ecosystem functions and services (Wagg et al., 2014), and the resistance and resilience capacity of the system after changes (de Vries et al., 2012). In addition, in the long run, also productivity can be decreased and rendered more dependent on external input than on natural internal functioning (Rickson et al., 2015).

Soil degradation is, therefore, a common problem in agriculture which can occur through human, but also through natural-induced soil threats (Rickson et al., 2015; Schwilch et al., 2016). The major threats to agricultural soils are loss of organic matter, erosion, contamination, landslides, sealing, salinization, and compaction (Glæsner et al., 2014; Jones et al., 2017). Human-induced soil degradation can speed up and exacerbate the process of natural-induced soil degradation (Rickson et al., 2015). Every year 12 Mha of agricultural land are degraded and/or lost (Rickson et al., 2015), and once degraded, soil regeneration is a very slow process (Amundson et al., 2015; Lal, 2015).

In the last decades, farmers, land managers, society, governments and scientists have felt the urge to stop and counteract the pressure that humans exert on natural resources, including soils (Montanarella, 2015; Baveye et al., 2016a). In this context, the development and the adoption of alternative soil practices that aim to maintain or increase agricultural

productivity and environmental resilience have become increasingly important (Bennett et al., 2010a; Schwilch et al., 2016; Barão et al., 2019). Diverse alternative agricultural management practices are available and show high potential for sustaining environmental resilience and foster soil protection, e.g. cover crops, reduced tillage, application of organic matter, organic and integrated farming, agroforestry, intercropping, mulching etc. (White et al., 2012). Reduced tillage and organic matter additions are two common soil management practices that positively affect multiple soil characteristics, and can help to counteract soil degradation in agricultural systems (White et al., 2012; Bai et al., 2018). For example, these practices can enhance soil carbon storage, thus having a positive impact on climate regulation, erosion control, water and nutrient retention and biodiversity (Lal, 2004; Diacono and Montemurro, 2010; Gattinger et al., 2012).

From the above mentioned considerations, it follows that the assessment and the monitoring of soil quality as affected by agricultural management is a bearing element of fundamental re-design of agricultural systems (Smith et al., 2016; Bai et al., 2018; Schwilch et al., 2018). In this respect, the monitoring of long-term field experiments that compare the effect of different and/or contrasting soil management practices and intensities is a precious resource which can help in this task (Körschens, 2005; Bai et al., 2018; Johnston and Poulton, 2018; Sandén et al., 2018).

1.3 How to measure soil quality?

Soil quality depends on soil **parameters** that together determine the capacity of the soil to perform processes and provide soil-based ecosystem services (Dominati et al., 2010). Soil quality, therefore, can be assessed by measuring the soil's chemical, physical and biological parameters (Bünemann et al., 2018) as a status or as a (rate of) change induced by a disturbance (Bone et al., 2014a). These soil parameters are considered **soil quality indicators**, but only if they match several criteria (Ritz et al., 2009b; Faber et al., 2013). First of all, these indicators should be *well correlated with soil functions* (Larson, 1994), preferably with *multiple* soil functions (Bone et al., 2010a). Second, they should be *sensitive to soil management and threats*, and *interpretable*. Third, from a more practical perspective, they should be reproducibly measurable in different laboratories. *Ease* and *cheapness* of measurement are often added from a practical perspective. Multiple parameters have to be measured when assessing and monitoring soil quality, because single properties will not adequately address the complexity of the soil compartment (Kibblewhite et al., 2008b; Griffiths et al., 2018b). In early soil quality assessments, mainly chemical and physical soil properties were taken into account (Adhikari and Hartemink, 2016). In Chapter 2 (Bünemann et al., 2018), we show that soil chemical and physical indicators are still the most measured parameters in soil quality assessments up to now, while biological

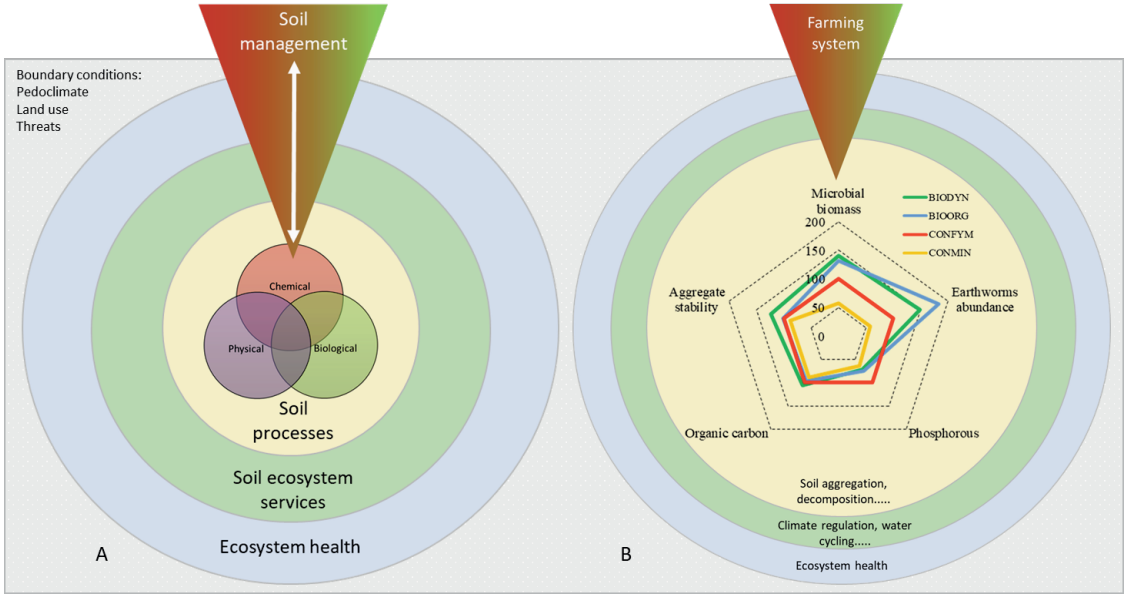


Figure 1.1. Figure 1.1A illustrates a schematic overview of the effect of agricultural management on soil parameters, processes, ecosystem services and finally ecosystem health within boundary conditions (pedoclimate, land use and soil threats). The white arrow indicates the possibility of adaptive management based on the effect of management on the soil system. Figure 1.1B illustrates an example of the changes that can occur in soil indicators in response to different farming system practices after 21 years of implementation in the DOK long-term field trial (BIODYN= biodynamic farming, BIOORG= organic farming, CONFYM= conventional farming with mineral fertilizer plus farmyard manure, CONMIN= conventional farming with only mineral fertilization). The spider diagram shows the effects of the farming practices relative to CONFYM (100%). Modified from Mäder et al. (2002).

parameters are underrepresented. This is likely due to the fact that soil biology is a complex and recently developed discipline, lacking, in many cases, standardization for lab protocols and sampling. More recently, it has been widely recognized that the composite use of chemical, physical and biological parameters is crucial to effectively assess soil quality in its entirety (Lehman et al., 2015a; Zornoza et al., 2015; Paz-Ferreiro and Fu, 2016) (**Figure 1.1A**).

Soil quality indicators can be measured directly in the field (visual assessment) or in the laboratory (analytical indicators). In this thesis I address analytical indicators.

1.3.1 Soil biological indicators

The soil biota have a primary role in many soil processes that determine soil quality (Brussaard et al., 1997; Adhikari and Hartemink, 2016; Bünemann et al., 2018; **Table S6** of Chapter 2). For this reason biodiversity *per se* is regarded as a soil-based ecosystem service, and soil biodiversity loss is considered a soil threat (Adhikari and Hartemink,

2016). Soil biota are tightly linked with physical and chemical parameters, and have the potential to act as an integral indicator of soil quality. In addition to their relevance for soil processes, biological parameters are more easily and quickly influenced than most chemical or physical parameters (Mijangos et al., 2006; van Leeuwen et al., 2015; Bai et al., 2018). Therefore, in the recent decade, soil biological indicators have been increasingly considered in soil quality assessment and monitoring schemes (Barrios, 2007; Bispo et al., 2009; Gardi et al., 2009; Ritz et al., 2009b; Faber et al., 2013; Stone et al., 2015; Krüger et al., 2018). However, the use of sol quality indicators does not come without drawbacks. Their dynamics can be very variable (de la Rosa, 2005), depending on season, weather and other factors, hampering the establishment of reference values that are essential for their interpretation, which is, therefore, not always straightforward. In addition, establishing a direct link between biological indicators and functions is challenging, also because of the difficulties related with the determination of the active part of organism populations and communities (Duraisamy et al., 2020).

Soil organic matter (or carbon), which I consider a biochemical parameter, is one of the most, if not 'the' most, important and central soil property (Bastida et al., 2008; Keesstra et al., 2016). Soil organic matter is important for sustaining soil organisms with all the processes they perform, and creating and maintaining soil structure, holding water and nutrients (Reeves, 1997b; Adhikari and Hartemink, 2016). Soil organic matter loss is, therefore, considered another soil threat (Amundson et al., 2015). Keesstra et al. (2016) explicitly mention that an urgent task for the scientific community is to raise awareness on soil organic matter as a key soil attribute. Due to the large organic matter pool in soil, total organic carbon is relatively insensitive in the short term to a management change, whereas labile carbon fractions are considered more sensitive (Haynes, 2005b).

Because of their sensitivity, their key role in soil processes, their underrepresentation in soil quality assessment schemes and the rapid development of measurement methods, this thesis focuses on biological and biochemical parameters. Approximately a hundred biological soil quality indicators have been found in the literature (Bispo et al., 2009). In Table 2.4 of Chapter 2 (Bünemann et al., 2018), the predominant soil biological indicators are reported along with measurement methods, links to soil functions and pros and cons. Here, the indicators are characterised at individual, population, community, and at ecosystem level (Visser and Parkinson, 1992). At individual, population and community level, the presence, abundance, diversity or community structure of specific organisms/ groups of organisms that govern processes are measured (Visser and Parkinson, 1992). At ecosystem level the processes performed by organisms or functional characteristics that contribute to the processes (e.g. functional genes) are measured. Among these indicators, some are more directly linked to soil processes than others, in particular

indicators at ecosystem level, for example soil respiration, nitrogen mineralization and enzymatic activities. Despite the enormous amount of literature and studies on soil quality assessment, there is not yet a consensus on (how to arrive at) the best combination of indicators that can efficiently assess how land use and management, together with soil type and climatic conditions influence soil quality. In this respect, I argue that the best universal combination of soil quality indicators does not exist, but that the most effective combination depends on the soil threats, functions or ecosystem services that are relevant for a specific system.

1.3.2 Novel soil quality indicators

Technological and knowledge advances in the field of soil biology, biochemistry and soil sensors, such as measures of total organic carbon quality, molecular methods and spectroscopy, offer the possibility to develop novel soil quality indicators (Bastida et al., 2008; van Elsas and Boersma, 2011; Black and Mele, 2015; Bouchez et al., 2016). Novel soil quality indicators can overcome limitations of traditionally used indicators, being faster to assess, more sensitive to management, and/or delivering more information about soil processes (Duraisamy et al., 2020). Ultimately, novel soil quality indicators can help scientists, farmers and other land managers to better discriminate management effects on soil, and to assess more precisely soil processes, also the ones that up to now have been difficult to assess. We reviewed novel soil quality indicators in Chapter 2 section 2.4.4 (Bünemann et al., 2018), and I refer to this section for a more detailed overview. Based on this literature review, I selected four indicators to be explored in the current thesis, as presented below.

1.3.2.1 Soil labile organic carbon

I already mentioned that soil organic carbon is established as one of, if not 'the', most relevant soil quality indicators. However, the use of total soil organic carbon as soil quality indicator presents some main drawbacks: i) it is difficult to detect changes in total organic carbon in response to short-term management; ii) being a large pool of functionally different compounds its functionality is not straightforward (Haynes, 2005b; Chenu et al., 2015). Recent organic matter is more associated with soil biological activity and, together with organic matter of intermediate age, contributes to physical soil characteristics, while materials with longer residence times contribute to a larger extent to soil physicochemical reactivity and chemical properties (Wander, 2004; Hoyle et al., 2011; Branco de Freitas Maia et al., 2013). Soil labile organic carbon pools inform about total organic carbon *quality* because they represent the carbon more available for organisms, sustaining the processes they govern. These pools change more rapidly than total organic carbon, and

can be linked with specific processes (Wander, 2004; Haynes, 2005b; Strosser, 2010), and might give additional information on the state of the soil when included in soil quality assessments.

1.3.2.2 Soil disease suppressiveness

Soil disease suppressiveness is defined as the capacity of soils to promote plant health by suppressing pathogens, also when these are present in the soil (Cook, 2014). Soil disease suppressiveness is generally distinguished into i) general disease suppressiveness, which is due to the collective capacity of the microbial community to control the pathogen, and ii) specific disease suppressiveness, which is due to the action of a specific antagonist of the pathogen (Schlatter et al., 2017a). In many cases the suppressiveness is the result of the two mechanisms combined. Soil disease suppressiveness is an important function for productivity, is sustained by complex biological interactions in the soil and can be affected by soil management (Hornby, 1983a). Despite its high priority, so far it has been difficult to find proper soil parameters which can indicate soil suppressiveness and assess its changes due to soil management. Among the soil parameters suggested to have a link with soil suppressiveness, is the quality of the organic matter, and in particular labile organic carbon (van Overbeek et al., 2012; Cao et al., 2016; Dignam et al., 2018). However, the mechanistic relationship between soil suppressiveness and labile carbon has not yet been elucidated.

1.3.2.3 Soil free-living nematode communities

Soil fauna are an essential part of the food web contributing directly and indirectly to various soil processes (Gardi et al., 2009). In particular, soil free-living nematodes have been presented as ideal soil quality indicators (Ritz et al., 2009b; Griffiths et al., 2016; Waeyenberge et al., 2019) because i) they are ubiquitous, ii) they are present at multiple levels in the food web, integrating information on the organisms they feed and are fed on, iii) they are sensitive to changes in the environment, iv) they can be characterised in functional groups (trophic and life-strategy groups). Information about functional groups can be aggregated and used to calculate indices (e.g. Maturity index) which inform about food web structure and nutrient flows (Ferris et al., 2001). The study of nematode communities can, therefore, provide information on a taxonomic level about richness and diversity, and on a more functional level about the entire food-web. Up to now, nematode communities and nematode-based soil quality indices have mainly been assessed with traditional microscopic methods. Novel molecular methods offer the possibility to assess nematode communities more rapidly more in depth and cheaply (Geisen et al., 2018). However, it is unclear whether the well-established methods for the calculation of

nematode-based soil quality indices based on microscopic data can also be used when using molecular data.

1.3.2.4 Soil microbial catabolic profiles

Microorganisms are very abundant and diverse in the soil, are sensitive to disturbances and they are performing many of the soil processes relevant for soil quality. In particular, they have been found to have a primary role in the resistance of multiple processes to global changes (Delgado-Baquerizo et al., 2017a). When the focus of soil quality is on processes, and not on soil biodiversity *per se*, functional characterisation of the microbial community can be more relevant for soil quality assessment than black box (e.g. microbial biomass, basal soil respiration) or taxonomic approaches (e.g. community structure characterisation) (Krause et al., 2014; Song et al., 2014; Wood et al., 2015b; Bastida et al., 2016). Studying the microbial community level physiological profiling (CLPP), also called microbial catabolic profiling, is one of the most promising methods to measure soil microbial functionality in soil quality assessments, approaching *in situ* conditions. In addition, this method can also give information about microbial functional diversity. However, there is the need to investigate the suitability of CLPP as soil quality indicator for agricultural management.

1.3.2.5 Novel indicators, soil functions and soil-based ecosystem services

The novel soil quality indicators described above have been selected considering that soil quality assessment schemes should have a focus on functional assessment as in, for example, Lima et al. (2013), and that, preferably, indicators should be linked with multiple soil functions (Bone et al., 2014; **Figure 1.2**). I also aimed at taking into account the main different, but at the same time complementary, dimensions of soil biology: soil organic carbon, soil disease suppressiveness as an important soil ecosystem service which lacks appropriate indicators, soil fauna and soil microorganisms. With this selection, I also aimed to underline the importance of trophic interactions.

1.4 Research objectives

This research has been done in the context of the Horizon 2020 project iSQAPER (http://isqaper-project.eu/, interactive Soil Quality Assessment in Europe and China for Productivity and Environmental Resilience). The overall aim of iSQAPER is to assess soil quality to identify alternative agricultural practices that can be implemented by farmers to sustain agricultural production and, at the same time, environmental resilience. In this context, the main research objective of this thesis was developed: "Screening and evaluating a range of newly developed indicators of soil quality in long term trials"

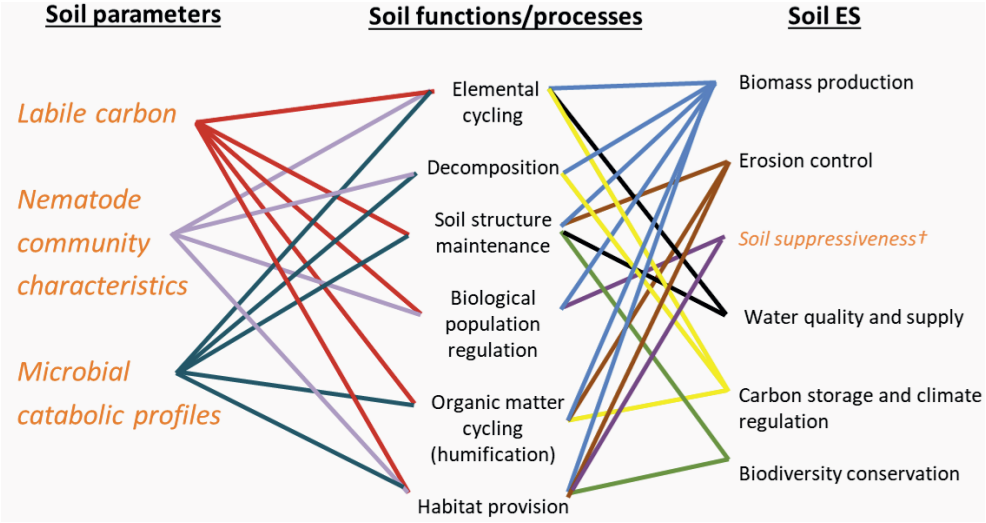


Figure 1.2. Schematic linkages between the novel indicators (soil parameters, in orange), soil functions/processes and ecosystem services (ES), modified from Bünemann et al. (2018) (Chapter 2). †originally 'pest and disease regulation'.

[quote from the iSQAPER proposal]. In addition, the focus of the thesis was required to be concentrated on biological indicators: "The focus will be, however, on enhancing biological soil quality assessment in the search for cost-effective indicators that respond more quickly and predictably to environmental and management stress as well as to soil remediation measures" [quote from the iSQAPER proposal].
In order to address the main objective, the following research objectives were developed:

I. Assess the sensitivity of the selected novel soil quality indicators to agricultural management, in particular to two common agricultural practices: tillage (conventional *vs.* reduced) and organic matter addition (low *vs.* high).
II. Assess the relationship between the novel indicators and traditional soil quality parameters which have been selected for the iSQAPER minimum data set (MDS) as indicators of soil functions, and elucidate the pros and the cons of the novel soil quality indicators.

After a thorough review of the literature about soil quality (Chapter 2; Bünemann et al., 2018), the selected soil quality indicators were:

- Soil organic carbon assessed as labile fractions (Chapter 3; Bongiorno et al., 2019b).
- Soil disease suppressiveness assessed with a bioassay (Chapter 4; Bongiorno et al., 2019c).

- Soil free-living nematode community assessed with molecular methods (Chapter 5; Bongiorno et al., 2019a).
- Potential soil microbial functionality assessed with community-level physiological profiling (CLPP) (Chapter 6; Bongiorno et al., submitted).

In Chapters 3 to 6, the objectives I and II are addressed for each of the indicators measured. The general hypothesis was that reduced tillage and high organic matter input will have a positive effect on the novel soil quality indicators compared to conventional tillage and low organic matter input. In addition, we hypothesised that the novel soil quality indicators will be positively correlated to the iSQAPER MDS parameters currently used as indicators for nutrient cycling, soil organic carbon sequestration, soil aggregation and habitat provision, and that the novel soil quality indicators will improve the ability to infer information about soil functionality as changed by agricultural practices in addition to, or substituting, the traditionally measured iSQAPER MDS parameters. In the general discussion of this thesis the results are synthesised and put in the perspective of future soil quality assessment (Chapter 7).

1.5 Thesis outline and experimental approach

A variety of experimental approaches, ranging from literature review, chemical analysis, molecular analysis to a greenhouse bioassay, have been used in order to address the research objectives outlined in the previous section. All the investigations have been performed in the same samples from 10 European long-term field experiments with two common conservation agriculture practices, viz. reduced tillage and organic matter addition, as main soil management measures. The long-term field experiments have been made available in the framework of the iSQAPER project with the purpose of studying the long-term effect of agricultural management on soil quality. Sampling was done in spring, before any agricultural management practices were performed to better allow the assessment of long-term soil management effects, avoiding the influence of short-term effects.

The various soil chemical, physical and biological parameters were measured in the same samples as indicators of soil functions (iSQAPER minimum data set – MDS) (for details see section 3.2.3 of Chapter 3, Bongiorno et al., 2019b; and Table 4,1 in Chapter 4, Bongiorno et al., 2019c).

In Chapter 2 (Bünemann et al., 2018), we reviewed soil quality concepts, their evolution over time, and soil quality indicators, including an overview on novel soil quality indicators, which was used as a base for the selection of the soil parameters studied in this thesis.

In Chapter 3, we measured five different soil labile carbon fractions: hydrophilic dissolved organic carbon (Hy-DOC), dissolved organic carbon (DOC), permanganate-oxidizable carbon (POXC), hot-water extractable organic carbon (HWEC), and particulate organic carbon (POMC), ordered here from the smallest to the largest proportion of the total organic carbon (Bongiorno et al., 2019b). We assessed their sensitivity to tillage and organic matter addition, and their relationship with the parameters measured in the iSQAPER MDS.

Based on previous evidence in the literature of the effect of soil management on the capacity of soils to suppress soil-borne plant pathogens, in Chapter 4 we assessed general soil disease suppressiveness. To measure soil disease suppressiveness we carried out a greenhouse bioassay with the model pathosystem *Pythium*-cress (Bongiorno et al., 2019c). Thereafter, we assessed the most important parameters in explaining soil disease suppressiveness by relating it with the labile carbon fractions assessed in Chapter 3 (Bongiorno et al., 2019b), and the other soil quality indicators measured in the iSQAPER MDS.

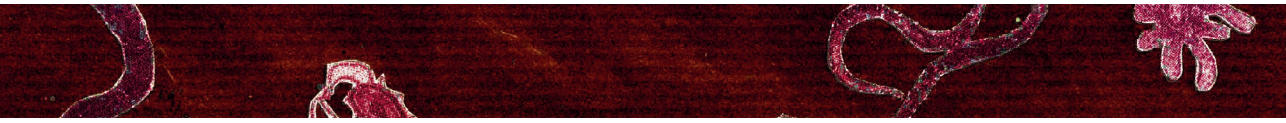
Chapter 5 deals with the assessment of the total abundance of soil free-living nematodes and their taxonomic community structure, with qPCR and amplicon sequencing, respectively (Bongiorno et al., 2019a). Nematode communities, and the food web indices calculated with the sequencing data, were tested for their sensitivity to tillage and organic matter addition and linked to labile carbon fractions, soil suppressiveness and the soil quality parameters measured in the iSQAPER MDS.

In Chapter 6, we investigated the effect of tillage and organic matter addition on the soil microbial catabolic profiles and functional diversity measured with MicroResp™, a community-level physiological profiling method (Bongiorno et al., submitted). In addition, as with the other experimental chapters, we linked the results of the microbial catabolic profiles with labile organic carbon fractions, soil suppressiveness, nematode communities and the parameters measured in the iSQAPER MDS.

In the final chapter, Chapter 7, I bring together the results from the previous chapters with a few additional statistical analyses, and I interpret them relative to one another but also to the broader body of literature on soil quality assessment. Moreover, I point to limitations of methods and indicators applied in my thesis and outline both possibilities and necessities for future research in the field of soil quality indicator development.


CHAPTER 2
2

Soil quality – A critical review



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Abstract

Sampling and analysis or visual examination of soil to assess its status and use potential is widely practiced from plot to national scales. However, the choice of relevant soil attributes and interpretation of measurements are not straightforward, because of the complexity and site- specificity of soils, legacy effects of previous land use, and trade-offs between ecosystem services. Here we review soil quality and related concepts, in terms of definition, assessment approaches, and indicator selection and interpretation. We identify the most frequently used soil quality indicators under agricultural land use. We find that explicit evaluation of soil quality with respect to specific soil threats, soil functions and ecosystem services has rarely been implemented, and few approaches provide clear interpretation schemes of measured indicator values. This limits their adoption by land managers as well as policy. We also consider novel indicators that address currently neglected though important soil properties and processes, and we list the crucial steps in the development of a soil quality assessment procedure that is scientifically sound and supports management and policy decisions that account for the multi-functionality of soil. This requires the involvement of the pertinent actors, stakeholders and end-users to a much larger degree than practiced to date.

2.1 Introduction

Soil quality is one of the three components of environmental quality, besides water and air quality (Andrews et al., 2002). Water and air quality are defined mainly by their degree of pollution that impacts directly on human and animal consumption and health, or on natural ecosystems (Carter et al., 1997; Davidson, 2000). In contrast, soil quality is not limited to the degree of soil pollution, but is commonly defined much more broadly as "the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Doran and Parkin, 1994a; Doran and Parkin, 1996). As Doran & Parkin (1994) state explicitly, animal health includes human health.

This definition reflects the complexity and site-specificity of the belowground part of terrestrial ecosystems as well as the many linkages between soil functions and soil-based ecosystem services. Indeed, soil quality is more complex than the quality of air and water, not only because soil constitutes solid, liquid and gaseous phases, but also because soils can be used for a larger variety of purposes (Nortcliff, 2002). This multi-functionality of soils is also addressed when soil quality is defined from an environmental perspective as "the capacity of the soil to promote the growth of plants, protect watersheds by regulating the infiltration and partitioning of precipitation, and prevent water and air pollution by buffering potential pollutants such as agricultural chemicals, organic wastes, and industrial chemicals" (National Research Council, 1993, as cited in Sims et al. (1997)). Soil quality can be assessed both for agro-ecosystems where the main, though not exclusive ecosystem service is productivity, and for natural ecosystems where major aims are maintenance of environmental quality and biodiversity conservation. Given the scope and readership of this journal, the "non-ecological functions" of soil *sensu* Blum (2005), such as the physical basis of human activities, source of raw materials, and geogenic and cultural heritage, are beyond the scope of this review.

Extrinsic factors such as parent material, climate, topography and hydrology may influence potential values of soil properties to such a degree (**Figure 2.1**) that it is impossible to establish universal target values, at least not in absolute terms.

Soil quality assessment thus needs to include baseline or reference values in order to enable identification of management effects. Soils often react slowly to changes in land use and management, and for that reason it can be more difficult to detect changes in soil quality before non-reversible damage has occurred than for the quality of water and air (Nortcliff, 2002). Therefore, an important component of soil quality assessment is the identification of a set of sensitive soil attributes that reflect the capacity of a soil to function and can be used as indicators of soil quality. Because management usually has only limited short-term effects on inherent properties such as texture and mineralogy, other indicators,

including biological ones, are needed. The distinction between inherent (static) and manageable (dynamic) attributes, however, is not absolute and also context-dependent (Schwilch et al., 2016). For example, stoniness as an inherent property is nevertheless manageable, e.g. by removal of stones from an area to facilitate tillage and to build separating walls between fields, or by addition of gravel and stones to improve friability, to accelerate soil warming in spring or decrease evaporation. Soil management by humans has even given rise to separate classes in the soil taxonomic system, such as Plaggic anthrosols, the plaggen soils of northwestern Europe (e.g., Blume and Leinweber (2004)), and Terric anthrosols, the Amazonian Dark Earths, also known as Terra Preta de Índio (Glaser and Birk, 2012).

The history of the concept of soil quality shows that it is rooted in two different approaches that either put more emphasis on the inherent soil properties or on the effects of human management. The oldest mention in the scientific literature is by Mausel (1971) who defined soil quality as "the ability of soils to yield corn, soybeans and wheat under conditions of high-level management. The choice of these crops to reflect soil quality in Illinois is due to their overwhelming agricultural economic dominance." This definition emphasises agricultural production and is linked to land evaluation (see below). A similar description was provided by SSSA (1987; cited in Doran & Parkin, 1994) as the "inherent attributes of soils that are inferred from soil characteristics or indirect observations". This definition is comparable to the more recent term soil capability, defined as the intrinsic capacity of a soil to contribute to ecosystem services, including biomass production (Bouma et al., 2017). The emphasis on inherent, more static soil properties was closely connected to soil taxonomy. It also took management for granted ("under conditions of high-level management"), without specifying those conditions. Larson & Pierce (1991) expressed uneasiness with the focus on agricultural productivity and proposed to disconnect soil quality from productivity. Doran & Parkin (1994) observed that definitions of soil quality included the capacity of soils to function sustainably, but likewise considered the focus on production to be too restrictive. They wanted a definition of soil quality to stress the main issues of concern regarding soil use. Besides productivity, they therefore included the ability of soils to contribute to environmental quality and to promote plant, animal and human health in their definition as cited above.

The concept of soil quality by Doran and Parkin (1994a) was heavily criticized in a series of papers (Sojka and Upchurch, 1999; Letey et al., 2003; Sojka et al., 2003). That criticism contained various elements. First, these authors claimed that the concept of soil quality could transform soil science from a value-neutral science into a value system and even referred to soil quality as promoting ideas of a politically correct soil. Second, they expressed discontent with the idea of a universal soil quality index, to which they

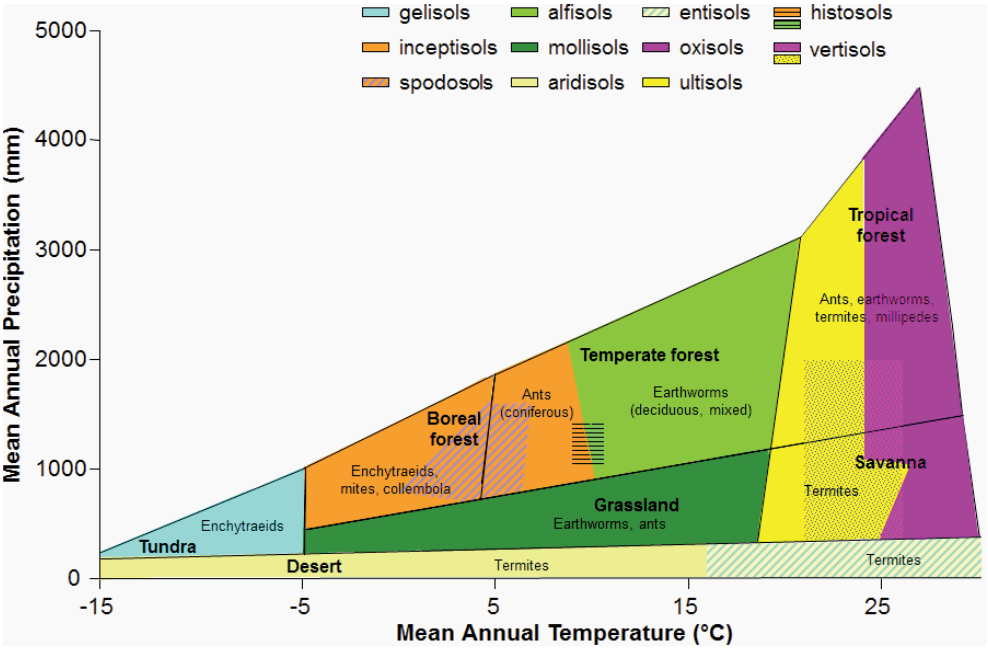

Figure 2.1. Abiotic and biotic factors constituting soil quality in the soils of the world (modified from Brussaard et al, 2012). Reproduced with permission from Oxford University Press (www.oup.com).

referred as institutionalizing soil quality. Third, they criticized the concept because of its bias towards certain soil types as a consequence of the focus on intrinsic properties. And finally, they criticized the definition because in its original form it puts too much emphasis and value on a limited number of annual crops that provide cheap food and that are heavily subsidized. Their proposal to replace the term soil quality management by the term quality soil management did not find support, but their criticisms did influence the further development of an operational concept of soil quality, in which management has become the central issue: agricultural productivity does not hold a privileged position any longer, trade-offs are explicitly recognized at the expense of a universally applicable index, and the role of soil scientists in relation to societal stakeholders who manage soils (farmers, owners of land for nature conservation, policy makers, etc.) has changed. A particular recommendation of Sojka and co-authors was to speak of soil use rather than soil functions, so that the responsibility to maintain the quality of the soil can be clearly assigned to the user of the soil. Soil quality assessment then provides the scientific tools for evaluation of the management of soil resources, considering also the societal demands of the various benefits that soils, if managed well, can provide to humankind.

The valuation of soil quality hence becomes connected to the valuation of the ecosystem services provided by soils. A further benefit of such a soil quality concept is that it raises awareness and enhances communication between stakeholders regarding the importance of soil resources (Karlen et al., 2001). Recently, there has been renewed interest in this educational aspect, either by focusing more on visual soil assessment (Ball et al., 2013) or by proposing interactive soil quality assessment tools, such as LandPKS (https://www.landpotential.org/) and the app currently being developed in the EU Horizon-2020 project 'Interactive Soil Quality Assessment in Europe and China for Agricultural Productivity and Environmental Resilience (iSQAPER - http://www.isqaper-project.eu/).

In this paper, we aim to critically review soil quality publications and assessment tools, especially with respect to soil quality indicators, in terms of commonalities, meaningful differences and omissions. To this end, the relevant definitions and terminologies are introduced in section 2, followed by an overview of approaches to soil quality assessment in section 3. The focus of this review is on analytical measurements. The most important approaches using visual soil evaluation in the field are only briefly presented, since visual soil assessments have been reviewed recently (Emmet-Booth et al., 2016). In section 4, the choice of soil quality indicators is discussed in-depth with respect to requirements of indicators and methods to select a minimum dataset. A compilation of the most frequently proposed indicators is followed by paragraphs on novel soil quality indicators with potential added value and on the interpretation of indicator values, including the potential aggregation into an operational soil quality index and its disadvantages. In the conclusions (section 5), we propose the crucial steps to be taken for successful soil quality assessment and analyze to what extent these have been implemented so far. Finally, fostering soil quality is considered in the wider context of enhancing environmental quality, embedded in an interactive process of co-creation of knowledge by scientists and other actors in urgent transitions towards sustainable use and management of natural resources (section 6).

2.2 Concepts related to soil assessment

2.2.1 Soil fertility, land quality, soil capability, soil quality and soil health

Various forms of soil assessment are encapsulated in different concepts. Apart from mining minerals, the main interest in soil has traditionally been in its potential for agricultural production. Assessments of the suitability of soil for crop growth may have been made even before the evidence of written records. Documentation can be found in ancient Chinese books such as "Yugong" and "Zhouli", written during the Xia (2070-1600 BC) and Zhou (1048-256 BC) dynasty, respectively (Harrison et al., 2010), and in the work

of Roman authors such as Columella (Warkentin, 1995). Ethnopedology also provides several examples of indigenous soil classifications that focus on indicators that allow judgement of the suitability of particular soils for various crops (e.g., Barrera-Bassols and Zinck, 2003). The suitability of soil for agricultural production is captured in the concept of *soil fertility*, originating from the German literature on "Bodenfruchtbarkeit" that is predominantly aligned to crop yields (Patzel et al., 2000). Accordingly, the FAO describes soil fertility as "the ability of the soil to supply essential plant nutrients and soil water in adequate amounts and proportions for plant growth and reproduction in the absence of toxic substances which may inhibit plant growth" (www.fao.org). Mäder et al. (2002) extend that scope in proposing that a fertile soil "provides essential nutrients for crop plant growth, supports a diverse and active biotic community, exhibits a typical soil structure and allows for an undisturbed decomposition". Nevertheless, the concept of soil fertility is generally operationalized chemically and partly physically in terms of the provision to crops of nutrients and water only.

To address physical and/or biological characteristics of soil, other concepts are more commonly used. One of the earliest is *land quality*, which integrates characteristics of soil, water, climate, topography and vegetation (Carter et al., 1997; Dumanski and Pieri, 2000) in the context of land evaluation, which aims to assess the use potential of land, based on its attributes (Rossiter, 1996). An early comprehensive elaboration of the concept is the FAO Framework for Land Evaluation (FAO, 1976). Soil survey is part of land quality assessment for land evaluation. It is done once or only repeated over large time intervals, relying heavily on field observations, supplemented with very few measured parameters (Huber et al., 2001). Land evaluation anticipates decisions on the optimal allocation of land for various uses and is, hence, the first step to sustainable land management. In countries with low population densities, the main purpose of land evaluation in the past was to identify fertile land for agricultural production, whereas in more densely populated regions such as Europe it was more targeted at identifying deficient factors in agriculture that could be remedied, in particular by manuring (van Diepen et al., 1991). However, land evaluation has also been used as part of a strategy to assess broader land use options (van Latesteijn, 1995). Similarly, *soil capability*, i.e. the intrinsic capacity of a soil to contribute to ecosystem services (Bouma et al., 2017), provides a neutral assessment of what soils can do and how their potential can be reached.

Since Mausel (1971) introduced the term *soil quality*, it has sometimes been used in the context of land quality and land evaluation (e.g. Eswaran et al., 1997). Whereas land quality and land evaluation primarily address the inherent soil properties that do not change easily and are often assessed for the entire profile, soil quality is more focused on the dynamic soil properties that can be strongly influenced by management and are

mainly monitored in the surface horizon (0-25 cm) of the soil (Karlen et al., 2003). However, when studying direct impacts of soil quality on water quality it is imperative that inherent soil properties in deeper parts of the soil profile are included in the assessment.

Typically, the concept of soil quality is considered to transcend the productivity of soils (Larson and Pierce, 1991; Parr et al., 1992) to explicitly include the interactions between humans and soil, and to encompass ecosystem sustainability as the basis for the benefits that humans derive from soils as well as the intrinsic values of soil as being irreplaceable and unique (Carter et al., 1997). The term soil quality in this broader sense was already used by Warkentin and Fletcher (1977). Recently, soil quality assessment is increasingly incorporated in land evaluation, as land evaluation procedures are now used in many different ways and for a range of purposes, including sustainable land management (Hurni et al., 2015), environmental risk assessments, monitoring of environmental change (Sonneveld et al., 2010) and land restoration (Schwilch et al., 2012). In the land-potential knowledge system LandPKS, general management options are based on long-term land potential (depending on climate, topography and inherent soil properties) and can be modified according to weather conditions and dynamic soil properties (Herrick et al., 2016). The integration of soil quality and land evaluation goes as far as developing soil natural capital accounting systems, stressing the importance of soils for human wellbeing (Robinson et al., 2017a).

In a program to assess and monitor soil quality in Canada (Acton and Gregorich, 1995), the term soil quality was used interchangeably with *soil health* and, in spite of the wider context in which it was presented, defined primarily from an agricultural perspective as "the soil's fitness to support crop growth without becoming degraded or otherwise harming the environment". The term soil health originates from the observation that soil quality influences the health of animals and humans via the quality of crops (e.g. Warkentin, 1995). Indeed, linkages to plant health are common, as in the case of disease-suppressive soils (Almario et al., 2014). Soil health has also been illustrated via the analogy to the health of an organism or a community (Larson and Pierce, 1991; Doran and Parkin, 1994a).

The debate about soil quality vs. soil health arose quickly after the concept of soil quality was criticized in the 1990s. In contrast to soil quality, soil health would "capture the ecological attributes of the soil which have implications beyond its quality or capacity to produce a particular crop. These attributes are chiefly those associated with the soil biota; its biodiversity, its food web structure, its activity and the range of functions it performs" (Pankhurst et al., 1997b). These authors further consider "that the term soil health encompasses the living and dynamic nature of soil, and that this differentiates it from soil quality". They therefore "adopt the view that although the concepts of soil quality and

soil health overlap to a major degree and that in many instances the two terms are used synonymously (....), soil quality focuses more on the soil's capacity to meet defined human needs such as the growth of a particular crop, whilst soil health focuses more on the soil's continued capacity to sustain plant growth and maintain its functions". Meanwhile, the debate subsided and partly changed focus. For example, Moebius-Clune et al. (2016) consider that soil quality includes both inherent and dynamic soil properties, and that soil health is equivalent to dynamic soil quality. The differential usage may also link to the observation of Romig et al. (1996), that, whereas soil quality is the preferred term of researchers, soil health is often preferred by farmers.

The differences between land quality and soil quality observed by Karlen et al. (2003) and between soil quality and soil health observed by Pankhurst et al. (1997) and Moebius-Clune et al. (2016) can be summarized in a transition in focus from land quality to soil quality and soil health going from inherent to dynamic soil properties. The website of the Natural Resources Conservation Service, USA (http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/) states that "soil health, also referred to as soil quality, is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans". We conclude that the distinction between soil quality and soil health developed from a matter of principle to a matter of preference and we therefore consider the terms equivalent. We further express this by explicitly including the soil biota/biodiversity and related soil functions and soil-based ecosystem services in figures 1-3.

Like in land quality assessment and land evaluation, approaches to soil quality and soil health go beyond the reductionist approach of measuring (indicators of) soil properties and processes. Although such measurements remain important from a practical perspective (Kibblewhite et al., 2008c), the concepts of soil quality and soil health also include the capacity for emergent system properties such as the self-organization of soils, e.g. feedbacks between soil organisms and soil structure (Lavelle et al., 2006), and the adaptability to changing conditions.

2.2.2. Linking soil quality to soil functions and ecosystem services

Ecosystem services are defined as "the benefits which humans derive from ecosystems" (Costanza et al., 1997b). With the early concept developed by Doran and Safely (1997), soil quality was addressing not only one ecosystem service such as provision of food, but also trying to represent and balance the multi-functionality of soil. This has recently been further embedded in the development of "functional land management", which assesses both the benefits and trade-offs of a multifunctional system for managing soil-based ecosystem services in agriculture (Schulte et al., 2014a) and a wider range of land uses (Coyle et al., 2016).

Among scientists, the concept of ecosystem services is often used in connection with the concept of soil functions. 'Function' is, however, variably used as a synonym for 1) process, 2) function*ing*, 3) role and 4) service (Glenk et al., 2012; Baveye et al., 2016b). Therefore, Schwilch et al. (2016) advise against using the term, but Baveye et al. (2016b) note that function "in a narrow and well-defined context (...) has been used in connection with soils for over 50 years, and has served as a conceptual foundation for an appreciable body of research and significant policy making, at least in Europe" (e.g., the Soil Thematic Strategy of the European Commission, 2006). Therefore, we concur with Baveye et al. (2016b) that "it makes sense to try to retain both "function" and "service" terminologies, as long as they can be articulated (...) with respect to soil properties and processes". In their seminal paper reconstructing how the notion that nature meets, or gets in the way, of the needs of people has pervaded concepts and theory in ecology vs. soil science, Baveye et al. (2016b) argue that mainstream ecology, by its emphasis on organisms, tended to neglect the soil, in particular the non-living soil, whereas mainstream soil science tended to avoid the term ecosystem, emphasizing the importance of soil properties and processes in landscape terms. In accordance with Glenk et al. (2012), we define soil functions as (bundles of) soil processes that underpin the delivery of ecosystem services. This definition will suffice for all practical purposes related to manageable soil functions, which can be used to address the gap between "what is" and "what can be", based on soil capability , i.e. "what soils can do" (Bouma et al., 2017), which is, in the context of this review, what living soils can do. Complementary to this bottom-up approach, soil functions can be used in a top-down approach when identifying the gap between what is currently measured in soil assessment schemes and what should be measured in view of assessing the soil functions that are impacted by, or to be managed in view of current and upcoming policies (van Leeuwen et al., 2017), possibly through the use of environmental accounting systems increasingly adopted by policymakers, such as the soil natural capital accounting system proposed by Robinson et al. (2017a).

Just as ecosystem services are influenced by (bundles of) soil processes, the latter are in turn affected by soil threats. The EU Soil Thematic Strategy identified the main threats to soil quality in Europe as soil erosion, organic matter decline, contamination, sealing, compaction, soil biodiversity loss, salinization, flooding and landslides (European Commission, 2002; Montanarella, 2002). Soil threats have been emphasized in order to inform risk assessment exercises indicating (geographical) areas where soil functioning is potentially hampered (van Beek et al., 2010). Different schemes linking soil-based ecosystem services and soil functions have been developed (Kibblewhite et al., 2008c; Haygarth and Ritz, 2009; Tóth et al., 2013), but none of them includes soil threats. The scheme presented by Kibblewhite et al. (2008c) and modified by Brussaard (2012b) was

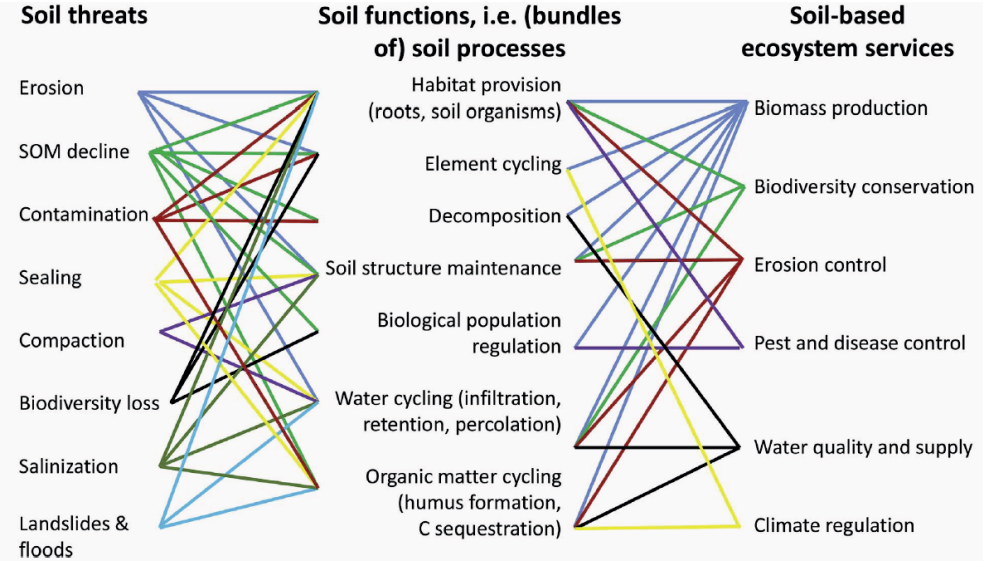

Figure 2.2. Linkages between soil threats, soil functions and soil-based ecosystem services. Further developed from the scheme presented by Kibblewhite et al. (2008b) and modified by Brussaard (2012a)

developed as a conceptual basis for the iSQAPER project, including soil threats as affecting the various soil functions and associated ecosystem services (**Figure 2.2**).

The soil functions in **figure 2.2** equate almost entirely to the "intermediate services" defined by Bennett et al. (2010b), which are similar to the soil processes presented by Schwilch et al. (2016). The ecosystem services in this scheme can be seen as a soil-related sub-set of the ecosystem services mentioned in the Common International Classification of Ecosystem Services (CICES - http://biodiversity.europa.eu/maes/common-international-classification-of-ecosystem-services-cices-classification-version-4.3), currently elaborated in the **Mapping and Assessment of Soil Ecosystems and their Services (MAES-Soil) Pilot project (**https://webgate.ec.europa.eu/fpfis/wikis/display/MAESSoil/MAES+Soil+Pilot).

It has been argued that soil quality can indeed only be assessed in relation to one or several soil functions, ecosystem services or soil threats (Sojka and Upchurch, 1999; Volchko et al., 2013; Bouma, 2014; e.g. Baveye et al., 2016b). Therefore, clear definitions of these terms as well as firmly established associations with soil quality indicators are the basis of any functional soil quality concept.

As soil quality plays a role in decision-making in the face of soil threats, the DPSIR (driver–pressure–state–impact–response) framework (EuropeanEnvironmentAgency, 1998) has frequently been adopted for use in EU policy to support decision-making and as a means to bridge the science-policy gap (Tscherning et al., 2012). Applying the DPSIR framework to soil (**Figure 2.3**), "drivers" are pedoclimatic conditions and land use policies,

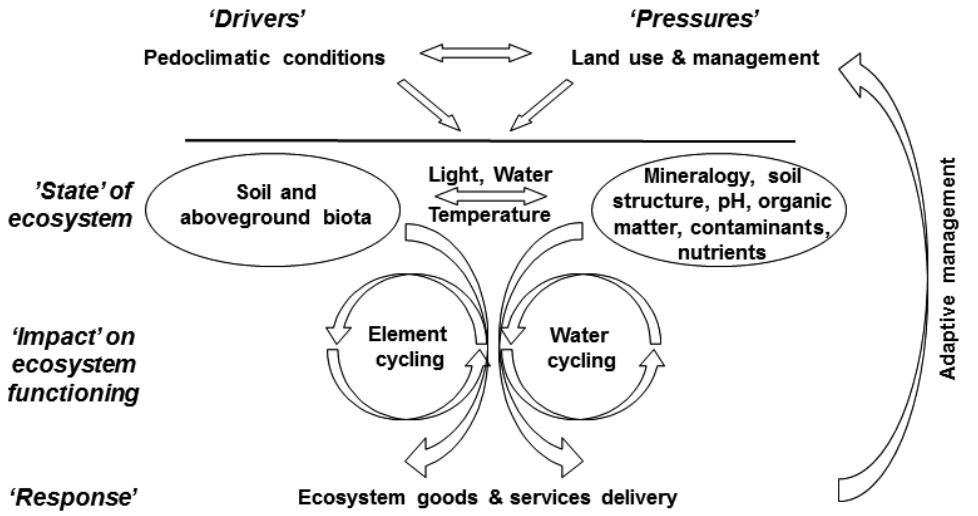

Figure 2.3. The Driver-Pressure-State-Impact-Response framework applied to soil. Modified from Brussaard et al. (2007). Permission for reproduction granted by Elsevier.

while "pressures" are land use and management and the associated soil threats. Pressures and drivers and their variabilities and interactions determine the "state" of the soil, with subsequent "impact" on soil and ecosystem functioning, and the "response" in terms of the delivery of ecosystem goods and services. Subsequent adaptive management may be re-active to observed deterioration of soil functioning or pro-active to reach transitions to newly desired soil functioning. To assess any changes in the status of soil quality, assessment tools are needed, and these are the subject of sections 3 and 4.

2.3 Approaches to soil quality assessment

A plethora of soil quality assessment and monitoring tools have become available since the 1990s. Here, we give an overview of the main developments in different countries, before addressing aspects of soil quality indicators in more depth in section 4.

2.3.1 Analytical approaches to soil quality

National assessments of soil quality are often based primarily on analytical approaches (Table 2.1). One of the earliest national programs to assess and monitor soil quality was started in Canada in 1988 (Acton and Gregorich, 1995), using benchmark sites to assess changes in soil quality over time, especially in relation to the soil threats erosion, compaction, organic matter loss, acidification and salinization (Wang et al., 1997). While the Canadian soil quality monitoring program as such was not consistently continued, the data are still partly used in the assessment of agri-environmental indicators that cover

soil, water and air quality (Clearwater et al., 2016). At a coarser scale, a GIS-based approach to characterize primarily inherent soil quality was presented by Macdonald et al. (1998).

Two major soil quality assessment approaches focusing at the plot scale were developed in the USA (**Table 2.1**).

The Soil Management Assessment Framework (SMAF) developed at the Soil Quality Institute (Andrews and Carroll, 2001; Karlen et al., 2001; Andrews et al., 2004; Wienhold et al., 2004; Wienhold et al., 2009) is rather unique in its flexibility in the selection of indicators. Based on a clear definition of the main ecosystem service(s) or management objective(s) to be addressed, a set of indicators is selected out of 81 potential indicators using selection rules. The user can disregard or alter the proposed minimum dataset as desired, although that limits comparability between sites. The interpretation of an indicator value is based on scoring curves and an additive soil quality index can be derived. The Cornell Soil Health Test (Idowu et al., 2008; Moebius-Clune et al., 2016) is much more standardized and targeted directly at land users, offering various soil health testing packages for farmers, landscape managers and others, and supplying them with management advice together with the results..

In New Zealand, a nationwide survey of seven soil quality indicators at 511 sites aimed at establishing benchmark values across all major soil types and land-uses (Lilburne et al., 2002; Sparling and Schipper, 2002; Lilburne et al., 2004; Sparling and Schipper, 2004). Based on these data, an online tool called Sindi (soil indicator assessment) was developed (Lilburne et al., 2002) that allows the comparison of measurements of soil properties in a given soil type with the information in the database.

In Australia, a consortium of public and private partners provides fact sheets and regional, soil type-specific critical threshold values of a range of soil quality indicators for impact on agricultural production, supplemented by land use-specific distributions of measured indicator values (Gonzalez-Quiñones et al., 2015). Hence, individual farmers can compare their own data for every indicator with the range of values known for similar circumstances in the region. Supplementary general information is also provided that can be used to modify management for environmental goals such as carbon sequestration and minimizing nutrient losses to the environment.

In Europe, many national approaches to soil quality assessment were developed. Those focusing on soil biodiversity rather than on general soil quality were reviewed by Pulleman et al. (2012). The French "soil quality observatory" was started in 1986 and included 11 sites (Martin et al., 1998). The more recent soil quality monitoring system (RMQS) program is based on a 16 x 16 km grid of the French territory and feeds into the French Information System on soils (Arrouays et al., 2003; Antoni et al., 2007). In the UK, the first approach to soil quality monitoring (Loveland and Thompson, 2002) had a focus

Table 2.1. Major soil quality assessment approaches based on analytical indicators according to geographic origin (North America, Europe, China): Objectives, target group, scale, interpretation approach, website

Country	Reference(s)	Name (if any)	Objectives	Target group (assumed)	Spatial scale	Interpretation	Website (if any)
Canada	(Acton and Gregorich, 1995; Wang et al., 1997)	National soil quality monitoring program	Assess status of and trends in soil health	Not stated (policy)	23 benchmark sites (5-10 ha each) across Canada	Mainly trend analysis	
	(Macdonald et al., 1998)		Assess inherent soil quality and susceptibility to change	Not stated (policy)	Regional and national	Rating procedures with respect to 4 soil functions	
USA	(Karlen et al., 2001; Andrews et al., 2004; Wienhold et al., 2004; Wienhold et al., 2009)	Soil management assessment framework (SMAF)	Evaluate management practices, educate about soil quality	Land managers, advisors, general public	Plot scale	Scoring curves, additive index	http://www.soilquality.org
	(Idowu et al., 2008; Moebius-Clune et al., 2016)	Cornell Soil Health Test	Assess soil health, address soil degradation, increase productivity	Farmers	Plot scale	Scoring curves, overall score	http://soilhealth.cals.cornell.edu
Australia	(Gonzalez-Quiñones et al., 2015)	Soil Quality Website	Benchmark sites, soil quality monitoring and education	Farmers	National and regional	Target values; threshold values wherever possible	http://soilquality.org.au/
New Zealand	(Schipper and Sparling, 2000; Sparling and Schipper, 2002; Lilburne et al., 2004; Sparling et al., 2004)	"500 soils project", soil indicator assessment (Sindi)	Assess soil quality for environmental reporting	Government; Sindi: regional council staff, landowners	511 sites across New Zealand, x soil types, 10 land uses	Comparative (compared to database) or according to target ranges	https://sindi.landcareresearch.co.nz
France	(Martin et al., 1998; Arrouays et al., 2002; Arrouays et al., 2003; Antoni et al., 2007)	Observatoire de la Qualité des Sols (OQS), Réseau des mesures de la qualité des sols (RMQS)	Assess soil quality for environmental protection, food security and sustainable management practices	Not stated (policy)	11 sites (OQS) 2000 sites (RMQS)	Mainly trend analysis	

Continue

UK	(Loveland and Thompson, 2002; Merrington, 2006)		Assess soil function of environmental interaction	Policy	National	Trigger values	
Ireland	(Bondi et al., 2017)	Soil quality assessment research project (SQARE)	Assessment of soil functions	Farmers	Plot (38 farms)		https://www.teagasc.ie/environment/soil/research/square/
The Netherlands	(Wattel-Koekkoek et al., 2012)	National Soil Quality Monitoring Network	Assess soil quality and land-use effects	Not stated (policy)	200 locations	Target values	
EU	(Huber et al., 2001)	European Soil Monitoring and Assessment framework	Provide objective, reliable and comparable information at European level	Policy			
	(Huber et al., 2008; Kibblewhite et al., 2008a; Stolte et al., 2016)	ENVASSO, RECARE	Assess soil degradation	Policy			http://esdac.jrc.ec.europa.eu/projects/envasso http://www.reca-re-project.eu/

on forestry and semi-natural soils. After further elaboration, a minimum dataset of only seven measurements was proposed (Merrington, 2006). In addition, Countryside Survey has been monitoring a few soil properties such as pH, soil organic carbon and some aspects of soil biodiversity (Black et al., 2003) since 1978 (http://www.countrysidesurvey.org.uk). In Ireland, recent work on the assessment of soil functions at grassland farms combines a full soil profile description and visual soil assessment with determination of a suite of analytical indicators (Bondi et al., 2017). In The Netherlands, a set of indicators for soil ecosystem services developed by RIVM (National Institute for Public Health and the Environment) was used in two five-year measurement cycles in 200 sites of the Dutch soil quality monitoring network (Wattel-Koekkoek et al., 2012). Target values and ranges for agronomic land use are based on median values of the monitoring network and on judgement of a group of soil experts. Also in the Netherlands, a large Public Private Partnership 'Sustainable Soil' is developing a soil quality assessment system in which a set of soil chemical, physical and biological indicators is related to target values and ranges for integral advice on soil management (www.beterbodembeheer.nl).

Given the plethora of soil monitoring programs in Europe, a common European soil monitoring framework was proposed (Huber et al., 2001), which was based as much as possible on existing monitoring activities. Subsequently, the EU-FP6 project ENVASSO (ENVironmental ASsessment of Soil for mOnitoring) aimed at defining and documenting a soil monitoring system for implementation in support of a European Soil Framework Directive (Kibblewhite et al., 2008a), focused on the assessment of soil threats, which however never materialized. Nevertheless, three priority indicators for each soil threat (Huber et al., 2008) were identified, and this list was further revised and amended by the EU-FP7 project RECARE (Preventing and Remediating Degradation of Soils in Europe through Land Care) as shown in **supplementary table 1**.

The history of soil quality assessment in China was reviewed for an international readership by Teng et al. (2014). Due to increasing pressure to maintain and improve soil quality in China, the Chinese government in 2008 established the China Soil Quality Standardisation & Technology Committee (SAC/TC 404) that has been responsible for formulating and modifying soil quality standards in China, including terminology, indicators, criteria, soil sampling methods, analytical methods, standards for soil quality assessment, and remediation of contaminated soils (Chen et al., 2011). By 2010, 141 soil quality-related standards had been set up, partly adopted from ISO.

The flexible and context-specific approach to soil quality assessment of the SMAF as described above has inspired several recent studies that apply multivariate statistical methods to select the most relevant indicators, often based on assumed but not assessed connections between indicators and soil functions, and utilize scoring functions to arrive

at a soil quality index geared to the specific conditions (Velasquez et al., 2007; Armenise et al., 2013; Lima et al., 2013; Swanepoel et al., 2014; Tesfahunegn, 2014; Askari and Holden, 2015; Congreves et al., 2015; de Paul Obade and Lal, 2016). The drawback of such flexible approaches lies in the limited comparability between studies, even more than between different applications of the SMAF.

The compilation of major soil quality assessment approaches in **Table 2.1** shows the variation in objectives, target groups (though often not explicitly stated) and spatial scales. Most of these approaches remain at the plot/field/site scale. Recently developed sensor-based approaches show promise to expand soil quality assessment to the landscape level (e.g. Vågen et al., 2013). Importantly, explicit evaluation of soil quality with respect to specific soil threats, functions and ecosystem services has rarely been implemented, and few approaches provide clear interpretation schemes of measured indicator values. This limits their adoption by land managers as well as policy.

2.3.2 Visual assessment approaches to soil quality

The above approaches to soil quality assessment typically require analytical laboratory facilities. Approaches targeting farmers and stressing the educational aspect benefit from more empirical, qualitative indicators that can be easily assessed in the field, deliver immediate results, and facilitate communication between farmers and scientists (Beare et al., 1997).

In the Wisconsin Soil Health Program, for example, a soil health score card was developed that collects farmers' observations on soil and plants, and includes a few questions on animal health and water quality (Romig et al., 1996). In Europe, the GROW Observatory (http://growobservatory.org/) was established in 2016, which is developing simple tools to support soil management for farmers and soil stakeholders, such as simple field-based assessments and educational tools. Visual soil assessment (VSA) approaches have been developed in different parts of the world (**Table 2.2**). Most of these methods target mainly soil structure, sometimes in relation to productivity (Mueller et al., 2013; Abdollahi et al., 2015). The methods vary in material and time requirements, with spade methods being generally faster to perform than profile methods and thus being more suitable for farmers (Boizard et al., 2005). The method developed by Peerlkamp (1959), which was used in the Netherlands for 40 years, has recently been improved by simplification of the scoring scheme and inclusion of a visual key (Ball et al., 2007; Guimaraes et al., 2011) to further support the use of the method by non-experts of soil science. Straightforward interpretation is certainly an asset of visual soil quality assessment, but visual soil assessment alone cannot evaluate the status of ecosystem services driven by biological and chemical soil processes (Ball et al., 2017). Because visual soil assessment

Table 2.2. Comparison of major visual soil assessment methods (X signifies required material or performed observations)

Country	Australia	France	Australia	UK	New Zealand	Brazil/UK	Germany
Reference	**McKenzie (2001)**	**Roger-Estrade et al. (2004)**	**McGarry (2006)**	**Ball et al. (2007)**	**Shepherd et al. (2008)**	**Guimaraes et al. (2011)**	**Mueller et al. (2014)**
Stated objectives (assessment of …)	soil structure, suitability for root growth	soil structure	land degradation	soil structure	soil quality	soil structure	soil properties with respect to yield potential
Method name	SOILpak	Profil cultural	VS-Fast	Peerlkamp	VSA	VESS[1]	M-SQR[2]
Principle	spade	trench	spade	spade	spade	spade	pit
Material							
spade	X	X	X	X	X	X	X
plastic basin					X		
hard square board	X				X		
plastic bag or sheet				X	X	X	
knife	X			X	X	X	X
auger							X
water bottle					X		
tape measure or ruler			X	X	X	X	X
Time needed (min)	25-90	60-180	?	5-15	25	5-15	10-40
General observations							
soil layers, A-horizon			X				X
surface crusting or cover			X		X		
surface ponding					X		X
slope							X
soil erosion					X		

Continue

Soil physical properties							
soil texture			X		X		X
soil structure	X	X	X	X	X	X	X
soil consistence	X		X				
aggregate size distrib.			X	X	X	X	X
aggregate shape	X						
slaking/dispersion			X				
soil porosity	X			X	X	X	
soil colour	X		X		X		
soil mottles (no., colour)					X		
available water							X
water infiltration			X				
Soil chemical properties							
soil pH			X				
labile organic C			X				
Soil biological properties							
earthworms (no., size)			X		X		
potential rooting depth					X		X
root development	X		X	X		X	

[1] Visual evaluation of soil structure

[2] Muencheberg Soil Quality Rating

provides different information than laboratory approaches (Emmet-Booth et al., 2016) the combination of both would be advantageous (Pulido Moncada et al., 2014). Ultimately, the increased use of visual soil assessment is considered to be important in yield gap analysis and land management programs (McKenzie et al., 2015).

2.4 Soil quality indicators

2.4.1 Requirements for soil quality indicators

Various requirements for soil quality indicators have been identified in some (but by far not all) approaches to assessing soil quality (**Table 2.3**). All publications that list such requirements mention at least one conceptual condition such as that a chosen indicator must be related to a given soil threat, function or ecosystem service and be relevant. However, this is not of great use if soil quality assessment is not targeting a specific soil threat, function or ecosystem service.

Of the practical requirements, ease of sampling and measurement is almost always mentioned, and reliability and cost are also considered important. Practical considerations such as the disadvantage of indicators requiring undisturbed samples often play an important role in discarding otherwise suitable soil quality indicators (Idowu et al., 2008), which is a serious limitation from a scientific perspective. Where the measurement of a specific soil indicator is considered too expensive, too difficult or not possible (e.g. bulk density, due to the stoniness of the soil), pedotransfer functions may provide a proxy value through the measurement of other properties, for example carbon and texture for bulk density (Reidy et al., 2016). The application of pedotransfer functions was already considered useful in early soil quality publications (Larson and Pierce, 1994; Doran and Parkin, 1996; Doran and Safley, 1997) and has again been advocated more recently (Bone et al., 2010b), especially for complex soil properties such as hydrologic characteristics (Saxton and Rawls, 2006; Tóth et al., 2015). However, the inaccuracy of pedotransfer functions needs to be clearly stated.

Sensitivity to changes in management is mentioned frequently (**Table 2.3**), but there may be trade-offs with robustness to seasonal variation. Regarding the interpretation of the obtained values, comparability to data from other sampling campaigns is often desired. However, some indicators such as organic carbon (or soil organic matter) content and pH are often measured, whereas others such as bulk density or earthworm diversity are rarely assessed (Morvan et al., 2008). Moreover, the requirement to have clear (absolute) interpretation schemes for a given indicator is mentioned in only half of the publications (**Table 2.3**), even though assessment of soil quality cannot be put into practice without it.

Table 2.3. Considerations and criteria for soil quality indicators mentioned in various publications.

Criteria and considerations		Larson and Pierce (1994)	Doran and Parkin (1996)	Macdonald et al. (1998)	Burger & Kelting, (1999)[1]	Southorn and Cattle (2000)	Nortcliff (2002)	Merrington (2006)	Idowu et al. (2008)	Ritz et al. (2009a)	West et al. (2010)	Oberholzer et al. (2012)	Bone et al. (2014b)
Conceptual	Related to soil function and/or ecosystem processes;		x		x		x	x	x	x	x	x	
	Relevance, representation of key variables controlling soil quality, correlated to long-term response, allow evaluation of assessment criteria	x			x			x				x	x
	Significance at the appropriate scale			x		x							
	Integrate soil physical, chemical, biological properties		x	x									
	Allow estimation of soil properties or functions which are more difficult to measure directly		x										x
Practical	Ease of sampling and measurement (simplicity, practicality, single or repeated sampling and measurement, provide information in short timeframe)	x	x	x	x	x	x	x	x	x	x		x
	High throughput of analysis, wide applicability									x			x
	Amount of soil needed									x			
	Sample storage before analysis									x			
	Reliability and reproducibility of measurement	x				x	x		x	x		x	x
	Existence of a standard method of estimation (standard operating procedure)					x				x			
	Availability of reference material for quality control									x			
	Cost (sampling, hardware, analysis, labour)	x			x	x		x	x	x	x		x
Sensitivity	Spatial variation						x						
	Temporal variation (not influenced by short-term weather patterns)		x			x	x				x		
	Sensitivity to changes in management, or land use, response to perturbation as well as corrective measures	x	x	x	x	x	x	x	x		x	x	
Interpretation	Comparability with routine sampling and monitoring programs (context data available); part of standard tests; baseline available		x		x	x	x	x	x	x			
	Ease of interpretation, interpretation criteria available			x		x	x	x				x	x
	Archivability, capable of continuous assessment				x					x			
	Mappable trend indicators					x							
	Generic or diagnostic value			x		x							
	Not redundant			x									

[1] as cited in Bone et al. (2010b)

Finally, indications to what extent soil quality indicators actually fulfill the requirements listed in **Table 2.3** are often missing but would be needed to make informed choices in soil quality assessment programs.

2.4.2 Methods for selecting a minimum dataset

Increasing the number of indicators can increase collinearity as well as the complexity of the relationships between indicators and management options. Moreover, costs of measurements easily become prohibitive, especially if detailed soil biological parameters are included (O'Sullivan et al., 2017). For these reasons, the number of soil quality indicators that is actually analyzed on a given set of samples needs to be reduced to a minimum dataset.

In the first proposed minimum datasets, this selection was based on expert judgement (e.g. Doran and Parkin, 1994a). Subsequently, statistical data reduction by multivariate techniques such as principal component analysis (PCA), redundancy analysis (RDA) and discriminant analysis (Schipper and Sparling, 2000; e.g. Andrews and Carroll, 2001; Shukla et al., 2006; Lima et al., 2013), and multiple regression (Kosmas et al., 2014) became more common. After this initial data reduction, simple or multiple correlation analysis can further decrease the number of indicators (Andrews and Carroll, 2001; Kosmas et al., 2014), sometimes followed by the use of expert judgement for choosing only one out of two or more highly correlated soil properties (Sparling and Schipper, 2002). With these techniques, the number of indicators finally selected typically ranges between 6 and 8. Because soil properties that are relevant for soil functioning but do not show much variation in a given study will not be included in the minimum dataset, validation of the minimum dataset is important, for example by testing its relation to predefined and independently measured management goals (Andrews and Carroll, 2001).

A participatory approach of selecting soil biological indicators from a long list of potential indicators was presented by Ritz et al. (2009a). Potential indicators were scored by scientists and end-users in a "logical-sieve" approach, which allowed several iterations. The different requirements for an indicator (**Table 2.3**) were weighted: reproducibility was considered absolutely essential, whereas the existence of a standard protocol had the lowest weight. A modified version of this method was applied by Stone et al. (2016b) to establish the top 10 biodiversity indicators of soil quality (defined as the ability to perform key soil processes) across the agricultural area of European member states for use in future monitoring.

Finally, the most important soil quality indicators can also be inferred from participatory conceptualization of how complex systems function. For example, Troldborg

et al. (2013) and Aalders et al. (2011) established a Bayesian Belief network defining which factors are most influential in determining the risk of compaction and erosion, respectively. Hence, the selection of a minimum dataset derived from a larger set of soil quality indicators is a necessary step in soil quality assessments because of financial and time limitations and to avoid collinearity. Methodological transparency is imperative to allow wide application of minimum dataset selection.

2.4.3 Frequently proposed soil quality indicators

To identify the most frequently proposed (combinations of) soil quality indicators, we summarized 62 publications (**supplementary table 2**) in which 65 minimum datasets of measured soil properties have been proposed. Due to the plethora of methods and terms, a certain aggregation of measured indicators into categories was required, e.g. aggregate stability, shear strength, tilth and friability, structure, consistence and slake test were merged in a category called structural stability (**supplementary table 3**). We included both peer-reviewed journal articles on soil quality assessment approaches and reports on national monitoring programs, aiming at global coverage. Considering that soil quality assessment includes many steps, from the definition of objectives via the selection of indicators to the interpretation of obtained indicator values, we only included studies that address more than one of these steps and thus have a certain conceptual and generalizable nature. Consequently, studies that are entirely limited to the comparison of a set of indicators in different management systems were excluded. Even though we may have missed some publications, especially from national assessment schemes, we noted that increasing the number of evaluated datasets from 45 to 65 during the compilation hardly changed the outcome**.** Therefore, we are confident that our evaluation shows a valid picture of which soil quality indicators are most used.

Total organic matter/carbon and pH are the most frequently proposed soil quality indicators (**Figure 2.4)**, followed by available phosphorus, various indicators of water storage and bulk density (all mentioned in > 50% of reviewed indicator sets). Texture, available potassium and total nitrogen are also frequently used (> 40%).

The average number of proposed indicators is 11 (**supplementary tables 4 and 5**), which is probably more than is feasible from a practical as well as a financial viewpoint under most circumstances. Therefore, a trend towards smaller indicator sets in recent years can be seen. However, the development of novel indicators, which can be applied on a high number of samples in a fast and cheap way, could change the picture in the future.

In most publications, at least one indicator of each category (physical, chemical and biological) is included. These categories are typically represented automatically when all

soil functions or soil-based ecosystem services are addressed. However, soil biological indicators were missing from 40% of the reviewed minimum datasets.

Soil physical indicators, especially those related to water storage, were frequently proposed in the early assessment schemes and again in the last 5 years, while they were less common in between (**supplementary table 4**). Among the soil chemical indicators, soil organic carbon content, pH, available P and K, total N, electrical conductivity, cation exchange capacity, and mineral N were proposed more often than all other indicators. Likewise, soil respiration, microbial biomass, N mineralization and earthworm density were more frequent among the biological indicators than the other 10 indicators that have been proposed at least once (**supplementary table 5**).

The explicit mentioning of extrinsic factors (**supplementary table 5**) such as climate, management or site data is surprisingly rare. In particular, yield, plant nutrient status and other measures of ecosystem services are very often not included. This means that soil quality assessment is typically not explicitly linked to ecosystem services or soil threats. An example of how to establish linkages between soil properties, soil functions and ecosystem services via correlations can be found in van Eekeren et al. (2010). Recent publications advocate indicators that are applicable to several soil processes (Bone et al., 2010b). In Lima et al. (2013), for example, earthworms serve as indicators for both water and nutrient cycling. However, many of the other publications lack a clear conceptual and/or mechanistic relationship between indicators and soil functions and ecosystem services.

2.4.4 Novel soil quality indicators

Adoption of additional or novel soil quality indicators into minimum datasets is of interest if they have clear added value from the perspective of the management goals for a particular situation. Recent developments in soil science, especially in soil biology, but also in spectroscopy and other fields, hold promise for future soil quality assessment schemes. Below, we briefly review these developments, from biological and biochemical indicators to data capture and high-throughput approaches that have the potential to change soil quality assessment approaches quite substantially.

Soil organisms play a central role in soil functioning (**supplementary table 6**). Therefore, adding biological and biochemical indicators can greatly improve soil quality assessments (Barrios, 2007). Moreover, the assessment of biological indicators of soil quality is required to connect abiotic soil properties to (changes in) soil functions in terms of biochemical and biophysical transformations and (potential) aboveground vegetation performance (Lehman et al., 2015b). Nevertheless, soil biological indicators are still underrepresented in soil quality assessments and mostly limited to black-box measurements such as microbial biomass and soil respiration (**Figure 2.4, Table 2.4).**

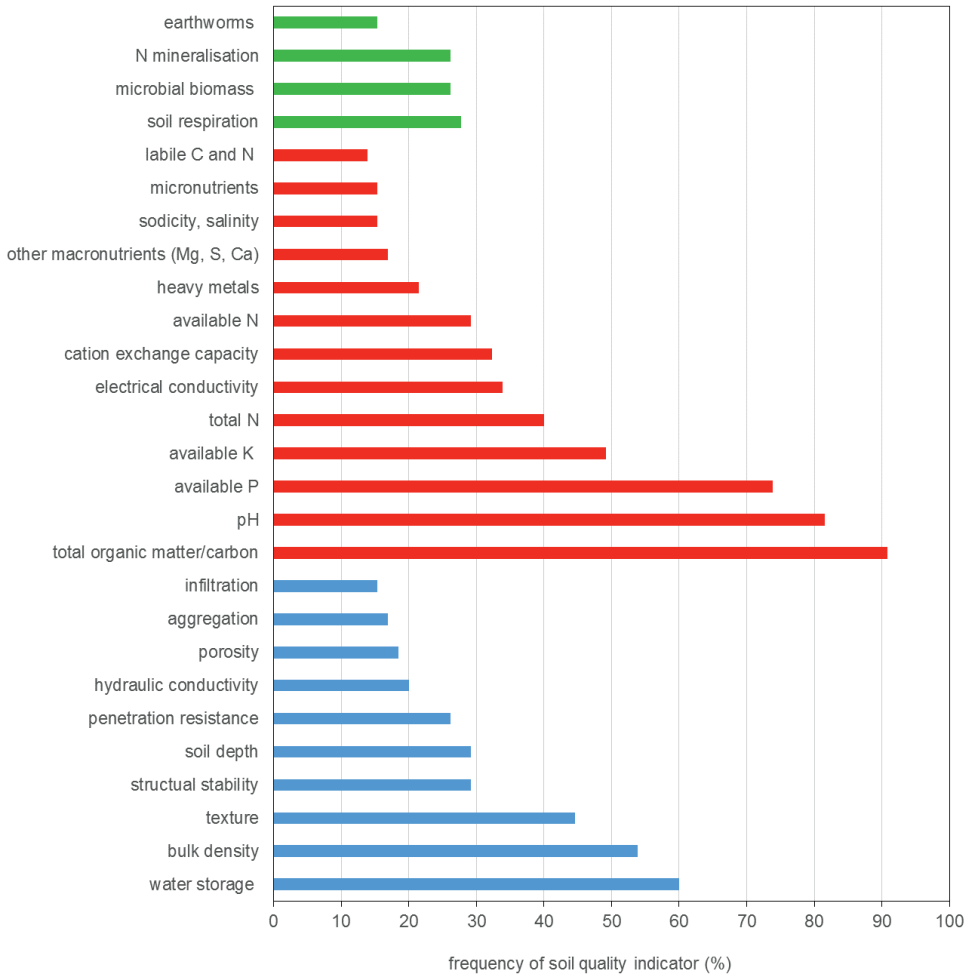

Figure 2.4. Frequency of different indicators (min. 10%) in all reviewed soil quality assessment approaches (n=65). Soil biological, chemical and physical indicators shown in green, red and blue, respectively. Publications dealing exclusively with forest soils (e.g. Zhang, 1992; Schoenholtz et al., 2000) or focusing on biological indicators only, without also looking at chemical and/or physical indicators (Filip, 2002; Parisi et al., 2005; Ritz et al., 2009a), were not included in this compilation. If the same authors proposed the same set of indicators in more than one publication, then only the first was considered. In two publications (Andrews et al., 2002; Biswas et al., 2017), different sets of indicator were proposed. Thus, the total number of reviewed publications was 62.

Despite clear potential, more specific indicators such as those based on nematodes (Stone et al., 2016a), (micro)arthropods (Rüdisser et al., 2015) or a suite of soil biota (Velasquez et al., 2007) have rarely been suggested, possibly because they require specific knowledge and skills. This situation is unfortunate because soil biota are considered the most sensitive indicators of soil quality due to their high responsiveness to changes in

Table 2.4. Soil biological indicators, methodologies, related main soil functions, and advantages/disadvantages at different scales.

INDICATOR	METHODOLOGY	MAIN SOIL FUNCTIONS	PROS	CONS
Individual, population and community level				
Presence, richness, abundance of individual soil organisms(for details see supplementary Table 6)	Traditional handsorting and microscopic methods; molecular quantitation (qPCR).	Element, organic matter and water cycling, biological population regulation, soil structure maintenance	Taxonomic and functional level.	Not always linked directly with functions. Difficult to apply to fauna, e.g. protozoa, mites and collembola.
Microbial biomass and fungal biomass, fungal:bacteria ratio	Direct counting, chloroform fumigation extraction, SIR, PLFA, molecular quantitation.	Element and organic matter cycling, decomposition, soil structure maintenance	Sensitive and well related with other soil quality indicators.	Spatially variable, difficult interpretation, contradictory results. Unclear direct link to functionality.
Indices based on faunal communities (e.g. Maturity Index, Enrichment Index, Channel Index, Structural Index for nematodes)	Counting and identification of specific groups of organisms	Element and organic matter cycling, biological population regulation, decomposition	Sensitive. Taxonomic and functional level.	Time-consuming and costly. Specialist required for morphological identification.
Community composition	Manual counting and identification	Element and organic matter cycling, biological population regulation, habitat provision, decomposition, soil structure maintenance	Division in functional groups can give an indication of functions.	Time-consuming, expertise required. Not indicative of active biota.
	PLFA		Correlated with other measurements. Good indicator of active microbial biomass. Integrated information on the microbial community.	Time-consuming. No direct link with functions. Coarse resolution.
	Fingerprinting methods (e.g. DGEE, T-RFLP, A-RISA, ARDRA, TGGE), microarrays		Greater phylogenetic resolution.	No direct link with function. Difficult comparison between studies due to great variety in methods. Difficulties to extract and amplify DNA.
	Sequencing (metabarcoding)		Detailed view of diversity. Enormous amounts of data. Detects less abundant organisms. Permits discovery of new diversity.	Taxonomic genes no direct link with functions. Difficulties to extract and amplify DNA. Costly. Problems related with handling of large datasets and analyses. Dependent on libraries. No standard methodology.
	Community Level Physiological Profiling (Biolog™, MicroResp™)	Element and organic matter cycling, decomposition, habitat provision	Insight into functionality of the community. MicroResp™ closer to *in situ* conditions, shorter time of measurements.	Many replicates needed because of variability and "border effects".

Continue

Ecosystem level				
Soil respiration, nitrogen mineralization, denitrification, nitrification	CO_2 evolution, N_2O emission, NO_3 produced.	Element, organic matter and water cycling, decomposition, habitat provision	Sensitive and ecologically relevant.	Highly variable and fluctuating. Relatively laborious.
Potentially mineralizable nitrogen	Anaerobic incubation.		Good correlation with MB and total soil N.	Relatively laborious.
Metabolic quotient (qCO_2), microbial quotient (Micrc/Soilc)			Sensitive, simple and inexpensive.	Difficult interpretation: confound disturbance with stress.
DNA and protein synthesis.	Thymidine and leucine DNA incorporation.		Reflection of active microbial biomass.	No stardardized procedure.
Enzymatic activities	Extraction of enzymes in the soil and incubation with various substrates.	Element and organic matter cycling, decomposition, , biological population regulation	Closely related to important soil quality parameters. Very sensitive. Simple and inexpensive methods.	Standard procedure not available. Contradictory results, complex behaviour and variable for each enzyme. Potential activity.
Functional genes and transcripts	FISH, Microarrays, meta-transcriptomic, qPCR, metagenome analysis.		Closer link to functionality. FISH and microarrays can give an idea of active microorganisms. High sensitivity and throughput.	Restricted to known gene sequences. Genes and transcripts might not be expressed. Difficulties linked with RNA extraction. Costly.
Metabolomics and metaproteomics	Assessment and quantitation of metabolites and proteins in the soil.	Element and organic matter cycling, decomposition, , biological population regulation, soil structure maintenance	Closer link to functionality.	Field in development. Difficult extraction of metabolites and proteins.
Stable isotope probing	Incorporation of ^{13}C- or ^{15}N-labelled substrates into DNA, RNA, PLFA, proteins	Element and organic matter cycling, decomposition	Permit to establish link between biodiversity and functions. Allow in situ analysis of active microbial population.	Field in development. Time involved in the assimilation of the substrates.

Table compiled from (Visser and Parkinson, 1992; Stenberg, 1999; Neher, 2001; Nielsen and Winding, 2002; Torsvik and Ovreas, 2002; Brussaard et al., 2004a; Gil-Sotres et al., 2005; Parisi et al., 2005; Saleh-Lakha et al., 2005; Bastida et al., 2008; Trasar-Cepeda et al., 2008; Bloem et al., 2009; Brussaard, 2012b; Blagodatskaya and Kuzyakov, 2013; Cardoso et al., 2013; de Groot et al., 2014; Lehman et al., 2015b; Orgiazzi et al., 2015; Rocca et al., 2015; Watzinger, 2015; Bouchez et al., 2016; Schloter et al., 2017).

environmental conditions (Nielsen and Winding, 2002; Bastida et al., 2008; Kibblewhite et al., 2008c; Bone et al., 2010b). In particular, there is an urgent need for indicators of soil–borne diseases (Kyselková et al., 2014; Liu et al., 2016b; Trivedi et al., 2017). In this context, soil suppressiveness, defined as the property of a soil to naturally reduce plant disease incidence (Hornby, 1983b), is of interest. Specific soil suppressiveness is the result of the presence of specific antagonists to pathogens, while general soil suppressiveness is based on the collective capacity of soil and plant microbiomes to act complementarily against pathogens (Schlatter et al., 2017b). Both combined are governing soil suppressiveness as a whole (Yadav et al., 2015). Several soil abiotic and biotic parameters have been suggested to underlie suppressiveness, such as soil pH, specific cations such as Mg and K, soil total N content, microbial biomass and activity, diversity and structure of microbial communities and specific microbial taxa in the case of specific suppressiveness (Janvier et al., 2007a; Wu et al., 2015), but without validation.

Recent rapid developments in soil biology have prompted the feasibility of indicators based on genotypic and phenotypic community diversity (Nielsen and Winding, 2002; Ritz et al., 2009a; Hartmann et al., 2015; Kumari et al., 2017). Molecular methods focusing on DNA and RNA hold great potential to perform faster, cheaper and more informative measurements of soil biota and soil processes than conventional methods (Bouchez et al., 2016). Consequently, they may yield novel indicators that could substitute or complement existing biological and biochemical soil quality indicators in regular monitoring programs (Hartmann et al., 2015; Hermans et al., 2017). In the participatory approach used by Stone et al. (2016b), seven out of ten selected indicators were indeed based on molecular methods, with 'molecular bacteria and archaea diversity' on top. In addition, recent data analysis approaches such as network analysis, structural equation modelling and machine learning could facilitate the establishment of links between indicators and functions (Allan et al., 2015; Creamer et al., 2016a). For example, Karimi et al. (2017) proposed microbial networks as integrated indicators of environmental quality that can overcome the lack of sensitivity and specificity of taxonomic diversity indicators. However, the prediction of process rates from the presence and quantity of genes and transcripts is yet to be clearly established (Rocca et al., 2015). Results gathered with these molecular techniques are also faced with biases introduced by sample contamination, PCR reaction, choice of primers and OTU definition and taxonomic assignment techniques (Abdelfattah et al., 2017; Hugerth and Andersson, 2017; Schloter et al., 2017). The analysis of the "big data" generated with sequencing also poses a serious challenge in terms of time, computing capacities and interpretation, since a large proportion of soil organisms yet remains to be characterized in taxonomic and functional terms (Schloter et al., 2017; Bouchez et al., 2016). Other molecular techniques such as metabolomics (Vestergaard et

al., 2017) and metaproteomics (Simon and Daniel, 2011) may yield potentially suitable soil quality indicators because the measurements are directly linked to ecosystem processes (Bouchez et al., 2016). These technologies have benefits but are limited in their application by the difficulty to extract metabolites and proteins from soil and to choose representative samples (Bouchez et al., 2016). Stable Isotope Probing (SIP) in conjunction with phospholipid fatty acid analysis (PLFA) and DNA probing could also help to link soil biodiversity to soil processes (Wang et al., 2015; Watzinger, 2015). Finally, for a meaningful integration of indicators based on molecular methods into soil quality assessments, standardized techniques and a reference system are still lacking and will have to be established (Bouchez et al., 2016).

Although total soil organic matter is ubiquitous as a soil quality indicator (**Figure 2.4**) changes in response to management and land use are difficult to detect since the total pool is large (Haynes, 2005a). Moreover, due to the structural and functional heterogeneity of total soil organic matter, its relevance in soil processes is not unequivocal. Therefore, qualitative information on soil organic matter may be more informative in soil quality assessments. Pools of soil organic matter such as labile or active carbon are typically more sensitive to disturbance than total soil organic matter and can give a better indication about soil processes (Gregorich et al., 1994a). Suggestions to measure this fraction include: particulate organic matter (Cambardella and Elliott, 1992), permanganate-oxidizable carbon (Weil et al., 2003b), hot water-extractable carbon (Ghani et al., 2003a) and water-soluble carbon, also called dissolved organic carbon (Filep et al., 2015). Despite their sensitivity to management and strong correlations to other parameters that are more difficult to measure, their relationship with soil processes is not well understood, partly because it is not clear which part of the organic matter they represent. Other methods to characterize (quality and quantity) of total soil organic matter such as thermal and spectroscopic methods are rapidly developing (Clemente et al., 2012; Derenne and Quénéa, 2015; Mouazen et al., 2016) and hold promise for soil quality assessments.

Additionally, soil sensing approaches such as spectroscopic techniques, e.g. near-infrared spectroscopy and remote sensing, offer the opportunity to measure various soil chemical, physical and biological parameters in a fast and inexpensive way (e.g. Cecillon et al., 2009; Kinoshita et al., 2012; Paz-Kagan et al., 2014; Gandariasbeitia et al., 2017). Sensors can be used directly in the field or in the laboratory (McKenzie et al., 2017), and commercial providers increasingly offer spectroscopy-based analyses (e.g. www.soilcares.com, www.eurofins.com). Combining laboratory-based visible and near-infrared spectroscopy with *in situ* measurements such as electrical conductivity and penetration resistance may be particularly useful (Veum et al., 2017). Spectroscopic techniques, however, also face limitations that hamper their routine use in soil quality assessment. First, when applied

to the soil surface in the field, information is gained only about the first millimeters of the soil. Second, sample characteristics such as moisture content, particle size distribution and roughness of the soil surface can influence the outcome of the analysis (Stenberg et al., 2010; Baveye and Laba, 2015). Third, a calibration step is used to relate the spectral information to soil characteristics (Gandariasbeitia et al., 2017) and the prediction is as good as the calibration data set. Several studies showed that calibration efficiency varies between studies and parameters considered (Islam et al., 2003); Kinoshita et al., 2012). Through their nature, spectroscopic estimates are always less precise than traditional analytical methods (Islam et al., 2003). Creation of freely-available databases that can be used for proper calibration and prediction of soil properties are essential for realizing the full potential of these techniques. These databases should involve both NIR spectra and results from wet chemistry and biological methods.

X-ray tomography is another non-destructive technique that can be used for soil structural analysis and can shed light on processes integrating soil physical and biological properties (Helliwell et al., 2013). It avoids some drawbacks of spectroscopic techniques, namely the fact that it scans a 3D image of the soil instead of only scanning its surface. Nevertheless, this technique is still a long way from routine application for soil quality assessment.

Such novel indicators potentially allow a more detailed assessment of soil processes. At the same time, some of the techniques may be developed into high-throughput soil analysis to shed light on the spatial and temporal variability of soil parameters and determine soil quality across different scales for application in precision agriculture, monitoring programs and life cycle assessments (Ge et al., 2011; Viscarra Rossel et al., 2017). The rapid evolution of these techniques and the decreasing costs associated with them will facilitate this development. However, the practical operability of these indicators by different stakeholders needs to be taken into account. The various limitations described above still seriously hamper application of such novel indicators in routine soil quality assessments. In addition, the absence of standard operating procedures (SOPs) and accepted threshold values, especially for molecular methods, make the comparison and the interpretation of the results challenging (Callahan et al., 2016). The final and most important limitation to the interpretation of these novel soil quality indicators is the lack of functional linkages with soil processes and management implications.

Although use of novel indicators directly by farmers would be an advantage, most farmers are willing to send samples to the laboratory as long as the analysed indicators are meaningful and responsive to management (Bouchez et al., 2016). For policy makers operating or setting up soil quality monitoring schemes, the introduction of novel indicators would also be aided by relating them to existing ones that may be phased out

when performance (or cost-efficiency) of novel indicators is superior. At the moment, however, most novel soil quality indicators still belong to the research domain, and many technological, practical and interpretation related issues need to be overcome.

2.4.5 Interpretation of indicator values

An indicator is only useful if its value can be unequivocally interpreted and reference values are available. Reference values for a given indicator could be either those of a native soil, which may however not be suitable for agricultural production, or of a soil with maximum production and/or environmental performance (Doran and Parkin, 1994a). In the Netherlands, for example, ten reference soils for good soil biological quality were selected out of 285 sites that had been monitored for over ten years (Rutgers et al., 2008). These reference soils represent specific combinations of soil type and land-use (e.g. arable land on clay soil). Soil quality indicators at a given site could thus be compared to those at the reference site as well as to the mean value, and 5% and 95% percentiles of all sites under a given land-use, with the percentiles given as a means to express the frequency distribution. An important drawback of this approach is that the reference may not be at an optimum in all parameters (Rutgers et al., 2012).

Acceptable values for an indicator can also be defined as those at which there is no loss or significant impairment of functioning (Loveland and Thompson, 2002). In the context of pollution, thresholds of contamination are often used (Chen, 1999). Likewise, Arshad and Martin (2002) list threshold levels for soil quality indicators, but this is rarely found in other publications on soil quality assessment. For plant nutrients, most agricultural advisory services use thresholds of available reserves below which plant production may become nutrient-limited, while maximum values are related to the risk of losses (Allen et al., 2006; Schoumans et al., 2014). Indicator thresholds for other soil functions are absent from most soil quality assessment approaches.

A more advanced way to evaluate soil quality indicators is the establishment of standard non-linear scoring functions, which typically have the shapes i) more is better, ii) optimum range, iii) less is better, or iv) undesirable range, with i-iii being most common in soil science. The shape of such curves is established based on a combination of literature values and expert judgement (Andrews et al., 2004). When scoring curves are based on regional data, such as in the Cornell Soil Health Assessment (Moebius-Clune et al., 2016), then scores are relative to measured values in the respective region. Each indicator measurement is transformed to a value between 0 and 1 (or 0 and 100) using a scoring algorithm (Karlen and Stott, 1994), with a score of 0 being the poorest (lower threshold) and a score of 1 (or 100) the best (upper threshold). The baseline value equals the midpoint between threshold values. Validation of scoring curves is possible if datasets

with measurements of the given soil quality indicator and a related soil process are available.

Obviously, acceptable target ranges of soil quality indicators need to be soil- and land use-specific, and they depend not only on targeted soil functions, but also on both spatial and temporal scale of soil quality assessments, with regional target ranges typically being narrower than national ones (Lilburne et al., 2004; Wienhold et al., 2009). In addition, acceptable ranges of a soil quality indicator for one property or process are often highly dependent on the value of another soil property or process, e.g. dependence of microbial biomass or soil organic carbon on soil texture (Candinas et al., 2002; Johannes et al., 2017).

It has been claimed that the interpretation of soil quality indicators, i.e. the establishment of target or workable ranges, will always remain contentious, which is partly due to a lack of data, partly due to the curvilinear pattern that many indicators follow and partly because the use of expert judgement is contentious itself (Merrington, 2006). A comparative approach in which indicator values or scores of a given sampling point are put in relation to other sampling points may be the most intuitive and flexible basis for interpretation, since it gives a relative assessment (e.g. top 25%) and allows continuing evolution of the system. This approach is being implemented in the iSQAPER project, where the variation in soil quality indicator values within pedo-climatic zones is determined. Ranges are defined for specific land uses (e.g. arable land, grassland), and benchmark scores based on relative frequency are given. This approach may also introduce modular extensions of indicators that are only relevant in specific contexts, where stakeholders can relate to them. Decision trees based on environmental conditions, management systems and relevance of ecosystem services can guide the selection of specific indicators.

2.4.6 Deriving a soil quality index and alternatives

Many studies on soil quality have searched for a way to aggregate the information obtained for each soil quality indicator into a single soil quality index, even though this was deemed impossible by Sojka and Upchurch (1999). For example, Velasquez et al. (2007) summed the contributions of each of five sub-indicators (hydraulic properties, chemical fertility, aggregation, organic matter and biodiversity) to derive the general indicator of soil quality (GISQ). In the SMAF, an additive index yields a number between 1 and 10 (Andrews et al., 2004). However, if assessed soil functions or ecosystem services rank very differently in importance, then some kind of weighting is mandatory.

For example, in the recent Canadian monitoring of soil quality within the agri-environmental indicator assessment, a soil quality compound index is calculated as the weighted average of the performance indices for erosion, soil organic carbon content, trace elements and soil salinization (Clearwater et al., 2016). Another example is the multi-

Table 2.5. Example of weighting of soil functions and associated indicators (Lima et al., 2013)

Soil function	Weight	Indicator level 1	Weight	Indicator level 2	Weight
Water infiltration,	0.33	Available water	0.25		
storage and supply		Mean weight diameter	0.25		
		Earthworms	0.25		
		Correlated indicators	0.25	Soil organic matter	0.50
				Bulk density	0.50
Nutrient storage,	0.33	Available water	0.25		
supply and cycling		Earthworms	0.25		
		Soil organic matter	0.25		
		Micronutrients	0.25	Manganese	0.33
				Copper	0.33
				Zn	0.33
Sustain biological activity	0.33	Soil organic matter	0.50		
		Earthworms	0.50		

objective approach based on principles of systems engineering proposed by Karlen and Stott (1994). The main soil functions are weighted according to their importance for the overall goal in soil quality management at a given site, and an overall rating of soil quality with respect to the predefined goal is obtained by summing the weighted soil functions. An exemplary application of this approach can be found in Lima et al. (2013), who used SIMOQS (Sistema de Monitoramento da Qualidade do Solo) software developed in Brazil to calculate a soil quality index (**Table 2.5**).

Visual soil assessments are also often summarized in an overall soil quality rating (McGarry, 2006; Shepherd et al., 2008; Mueller et al., 2014). Typically, the scores for the different indicators are summed up, with some weighting applied. In the Muencheberg Soil Quality Rating, the weighted sum of the basic indicators is multiplied with values for hazard indicators such as contamination, acidification and flooding (Mueller et al., 2014).

Instead of deriving an overall soil quality index, colour coding for different indicators alone or aggregated according to soil functions is more meaningful. For example, in the outputs the Cornell soil health test, in Sindi, and in the Australian soil quality monitoring framework a traffic light system of 3-5 colours indicates low, adequate or excessive values for a given indicator. Other graphical presentations such as amoeba diagrams (or spider diagrams) can likewise convey more information on trade-offs and synergies than a single number or index (Rutgers et al., 2009; Rutgers et al., 2012).

The ultimate purpose of a soil quality index is to inform farmers and other land managers about the effect of soil management on soil functionality. An aggregated

presentation of the outcome of soil quality assessments, especially by graphical means, can indeed be useful also for educational purposes and for communicating to society as a whole the consequences that human decisions can have on soil-based ecosystem services.

2.4.7 Stakeholder involvement

Because the reviewed literature is often not clear (enough) on who were the main developers and who are the main end users of the soil quality assessment schemes (**Table 2.1**, **Table 2.2**), we asked (by e-mail) 17 scientists who stood at the cradle of such schemes, or can currently act as spokespersons for them, to answer the following questions:

1. Who were the three main stakeholders, in order of importance, who were *involved in the development* of the soil quality assessment scheme?
2. Who are the three main stakeholders, in order of importance, *using* the soil quality assessment scheme?
3. Can you guide us to published or internet-accessible information (if any) on the extent of use and on user feedback?

We received answers from 11 countries: Australia (2 programs), Brazil, Canada, China, England, France, Germany, the Netherlands, New Zealand, Scotland and USA. The main *developers* of soil quality assessment schemes turned out to be scientists (8x) and government agencies (3x), while farmer organizations were top-ranked only once. The second position was taken by a mix of scientists (3x), (regional) government agencies (3x) and agricultural advisors (2x). Third positions were filled in only 5x, with various stakeholders. When it comes to *end users*, government agencies and consultants/agricultural advisors are top-ranked (each 4x), and farmers 2x. In second position are scientists (4x), (regional) authorities (3x), farmers/land managers (2x) and students (1x). Hence, not unexpectedly, scientists play a leading role in the development of soil quality assessment schemes. Remarkably, however, farmers/land managers, consultants/agricultural advisors and other stakeholders usually play an insignificant role in development, whereas they turn out to be important end users of the schemes. Quantitative data on the use of the assessment schemes is available in only four cases and user feedback data are equally scarce.

2.5 Conclusions

Our review has revealed how soil quality assessment has changed through time (**Figure 2.5**) in terms of objectives, tools and methods, and overall approach.

A number of steps are to be taken in soil quality assessment (**Figure 2.6**), elements of which are addressed to very different degrees in the large number of approaches that have been developed during the past three decades and reviewed in this article. An elementary start is a clear definition of the **objectives**, i.e. whether soil assessment is meant as a basis for management recommendations, seen as an educational tool, or as part of a monitoring program. Likewise, **target users** should be named and involved from the beginning in order to increase adoption of the developed assessment approach. Such approach has been taken in the Horizon 2020 project LANDMARK, where the assessment of soil functions and indicators has in the first place been derived through stakeholder workshops (http://landmark2020.eu/work-package/work-package-1/). The application of stakeholder- based assessment requires different tools for different knowledge. For example, visual soil assessment tools are targeted at farmers for understanding the status of soil structure in the field, whereas more detailed knowledge on productivity requires laboratory measurements, which are, e.g., offered to farmers in the Cornell soil health assessment (Moebius-Clune et al., 2016) and by recently developed commercial soil testing services based on spectroscopic methods (see section 4).

The **selection of soil quality indicators** needs to be based on mechanistic linkages between indicators and soil functions or ecosystem services that have sometimes been proposed (Creamer et al., 2016a) but rarely established firmly through experimental validation (e.g. van Eekeren et al., 2010). A clear definition of the targeted soil function(s) will determine the soil depth that is to be evaluated, since some soil functions are mainly related to the topsoil, whereas others are related to the entire soil profile. An asset of a novel soil quality framework would be the possibility to choose indicators based on the targeted soil threats, soil functions and ecosystem services, which is deemed possible by using the logical-sieve method (Stone et al., 2016b). Conceptually, soil threats, functions and ecosystem services are all linked (**Figure 2.2**), and concepts focusing on either of these can thus be reconciled, if it is recognized that the targeted soil function or ecosystem service and associated choice of indicators are scale-dependent (Schulte et al., 2015; Norton et al., 2016). (Multi-)functionality should clearly be integrated in future approaches to soil quality, such as that of functional land management (Schulte et al., 2015) applied in the LANDMARK project.

The possibility to choose between **substitute or proxy indicators** (**Figure 2.6**) would be highly beneficial but is so far rarely offered. The use of parallel independent lines of evidence in ecological risk assessment (Rutgers and Jensen, 2011) and the inclusion of

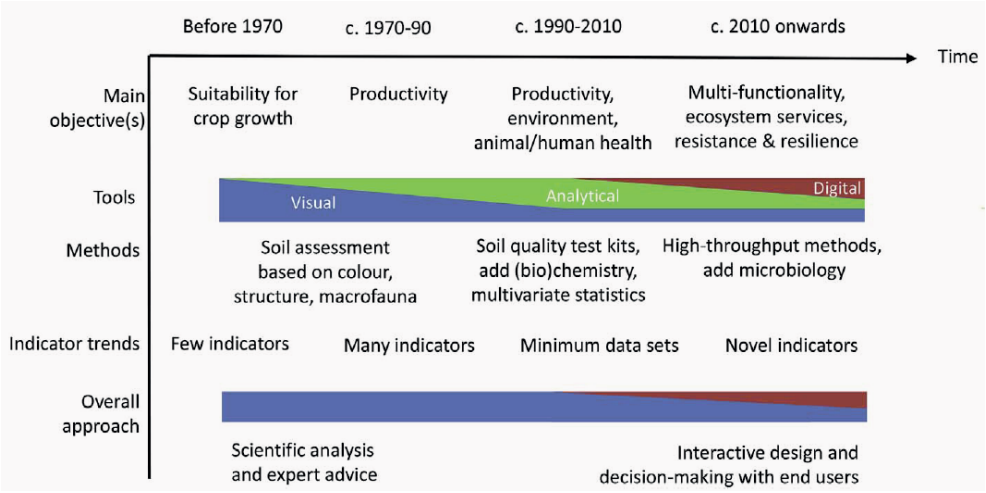

Figure 2.5. Main objectives, tools and approaches of soil quality assessment through history

both qualitative and quantitative information in classical land evaluation (Sonneveld et al., 2010) could be models for that. Besides soil indicators, whether obtained using field assessments, analytical methods, high-throughput approaches or pedotransfer functions, also non-soil factors such as climatic and site conditions and non-soil indicators such as plant performance and aboveground biodiversity, landscape and socio-economic indicators (e.g. Culman et al., 2010b; Jackson et al., 2012) should be considered.

The **interpretation** of the values of the proposed soil quality indicators needs to be well-defined. If no system for interpretation is provided, the indicators cannot be used in practice. For many soil properties, texture-dependent scoring curves need to be developed, which is possibly one of the greatest challenges. The increased availability of digital soil maps and soil survey data such as the LUCAS soil data available from the Joint Research Centre (http://esdac.jrc.ec.europa.eu/content/lucas-2009-topsoil-data) or global soil grids in 250M (https://soilgrids.org/#/?zoom=2&layer=geonode:taxnwrb_250m) provides an opportunity to establish such scoring curves or target values more easily from frequency distributions of a given soil property. However, if soils in a region are badly managed or were so in the past, such a frequency distribution may not include the optimum state. In this case, the principle of identifying reference sites with acknowledged good soil quality (Rutgers et al., 2008; Rutgers et al., 2012) would be more suitable, or could be combined with the scoring curve approach. Reference or threshold values are required both to use soil quality indicators to their full potential and to translate the interpretation into appropriate management and policy advice. The assessment of the (dis)agreement of

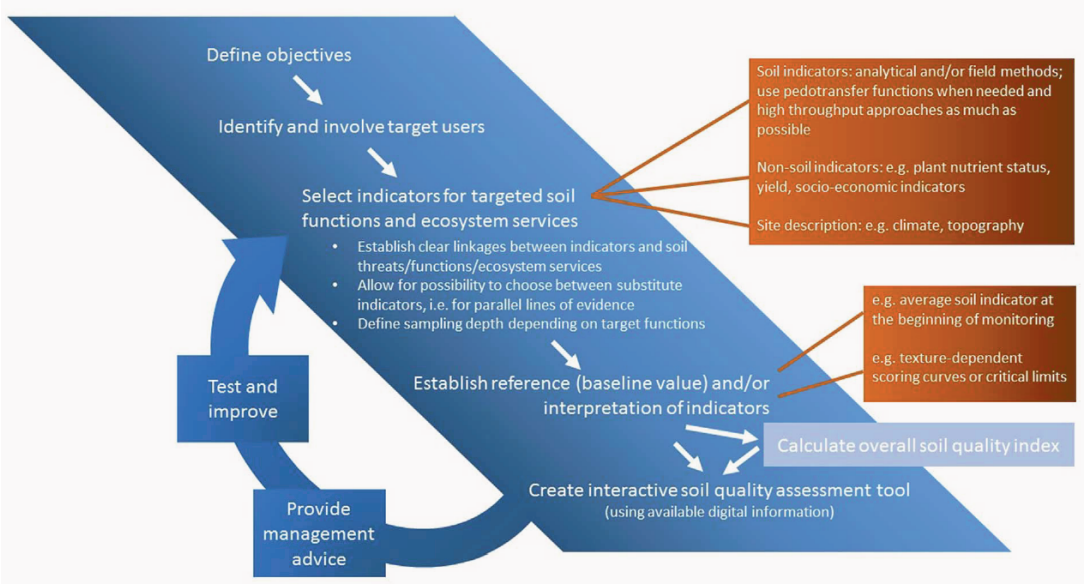

Figure 2.6. Main steps in the development of a soil quality assessment approach

results obtained from different lines of evidence (e.g. sets of indicators based on physical, chemical or biological parameters; see e.g. Velasquez et al., 2007) can be adopted from mathematical procedures developed in ecological risk assessment (Karlen et al., 2001; Rutgers and Jensen, 2011).

An overall **soil quality index** is often desired but actually not very meaningful, since soil quality is best assessed in relation to specific soil functions. Rather than calculating an overall index, a graphical representation of how well a given soil fulfils its various functions is much more effective in communicating with stakeholders, target users and the general public. In practice, different sets of soil quality indicators will be used with different weightings, depending on the set of soil threats and ecosystem services at stake according to the "stake-holders".Future soil quality assessment and monitoring can benefit from recent technological developments such as the SoilInfo App (http://www.isric.org/explore/soilinfo), mobile data capture including photographs and big-data approaches which are both used in the proposed LandPKS tool (www.landpotential.org), and high-throughput soil analysis approaches, such as visual and near-infrared spectroscopy. Future tools promise to be truly **interactive**, such as the soil quality assessment tool (SQAPP) that is being developed within the EU iSQAPER project.

Finally, soil quality assessment can become effective to improve the state of our soils only with inclusion of **management or policy advice**.

2.6 Outlook

Science plays an important part in the search, under prevailing pedo-climatic conditions (**Figure 2.1**), for indicators of the structural and process aspects of soil functioning that mediate the delivery of soil-based ecosystem services deemed important by actors and other stakeholders who exert(ed) pressures on the soil through land use and soil threats. The key terms here are 'actors' and 'stakeholders'. Terms such as 'soil function', 'ecosystem service' and, indeed, 'soil quality', are boundary concepts, i.e. concepts that enable researchers from different disciplines, policy-makers, and other stakeholders to develop a common language and integrate and derive knowledge relevant to their field (Schleyer et al., 2017). Beyond scientists, those who have an immediate stake in soil quality are land managers, i.e. farmers, managers of nature conservation areas, roadsides, banks of waterways and urban green areas, and the public at large. As soil quality management is also about societal negotiation in the face of unavoidable trade-offs between various soil uses, the very development of soil quality indicator schemes will benefit from the involvement of actors and other stakeholders with a view to implement adaptive land use and management (Barrios et al., 2006; Barrios et al., 2012).

Although, clearly, soil quality is not merely a natural science topic, in most of the reviewed assessment schemes farmers/land managers did not play a leading role. We suggest that intimate involvement of end users is a major point of attention, but it may still not lead to full implementation of the results. For example, in the Illinois Soil Quality Initiative, where farmers were involved in the development of soil quality assessment schemes, they were constrained in the necessary implementation of the results by socio-economic factors (Wander et al., 2002). Clearly, other actors play an important part. Industries that ultimately also depend on the soil, will be(come) important actors, too, such as food, fibre and fuel industries, and electricity production, manufacturing and fashion industries (Davies, 2017). Their interest is in sustained resource supply, which is at stake because of ongoing loss of soil functionality and increased variability in harvests and water supply associated with global climate change, partly induced by unsustainable land use and management. Land managers, industries and, indeed, investors and insurance companies and the public sector at large are increasingly aware of the associated monetary and societal costs and, *vice versa*, they understand the urgency of adaptive land management and re-design in the framework of food systems (Foresight, 2011) and a fossil-free and circular economy (Rockström et al., 2016).

To be part of such urgent transitions, soil scientists are challenged to engage as 'honest brokers' of knowledge who increase the decision space of actors (Pielke, 2007). This engagement of soil (quality) researchers should take into account the following points:

First, we should consider (fostering) soil quality an integral part of (enhancing) environmental quality in general, as argued by Döring et al. (2015). We should not consider soil quality in isolation, but as part of quality assessment and adaptation of systems, e.g. of agricultural systems such as mainstream *vs.* integrated *vs.* conservation agriculture (Stavi et al., 2016) or mainstream *vs.* integrated *vs.* organic agriculture (Mäder et al., 2002; Seufert and Ramankutty, 2017). This requires engagement with farmers of different philosophies from purely organic to industrialized, and with other players in food systems.

Second, we should recognize that the radical changes in agricultural practices, summarized as 'smart farming' (Walter et al., 2017), require novel soil quality assessment tools, both in de-intensifying mainstream agriculture and in intensifying ecological agriculture (Struik et al., 2014).

Third, our focus should not just be on informing adaptive land management in existing agricultural systems, but also on fundamental system re-design, summarized as regenerative agriculture (Rhodes, 2017), in the framework of the circular economy.

Fourth, engaging with societal goals such as the UN Sustainable Development Goals (SDGs) is not only important in itself, but strategic in stressing the importance of soil (quality) knowledge for society (Bouma, 2014). In turn, monitoring progress towards the SDGs will require soil quality monitoring too, e.g. through the UNCCD Land Degradation Neutrality goals and associated reporting mechanism (Akhtar-Schuster et al., 2017).

Finally, awareness of the power relationships in the context of scientific support to stakeholders is essential. Generally, existing institutions and power relations resist innovation. Hence, the challenge is to associate with initiatives and policies that can create a greater space for innovation and system re-design and strengthen actors' influence from lower up to higher levels (Giller et al., 2008).

The engagement we make a plea for may require painstaking efforts, from gradual but consistent improvements within existing legislative frameworks (e.g. Römbke et al., 2016; Ockleford et al., 2017) to developing fundamental alternatives to current land use practices (e.g. Montgomery, 2017; Rhodes, 2017). Such engagement will at the same time require unquestionable scientific independence in the co-creation of knowledge (Mauser et al., 2013). We suggest that such engagement is necessary for the improvement of existing schemes and the development of novel schemes for assessment and monitoring of soil quality, as well as for the evaluation of their use and usefulness for all actors involved.

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CHAPTER 3

3

Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe

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Abstract

Soil quality is defined as the capacity of the soil to perform multiple functions, and can be assessed by measuring soil chemical, physical and biological parameters. Among soil parameters, labile organic carbon is considered to have a primary role in many soil functions related to productivity and environmental resilience. Our study aimed at assessing the suitability of different labile carbon fractions, namely dissolved organic carbon (DOC), hydrophilic DOC (Hy-DOC), permanganate oxidizable carbon (POXC, also referred to as Active Carbon), hot water extractable carbon (HWEC) and particulate organic matter carbon (POMC) as soil quality indicators in agricultural systems. To do so, we tested their sensitivity to two agricultural management factors (tillage and organic matter input) in 10 European long-term field experiments (LTEs), and we assessed the correlation of the different labile carbon fractions with physical, chemical and biological soil quality indicators linked to soil functions. We found that reduced tillage and high organic matter input increase concentrations of labile carbon fractions in soil compared to conventional tillage and low organic matter addition, respectively. POXC and POMC were the most sensitive fractions to both tillage and fertilization across the 10 European LTEs. In addition, POXC was the labile carbon fraction most positively correlated with soil chemical (total organic carbon, total nitrogen, and cation exchange capacity), physical (water stable aggregates, bulk density) and biological soil quality indicators (microbial biomass carbon and nitrogen, soil respiration, and abundance of earthworms).

We conclude that POXC represents a labile carbon fraction sensitive to soil management and that is the most informative about total soil organic matter, nutrients, soil structure, and microbial pools and activity, parameters commonly used as indicators of various soil functions, such as C sequestration, nutrient cycling, soil structure formation and soil as a habitat for biodiversity. Moreover, POXC measurement is relatively cheap, fast and easy. Therefore, we suggest measuring POXC as the labile carbon fraction in soil quality assessment schemes in addition to other valuable soil quality indicators.

3.1 Introduction

Soil organic carbon (SOC) is one of the most widely used soil quality indicators together with pH and available P and K (Bünemann et al., 2018). It affects various soil chemical, physical and biological properties and plays a primary role in multiple soil functions in agricultural soils, such as nutrient cycling, soil aggregate formation, water retention and habitat provision for biodiversity (Reeves, 1997a). Soil organic carbon also plays an important role in climate regulation, with the potential of increasing carbon sequestration, offsetting fossil-fuel emissions and counteracting yield reduction created by extreme weather events (Lal, 2004). Despite the importance of SOC, its depletion is one of the main threats for agricultural soils. Agricultural measures that are aimed at increasing SOC stocks are therefore becoming a priority worldwide. For example, the "4 per Mille" Initiative (https://www.4p1000.org/) aims at implementing soil management practices such as reduced tillage and the use of cover crops, which can effectively increase SOC stocks (Lal, 2016). Such soil practices have the potential to increase carbon stocks directly via the addition of organic material but also indirectly through promoting aggregate formation, thus improving soil structure (Deb et al., 2015).

Soil organic carbon consists of multiple compounds, from simple to more complex molecules which can have different stability (Deb et al., 2015). Since changes induced by soil practices are often difficult to detect by total SOC measurement (Haynes, 2005a), measuring rapidly changing SOC pools, such as labile carbon pools, might be more informative to assess soil quality (Gregorich et al., 1994b; Wander, 2004; Quanying et al., 2014; Awale et al., 2017).

Labile organic matter in soil mainly originates from the decomposition of plant and faunal biomass, root exudates, and deceased microbial biomass (Bolan et al., 2011). Labile carbon is the SOC pool which is directly available for microbial activity and, hence, is considered to be the primary energy source for microorganisms (Chantigny, 2003; Haynes, 2005a). Addition of organic matter as fertilizer (Gattinger et al., 2012) and reduced tillage will likely increase labile organic carbon (Cooper et al., 2016). In addition, these practices have the potential to enhance carbon and nitrogen cycling as well as soil aggregation, which is one of the primary mechanisms through which organic carbon is sequestered in soil (Panettieri et al., 2015). Therefore, labile carbon has potential as an indicator of soil functions, in particular: nutrient cycling (measured e.g. by soil nutrient contents and C mineralization), soil aggregate formation (measured e.g. by water stable aggregates), carbon sequestration (typically derived from changes in total organic carbon content) and habitat provision for biodiversity (currently assessed by biological indicators such as microbial biomass and abundance of faunal groups).

Multiple labile carbon fractions have been defined in the last thirty years. They are discerned based on the nature of their fractionation methodology, which can be chemical, physical or biological (Haynes, 2005a). Labile carbon fractions determined by chemical fractionation are extracted from the soil with different chemical compounds. Dissolved organic carbon (DOC) represents the organic carbon in the soil solution that is extracted with water and passes a mesh with a pore size of 0.45 µm. Hydrophilic DOC (Hy-DOC) represents the more bioavailable part of the DOC (Bolan et al., 2011). DOC and Hy-DOC are small, mainly soluble fractions of total organic carbon (TOC), primarily comprised of root and microbial exudates, products of hydrolysis and leachates from organic matter. Particularly Hy-DOC can turn over very rapidly, while DOC fractions can also adsorb to mineral surfaces (Lundquist et al., 1999; Leinemann et al., 2018). Labile carbon can also be extracted with hot water (hot water extractable carbon, HWEC), which generally has higher concentration in soil than DOC (Ghani et al., 2003b). Permanganate oxidizable carbon (POXC, also referred to as Active Carbon), K2SO4 extractable C, and acid (H2SO4, HCl) hydrolysable C are based on the use of extractants other than water. Although the quantities of HWEC and POXC are similar and both fractions probably comprise carbon derived from dissolved organic matter and microbial biomass, they are most likely derived from different organic matter fractions. HWEC largely (45-60%) comprises carbohydrates and amides derived from soil microorganisms, enzymes, root exudates and lysates, while POXC contains also compounds like lignin and complex polysaccharides (Haynes and Beare, 1997; Ghani et al., 2003b). HWEC is mainly present in the soil solution or loosely bound to soil minerals, and is prone to short-term seasonal variation (Leinweber et al., 1995). Physical fractionation by particle size or density determines particulate organic matter carbon (POMC) which consists mainly of partially decomposed organic residues (Haynes, 2005a) and contains microbial biomass together with fresh plant residues and decomposing organic matter (Gregorich et al., 1994; Sequeira and Alley, 2011). Finally, microbial biomass carbon (MBC) and mineralizable C are also considered labile organic carbon fractions (also called biological fractions), and they are normally determined by soil fumigation and measurement of evolved CO_2 produced by microbial respiration in closed or open incubation systems (Vance et al., 1987; Haynes, 2005a).

Many studies have used labile carbon to assess the impact of agricultural management and land use change on soil quality (Mirsky et al., 2008; Ibrahim et al., 2013; Geraei et al., 2016; Awale et al., 2017). In addition, previous studies also compared different labile carbon fractions for their sensitivity to management (Dou et al., 2008; Culman et al., 2012; Geraei et al., 2016). However, still remains unresolved which labile carbon fraction is the most sensitive to management and can be usefully related to soil functions, and as such be used as a sensitive soil quality indicator. Different fractions have been suggested

as the most sensitive to soil management, and various methodologies and protocols have been applied, hampering comparisons between studies (Poeplau et al., 2018). Moreover, the linkage between labile carbon fractions and soil functions is often assumed and not established (Bünemann et al., 2018), and the generality of applying labile carbon fractions as soil quality indicators as well as the general application of harmonized methods for labile carbon fractions determination has never been assessed across different European pedoclimatic zones and agricultural management systems.

The general objective of this study was to facilitate the assessment of soil quality in agricultural systems by identifying a biochemical parameter that is sensitive to soil disturbance and linked with soil functions. The specific objective of our study was to assess the suitability of five different labile carbon fractions - dissolved organic carbon (DOC), hydrophilic DOC (Hy-DOC), permanganate oxidizable carbon (POXC), hot water extractable carbon (HWEC) and particulate organic matter carbon (POMC) - as soil quality indicators across different pedoclimatic zones. To do so, we tested the sensitivity of the labile carbon fractions to tillage and organic matter input in 10 European long-term field experiments. Monitoring of long term field experiments is essential in soil science for the generalization of conclusions about the effects of specific soil management on soil quality and soil functions (Debreczeni and Körschens, 2003). We assessed the relationship of the different labile carbon fractions with physical, chemical and biological soil properties linked to soil functions, in particular nutrient cycling, carbon sequestration, soil aggregate formation and soil as a habitat for biodiversity. We hypothesised that labile carbon concentrations would increase with reduced tillage and high organic matter input, being more sensitive than TOC. Moreover, we expected that labile carbon fractions would be positively correlated to chemical, physical and biological soil properties currently used as indicators for nutrient cycling, soil organic carbon sequestration, soil aggregation and habitat provision.

3.2 Materials and methods

3.2.1 Experimental sites and management

Ten European long-term field experiments (LTEs) with a minimum duration of 5 years were selected (**Figure 3.1**). Our selection covered different European climatic zones: Dfb and Dfc (continental climate with cold winters and warm summer without a dry season, or with cold winters and temperate summers without a dry season, respectively), Cfb and Csb (temperate climate with warm summer with or without dry season, respectively) and Bsk (arid cold steppe climate) (Köppen, 1918) (**Figure 3.2**, **Table S1**). Also, we covered

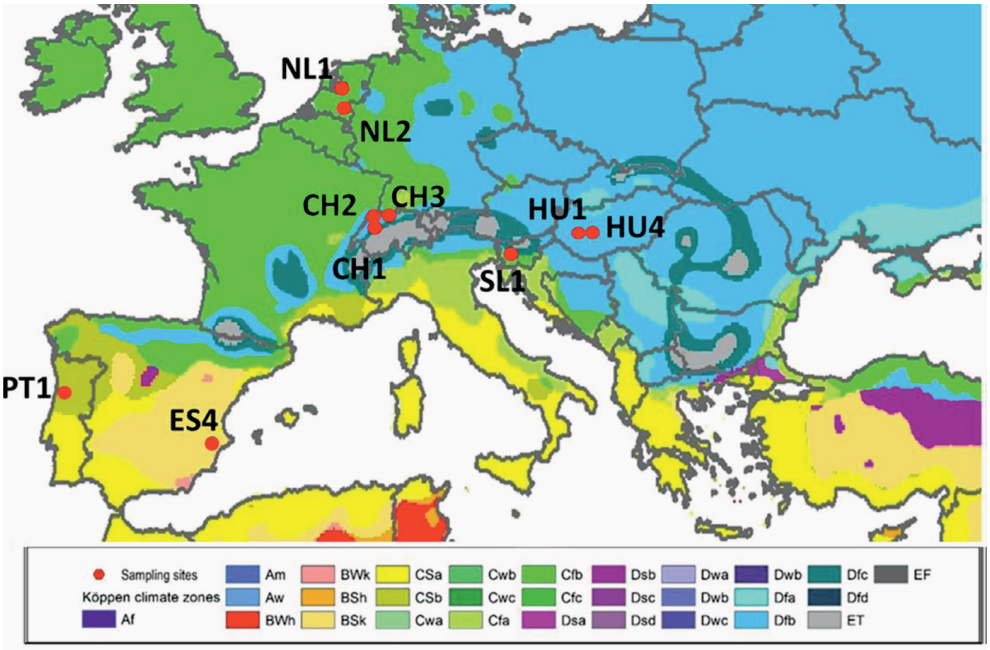

Figure 3.1. Map showing the location of the 10 European long-term field experiments (LTE, here denoted with red dots and called "Sampling site") used in the current study (Peel et al., 2007). The different colours on the map correspond to the Köppen climate zone classification. *CH1* Frick trial, CH2 Aesch trial, *CH3* DOK trial, *HU4* Keszthely trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* De Peel trial, *NL1* Basis trial, *PT1* Vitichar trial, *ES4* Pago trial.

different soil types (Vertic Cambisol, Haplic Luvisol, Fluvisol, Gleyic Podzol, Eutric Gleysol, and Eutric Cambisol (WRB, 2014).

Each LTE had unique management characteristics, but the main agricultural practices studied can be simplified as tillage (T) and organic matter addition (OM) (**Figure 3.2**, **Table S1**). The comparison of farming systems (organic or integrated vs. conventional) studied in three LTEs (CH3, ES4 and NL2) was allocated to the factor OM, even though the treatments differed in other aspects as well (e.g. pesticides input). For NL1, SL1, PT1 and HU1 the organic matter addition was categorised based on the type of organic matter addition (mineral or no organic matter addition vs. organic matter addition). The contrast in tillage was categorised as conventional tillage (ploughing to 20-25 cm depth, CT) versus reduced tillage (tillage to 0-10 cm, RT) and studied in six LTEs (CH1, CH2, HU4, NL1, NL2 and SL1). The level of OM addition was categorised as low organic matter input (LOW, no organic matter additions or only mineral fertilization) versus high organic matter input (HIGH, organic matter additions or organic matter additions with mineral fertilizer). At some sites, both treatment factors (i.e. T and OM) were implemented and at others only one of these (**Figure 3.2**). The layout of the LTEs followed different designs, including

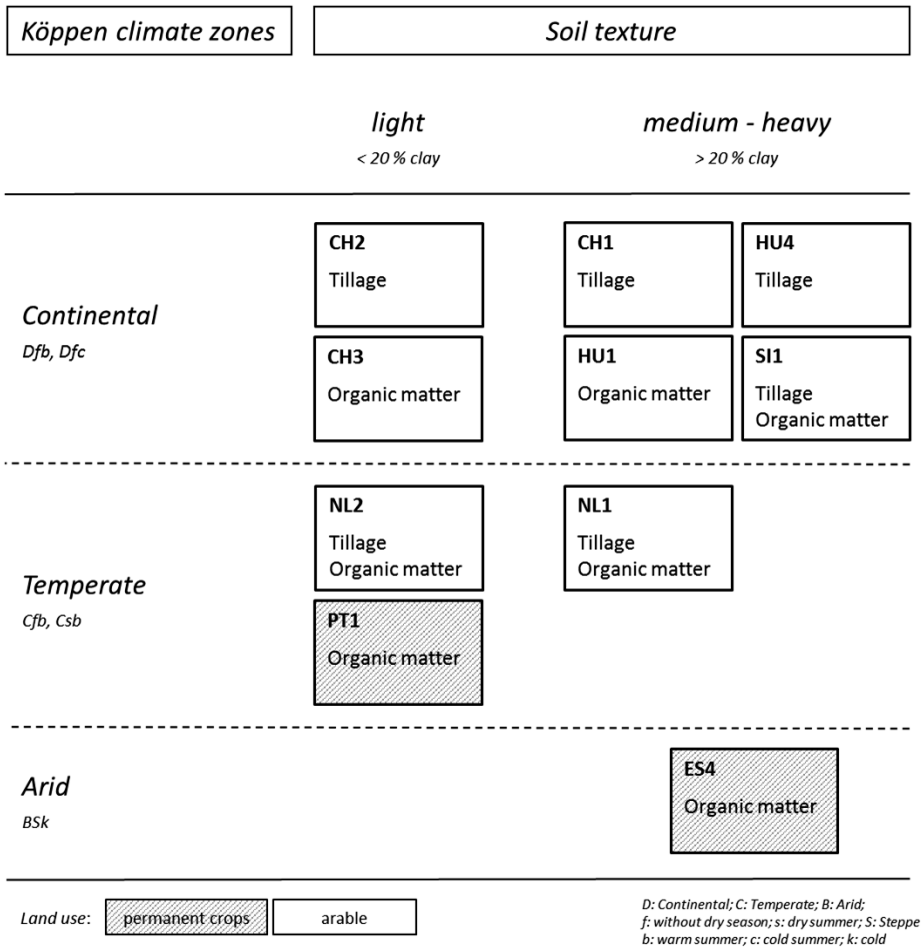

Figure 3.2. Main pedoclimatic characteristics and management practices (categorised in tillage or organic matter input, or a combination of the two practices) of ten long-term field experiments analysed in the current study. *T* tillage, *OM* organic matter addition. *CH1* Frick trial, CH2 Aesch trial, *CH3* DOK trial, *HU4* Keszthely trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* De Peel trial, *NL1* Basis trial, *PT1* Viticher trial, *ES4* Pago trial. For detailed information about the experiments we refer to Table S1 in the supplementary materials.

complete randomized block and split plot design, and per treatment 3 or 4 replicates were present (**Table S1**). Most LTEs had arable crop rotations, but two LTEs (ES4 and PT1) in drier climates had grapes as permanent crops.

3.2.2 Sampling procedure and sample handling

In total, 167 soil samples were collected in spring 2016 before any major soil management was applied to the fields. Each sample comprised 20 soil cores randomly collected in the central area of the plot to avoid border effects. In the trials with tillage as management

factor, samples were taken from two depths: 0-10 cm and 10-20 cm (**Table S1**). In the trials with organic matter input as the only management factor, samples were taken from the 0-20 cm layer. Shortly after collection, fresh soil samples were sent to Wageningen University (The Netherlands), Research Institute of Organic Agriculture (Frick, Switzerland), University of Trier (Germany) and University Miguel Hernandez (Alicante, Spain), and air-dried samples were sent to University of Ljubljana (Slovenia). Upon arrival, fresh samples were sieved at 5 mm and stored at 3˚C. The samples were used for measuring chemical, physical and biological parameters. All the analyses were performed within 6 months after sampling. A part of the samples was subsequently air-dried for POXC and POM-C analysis.

3.2.3 Chemical, physical and biological soil parameters

Various chemical, physical and biological soil parameters, selected to represent soil functions and general soil characteristics, were determined as follows. Total organic carbon (TOC) and total organic nitrogen (TON) were determined by elementary C and N analysis with combustion > 950º by a Vario Max Elemental Analyser. In case of calcareous soils, the samples were pre-treated with HCl to remove inorganic carbon. The pH was measured with a glass electrode WTW pH 538 in 0.01 M $CaCl_2$. Cation exchange capacity (CEC) was determined using a barium chloride solution buffered at pH 8.1. Plant available phosphorus (P_2O_5), plant available potassium (K_2O), and exchangeable magnesium, calcium, sodium and potassium ($Mg2^+$, $Ca2^+$, Na^+, K^+) were determined using ammonium-acetate extraction (van Reeuwijk, 2002). Available phosphorous-Olsen (P-Ol) was determined according to Olsen et al. (1954). These chemical parameters were measured as a *proxy* for the soil functions carbon sequestration and nutrient cycling.

Water-stable aggregates (WSA) were measured by a wet sieving method (Kemper and Koch, 1966) using an apparatus designed by Murer et al. (1993). Particle size distribution was determined by sieving and sedimentation (SIST ISO 11277:2011). Soluble salt and gypsum were removed and organic matter was destructed. Material between 0.063-2 mm was wet-sieved, while material <0.063 mm was determined by sedimentation. Water holding capacity (water content at field capacity, pF 2.5) was calculated using the particle size distribution characteristics and the organic carbon content as described in Tóth et al. (2015). Water stable aggregates and water holding capacity were measured as a *proxy* for the soil functions soil structure formation and water retention.

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined with the method of chloroform-fumigation extraction of Vance et al. (1987), using 0.01 M CaCl2 as extractant. Concentrations of dissolved C and N in fumigated and non-fumigated subsamples were determined with a Shimadzu TOC Analyzer (V CPN E200V), and MBC and MBN were calculated as the difference between fumigated and non-fumigated

subsamples, with conversion factors of 0.45 and 0.4 for incomplete extraction of microbial C and N, respectively (Vance et al., 1987). To assess basal soil respiration (SR), moist samples (approx. 60% of WHC) were incubated at 25ºC for 72 h in a thermostat bath where the bottles were connected to a respirometer (Micro-Oxymay, Columbus, OH, USA). The CO_2 rate was determined when it stabilized at 72 h from the beginning of the incubation. Metabolic quotient (qCO_2) and microbial quotient (qMic) were calculated as the ratio of soil respiration to microbial biomass carbon and the ratio of microbial biomass carbon to total organic carbon, respectively (Anderson and Domsch, 1990). Earthworms were collected in sampling plots of 30x30 cm with a mixed method consisting of hand sorting the top 30 cm and irritating with mustard solution (10 L per plot). The mustard solution comprised 6 g of dry powder mustard that was mixed with 1 L of water, and this solution was added to the excavated soil pit. In the lab, the earthworms were stored overnight at 15 °C in a jar with moist tissue, to allow them to void their gut. All individual earthworms were afterwards counted and weighed, and for the individuals that were damaged only the body parts containing the head were counted. Microbial biomass carbon and nitrogen, ecological indices and earthworm biomass and abundance were measured as *proxies* for the soil habitat function. Basal respiration was measures as a *proxy* for soil nutrient cycling.

The chemical and physical parameters were assessed at the University of Ljubljana, while microbial biomass was assessed at the University of Trier and basal soil respiration at the University Miguel Hernandez. Other physical and biological properties were assessed in the fields by the long-term field experiment owners. Soil bulk density (BD) was determined with calibrated sample cylinders of 100 cm^3 and special augers (Ø 0.05m, Eijkelkamp, NL) that were used to take undisturbed soil samples in one or two layers, depending on the tillage treatment. The soil bulk density was calculated as follows:

$$Bulk\ density\ [gcm^{-3}] = \frac{dry\ weight\ [g]}{ring\ volume\ [cm^3]}$$

The measurement of plant residue decomposition was based on the decomposition of green tea or rooibos tea in bags, as described by Keuskamp et al. (2013). Briefly, per plot, four tea bags of each tea type were weighed and buried 8 cm deep. After approximately 90 days, the tea bags were recovered, dried for 48 h at 70 °C and weighed. In CH1 and CH2, fine material entered the tea bags and influenced the results. Therefore, to get a more precise estimation, the content of the tea bags was combusted at 550ºC and the final weight after combustion (which consisted only of soil particles) was subtracted from the content weight before combustion. Penetration resistance was determined using penetrometer loggers, with different instruments used by the different LTE owners. Per plot, 10 probes were made of which the results were averaged. The soil resistance pressure was measured until 50 cm depth for every 5 cm.

3.2.4 Labile carbon measurements

Dissolved organic carbon (DOC) and Hydrophilic DOC (Hy-DOC)

Twenty g of field moist soil was used to extract dissolved organic carbon (DOC) as described in Van Agtmaal et al. (2017) and adapted as follows. Briefly, the samples were mixed with ultrapure water at a soil-to-solution ratio of 1:2 (dry wt/vol) in DOC-free polypropylene tubes, shaken for 1 hour, centrifuged for 20 minutes at 3750 rpm and subsequently for 10 minutes at 10000 rpm. The samples were then filtered at 0.45 µm with cellulose acetate Whatman® Puradisc membrane filters to obtain total DOC. Filters were pre-rinsed with ultrapure water and flushed with air to avoid any release of DOC during filtration. A fraction of the DOC obtained was subsequently acidified to pH 1 with 6 M HCl to extract the hydrophilic part of the DOC (Hy-DOC) using a simplified DOC fractionation scheme adapted from Van Zomeren and Comans (2007). During the fractionation the hydrophobic components of DOC present in solution (humic and fulvic acid, and hydrophobic neutrals) bind to an added insoluble polymeric adsorbent (Supelite™ DAX-8, Sigma-Aldrich). Only the hydrophilic part of the DOC remains in solution not binding to the resin and can subsequently be quantified. Briefly, the DAX-8 resin was added to the acidified solutions to reach a ratio of 1:5 (wt/vol). The solution was then shaken horizontally for one hour at 180 rpm, centrifuged for 5 minutes at 3750 rpm, and the supernatant containing the hydrophilic part of DOC was collected. The total carbon (C) concentration of both the DOC solution and the supernatant was determined on a TOC-5050A analyser (Shimadzu Corporation, Kyoto, Japan). DOC and Hy-DOC fractions were further analysed for specific ultraviolet absorbance (SUVA) to assess their aromaticity (Weishaar et al., 2003). To this end, 1.5 ml extracted DOC and Hy-DOC from each sample were analysed with a spectrophotometer (Genesys 10S UV-VIS, Thermo Fisher Scientific Inc., Waltham MA, USA) and ultrapure water was used as a blank. The aromaticity of the two fractions expressed by the SUVA (L g C^{-1} cm^{-1}) at 254 nm was calculated as described in Weishaar et al. (2003) and adapted by Amery et al. (2008):

$$SUVA = \frac{A_{254} * 1000}{b * [DOC]}$$

Where A_{254} is absorbance at 254 nm (dimensionless), b is the path length (cm) and DOC (or Hy-DOC) is the dissolved organic carbon concentration (mg L^{1}) of the solution.

Hot water extractable carbon (HWEC)

Hot water extractable carbon (HWEC) was determined according to the methodology of Ghani et al. (2003b). Briefly, 4 g of soil was mixed with 30 ml of deionized water in a 50 ml polypropylene centrifuge tube. The tube was shaken horizontally for 30 minutes at 150 rpm and centrifuged for 20 minutes at 3500 rpm. The supernatant obtained at this

stage (water-soluble carbon) was discarded. An additional 30 ml of deionized water was added to the sediments remaining in the tube and the tube was shaken for 10 seconds to suspend the soil in the water. Subsequently, the closed tubes were placed in an oven at 80°C for 16 hours. After this step, the tubes were shaken for 10 seconds in a vortex shaker and centrifuged for 20 minutes at 3500 rpm, and additionally for 10 minutes at 10000 rpm if necessary (to bring down the solid). The supernatants were filtered using 0.45 µm cellulose nitrate filter membranes and total carbon was determined on a TOC-5050A analyser (Shimadzu Corporation, Kyoto, Japan).

Permanganate oxidizable carbon (POXC)

The permanganate oxidizable carbon (POXC, also referred to as Active Carbon) was extracted and analysed following the procedure of Weil et al. (2003a) modified as follows. Briefly, 2.5 g of air-dried soil was weighed into a polypropylene tube and 18 ml of demineralized water and 2 ml of 0.2 M K_2MnO_4 was added. The tube was shaken for 2 minutes at 120 rpm and thereafter left undisturbed on a lab bench for 8 minutes to continue the oxidation reaction. Subsequently, 0.5 ml of solution was taken from the tube and placed in another tube with 49.5 ml of demineralized water, allowing the reaction to stop. The absorbance of each sample at 550 nm (Abs) was determined using a GENESYS 10S UV-VIS Spectrophotometer. Permanganate oxidizable carbon was calculated according to Weil et al. (2003a):

$$POXC(mg\ kg^{-1}) = [0.02\ mol\ L^{-1} - (a + b * \text{Abs})] * (9\ \text{kg C}\ mol^{-1})(0.02\ L\ solution\ Wt^{-1})$$

where 0.02 mol L^{-1} is the concentration of the K_2MnO_4 solution, *a* is the intercept and *b* is the slope of the standard calibration curve, 9 kg is the amount of carbon oxidized by 1 mol of MnO_4 changing from Mn^{+7} to Mn^{+4}, 0.02 L is the volume of the K_2MnO_4 reacting with the samples, and Wt is the mass of soil in kg used for the reaction.

Carbon from particulate organic matter (POMC)

The particulate organic matter was characterized as reported by Wyngaard et al. (2016) modified from Salas et al. (2003). Briefly, 10 g of dry soil samples was shaken for 15 hours with 30 ml of 1 M NaCl on a horizontal shaker. Subsequently the suspension was wet-sieved through a 53 µm sieve. The material on top of the sieve was transferred to a crucible and dried overnight at 105˚C. The samples were weighted (M1) and placed in a furnace at 550˚C for 4 hours before weighing them again (M2). The POM was calculated by loss of ignition, i.e. as the weight loss during combustion at 550˚C in the muffle furnace. The POMC was calculated dividing POM values for 1.724, assuming that the percentage of organic carbon in the POM was 58%. This conversion factor has been criticized and might

not be completely correct, but for the purpose of this study we needed an approximation and small differences in the C content of POM will not compromise the use of the calculated POMC (Pribyl, 2010).

Labile carbon and TOC stocks

Labile carbon and TOC stocks were calculated in the different layers taken into account in the study as:

$$C\ stock(Mg\ C\ ha^{-1}) = \left[BD\left(\frac{g}{cm^3}\right) * Soil\ depth\ (cm) * Labile\ C\ concentration\ (g\ kg^{-1})\right] * 100$$

Where *BD* is the bulk density expressed in g cm^{-3}, *soil depth* is the soil layer sampled, and *Labile C concentration* is the concentration of labile carbon measured in g kg^{-1}. For the LTEs where the two layers were sampled, C stocks were calculated in the two layers separately and then added to obtain the value of the stocks in the 0-20 cm layer.

3.2.5 Statistical analysis

All statistical calculations were carried out using R version 3.3.2 (R Development Core Team, 2013). For the linear mixed effects model, the packages *nlme* (Pinheiro et al., 2018) and *emmeans* (Lenth et al., 2018) were used, while for the correlation analysis the packages *car* (Fox and Weisberg, 2011) and *stats* were used.

The effects of soil management on the labile carbon fractions (presented either in mg kg^{-1}, percentage of TOC or as C stocks) per site across the 10 European long-term field experiments were assessed using linear mixed effects models. Mixed models were used to take into account the possible correlations introduced by the multi-site field experiments and to generalize the effect of the management practices across the different LTEs (Lucas and Weil, 2012; Bradford et al., 2013). The tillage and/or the soil organic matter addition and, if distinguished, the layer, their two-way and, if applicable, three-way interactions were used as fixed factors. Random effects of trials, blocks, main plots and subplots were introduced in the models to represent the experimental designs of the different trials. The effect of the soil pedoclimatic zone was not included in the fixed part of the model because we were interested in the management effects across the pedoclimatic zone. Three separate linear mixed effect models were applied to three subsets of the LTEs:

1- Tillage model. The primary factor of interest in this analysis was tillage, followed by xo assess the effect of tillage and layer, the LTEs CH1, CH2, NL1, NL2, SL1 and HU4 were used and the analysis was performed on data from both layers (0-10 cm and 10-20 cm). For these trials, the stratification ratio for the labile carbon fractions in RT and CT was calculated and analysed in the linear mixed effect model according to Franzluebbers (2002) :

$$Stratification\ Ratio = \frac{Labile\ carbon\ mg\ kg^{-1}\ in\ 0{-}10\ cm}{Labile\ carbon\ mg\ kg^{-1}\ in\ 10{-}20\ cm}$$

2- OM model. The primary factor of interest in this analysis was the OM addition, followed by tillage. For this analysis, the LTEs analysed were NL1, NL2, SL1, CH3, HU1, PT1, ES4 and the 0-20 cm layer was used. In the LTEs in which the two layers were sampled separately, the value of the 0-20 cm layer was taken as the average of the 0-10 and the 10-20 layers.
3- Stocks model. The factors of interest in this analysis were tillage and OM addition. For the analysis of the labile carbon stocks (Mg ha^{-1}) in the 0-20 layer, all ten trials were used.

The effect of agricultural management and the layer, if applicable, on the labile carbon concentrations was assessed in each long-term field experiment with linear mixed effect models.

The effects of tillage and fertilization and their interaction on the labile carbon fractions were addressed by performing F-tests (using the function *anova*) for the fitted linear mixed effect model. For all the studied variables, the model assumptions of normality and homogeneity of variances of the residuals were checked both visually (plotting sample quantiles versus theoretical quantiles and residuals versus fitted values) and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). Variables whose residuals did not meet these assumptions were log-transformed or square root-transformed and then used for analysis. If the transformation did not meet the criteria, the function *weights* was used in the linear mixed model effect formula to take into account the non-homogeneous variance structure introduced by the factors studied (Zuur, 2009). The function *emmeans* was used to estimate the marginal means and Tukey HSD post-hoc tests were used to assess significant differences between treatments when the F-tests indicated statistically significant effects. All test results were considered statistically significant at $p \leq 0.05$.

Spearman's rank correlation was used to examine the relationships between labile carbon fractions and biological, physical and chemical soil quality parameters across the LTEs. Correlation analysis was done on log-transformed or square root-transformed variables. The relationship between labile carbon fractions and soil parameters was validated using partial correlations, correcting for variation caused by the intrinsic differences of the LTEs (pedoclimatic zones). Partial correlations can, in fact, remove the effect of a variable (in this case the LTE) which might control the observed relationship between two variables. When partial correlations are applied, the relationship between two variables is independent from the controlling variable. In addition, we calculated the average correlation coefficients between the labile carbon fractions and the three indicators' groups (chemical, physical and biological), and the overall average correlation coefficient with the entire set of soil parameters.

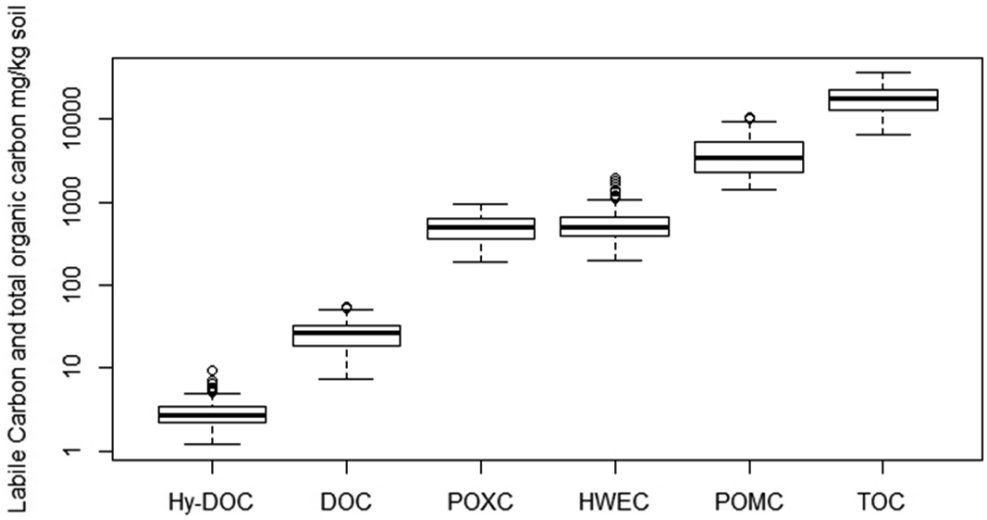

Figure 3.3. Box plot of the concentrations of hydrophylic dissolved organic carbon (Hy-DOC), dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), hot water extractable carbon (HWEC), particulate organic matter carbon (POMC), and total organic carbon (TOC) in mg kg^{-1} soil across all 10 LTEs (n=167). We report a logarithmic y-axis. The boxes represent the values between the 25th and the 75th percentiles, the thin lines represent the minimum and the maximum values and the thick line is the median. The open dots are outliers.

3.3 Results

The concentrations of DOC, Hy-DOC, POXC, HWEC and POMC differed widely between the LTEs, but their order of magnitude was consistent across the 10 LTEs (**Figure 3.3**, **Table S2**).

Hy-DOC was the least abundant fraction per unit of soil or per unit of total organic C (0.004-0.050 % of TOC), followed by DOC (0.06-0.40% of TOC), POXC (1.45-4.32% of TOC), HWEC (1.0-6.0% of TOC) and finally POMC (8-52% of TOC). In comparison, microbial biomass carbon was intermediate between DOC, POXC and HWEC (0.12-2.84% of TOC). POXC and HWEC were similar in their concentration and total share in the TOC. Among the labile carbon fractions and across all the LTEs, the fraction with the lowest coefficient of variation (calculated using all data points) was POXC (32%), followed by DOC (42%), Hy-DOC (43%), HWEC (51%) and POMC (52%). Most labile carbon fractions had lower concentrations in the lower than in the upper layer, with the exception of DOC, which was often higher in the lower layer. The LTEs HU1 and PT1 had the lowest concentrations of labile carbon across the different fractions. We did not find specific LTEs that had consistently higher or lower labile carbon fractions expressed as percentage of TOC. Table S3 shows the results of the analysis of the effect of the soil management on the labile carbon fractions for each of the LTEs.

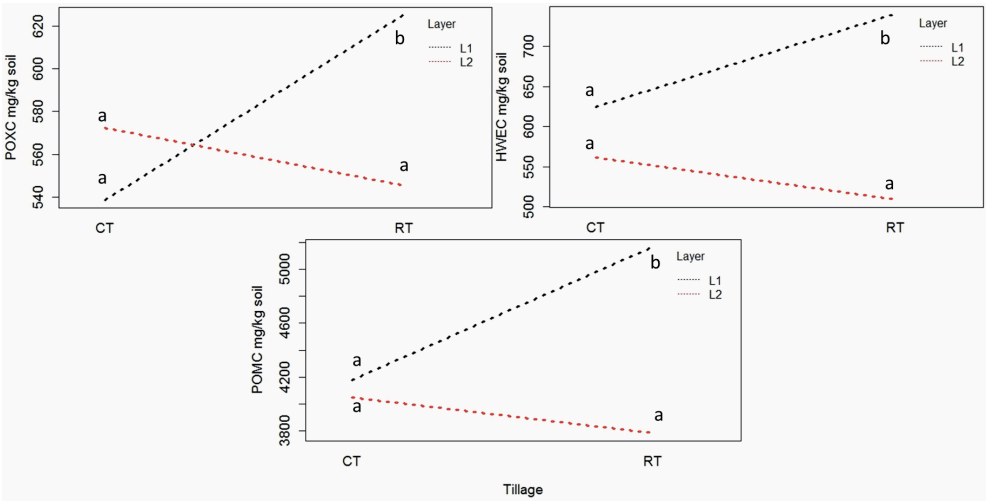

Figure 3.4. Interaction plots showing the 2-way interaction between tillage and layer (L1, L2) for the variables POXC, HWEC and POMC expressed in mg kg^{-1} soil for the tillage trials as analysed with mixed linear effects models (number of observations= 120). Different letters show the treatments which are significantly different ($p \leq 0.05$) according to Tukey post-hoc test for the 2-way interaction. POXC permanganate oxidizable carbon; HWEC hot water extractable carbon; POMC particulate organic matter carbon; CT conventional tillage; RT reduced tillage; L1 0-10 cm and L2 10-20 cm soil depth. Note that the y-axes do not start at zero.

3.3.1 Effect of tillage on the labile carbon fractions

The labile carbon fractions differed in their sensitivity to tillage (**Table 3.1**).

Looking at the *F* statistics, POXC and POMC (mg kg^{-1} soil) were the fractions most sensitive to tillage. However, there was a significant interaction between tillage and layer for POXC, HWEC and POM, since concentrations of these three fractions were higher under RT than CT in the upper layer only (**Figure 3.4**).

Accordingly, we found higher values of stratification ratio in RT than CT with both LOW and HIGH organic matter input for POXC, HWEC and POMC (**Table S4**). For Hy-DOC and DOC, the stratification ratio was higher in RT, but only with low organic matter input. For TOC only a trend ($p=0.057$) of higher values under RT was found. When expressed as percentage of TOC, the labile carbon fractions were not affected by the tillage treatment but only by the layer (**Table S5** and **Figure S1**). In the same way, the aromaticity of the DOC and Hy-DOC as measured by $SUVA_{254}$ was not affected by the tillage treatments across the sites, but DOC SUVA was affected by the layer (**Table S6** and **Figure S2**). The significant interaction that we found means that in the reduced tillage plots, the aromaticity of DOC was greater in the lower than in the upper layer.

Table 3.1. Effects of tillage (CT vs. RT), organic matter addition (LOW vs. HIGH), and layer (0-10 cm and 10-20 cm) on the labile carbon fractions for the tillage trials as analysed with mixed linear effects models (number of observations= 120). In the upper part of the table the estimated means and 95% confidence intervals (in parentheses) of Hy-DOC, DOC (mg kg^{-1} soil), POXC, HWEC, POMC and TOC (g kg^{-1} soil) under tillage and organic matter (OM) management are reported. Different letters following means have to be read per columns and per layer; they show treatments which are significantly different ($p \leq 0.05$) according to Tukey post-hoc tests for the three way interactions. In the lower part of the table, F statistics and p-values (values ≤0.05 are given in bold) for the main factors and their interactions are reported.

		Hy-DOC	DOC	POXC	HWEC	POMC	TOC
Layer 0-10 cm		(mg kg^{-1} soil)		(g kg^{-1} soil)			
CT- LOW		2.6ab (1.97-3.38)	22.1a (14.6-31.2)	0.50 (0.39-0.66)	0.53 (0.46-0.96)	3.7 (1.95-5.44)	20 (13.9-25.7)
RT- LOW		3.2b (2.43-4.17)	27.7ab (19.2-37.8)	0.63 (0.48-0.77)	0.72 (0.50-1.03)	4.9 (3.15-6.74)	21 (15.6-27.3)
CT- HIGH		3.2ab (2.41-4.66)	26.4ab (17.8-36.7)	0.55 (0.42-0.70)	0.66 (0.37-0.75)	4.4 (2.62-6.25)	23 (17.1-28.7)
RT- HIGH		3.1ab (2.36-4.17)	25.6ab (17.2-35.7)	0.60 (0.47-0.77)	0.71 (0.49-1.03)	5.1 (3.13-7.09)	24 (17.4-30.4)
Layer 10-20 cm							
CT- LOW		2.6ab (2.01-3.49)	27.5b (19.0-37.5)	0.53 (0.42-0.68)	0.53 (0.37-0.76)	3.5 (1.80-5.29)	19 (13.4-24.7)
RT- LOW		2.4a (1.85-3.18)	25.5ab (17.3-35.2)	0.51 (0.39-0.65)	0.48 (0.33-0.68)	3.3 (1.53-5.13)	19 (13.5-25.1)
CT- HIGH		3.5ab (2.61-4.66)	27.9ab (19.1-38.6)	0.57 (0.44-0.73)	0.57 (0.39-0.82)	4.3 (2.51-6.14)	19 (12.8-25.1)
RT- HIGH		3.5b (2.63-4.71)	29.4ab (20.3-40.3)	0.54 (0.43-0.71)	0.52 (0.36-0.76)	4.0 (2.06-6.02)	18 (12.6-24.3)
Tillage (T)	F p	0.33 0.56	0.45 0.64	3.81 **0.04**	0.95 0.33	7.29 **0.01**	0.09 0.75
OM	F p	10.16 **0.002**	0.82 0.37	2.72 0.09	2.38 0.13	11.25 **0.003**	6.7 **0.01**
Layer (L)	F p	0.12 0.72	5.5 **0.02**	3.81 **0.04**	22.7 **<0.0001**	15.4 **0.0002**	44 **<0.0001**
T X OM	F p	0.42 0.51	0.27 0.60	1.73 0.19	1.4 0.24	0.7 0.38	2.7 0.11
T X L	F p	3.33 0.07	2.25 0.13	19.18 **<0.0001**	9.5 **0.003**	28.3 **<0.0001**	2.2 0.14
OM X L	F p	9.57 **0.003**	0.28 0.59	1.03 0.31	0.09 0.76	0.28 0.59	12.5 **0.008**
T X OM X L	F p	5.15 **0.02**	7.46 **0.008**	2.67 0.10	1.91 0.17	0.58 0.44	0.009 0.92

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *TOC* total organic carbon, *LOW* low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage

3.3.2 Effect of OM addition on the labile carbon fractions

All labile carbon fractions were significantly higher in high OM compared to low OM input trials (**Table 3.2**).

In the analyses, the type of tillage applied to the plots was also taken into account. POXC, HWEC and POMC (mg kg^{-1}) were significantly increased in RT compared to CT plots. POXC, Hy-DOC and POMC (mg kg^{-1}) were the more sensitive labile carbon fractions (taking into account the *F* statistics). When labile carbon fractions were expressed as percentage of total organic carbon, only Hy-DOC, POXC and POMC were significantly higher in the high OM compared to low OM input trials (**Table S7**). In addition, the positive effect exerted by the high organic matter input on POXC was stronger in trials with CT. Aromaticity of DOC and Hy-DOC as measured by $SUVA_{254}$ was not affected by organic matter addition across all the sites (**Table S8**).

Table 3.2. Effects of organic matter (OM) addition (LOW vs. HIGH) and tillage (CT vs. RT) on the labile carbon fractions for the OM input trials in the 0-20 cm layer as analysed with mixed linear effects models (number of observations = 119). In trials where the 0-10 cm and the 10-20 cm layers were sampled separately, we averaged the C values over the two layers. In the upper part of the table the estimated means and 95% confidence intervals (in parentheses) of Hy-DOC, DOC (mg kg^{-1} soil), POXC, HWEC, POM-C and TOC (g kg^{-1} soil) under OM and tillage management are reported. In the lower part of the table F statistics and p-values (values ≤0.05 are given in bold) for the main factors and their interactions are reported.

		Hy-DOC	DOC	POXC	HWEC	POMC	TOC
Layer 0-20 cm		(mg kg^{-1} soil)		(g kg^{-1} soil)			
LOW-CT		2.2 (1.71-2.91)	23.4 (14.9-33.7)	0.40 (0.26-0.53)	0.38 (0.28-0.53)	2.4 (1.80-3.22)	16.2 (11.6-20.8)
LOW-RT		2.2 (1.63-3.00)	24.6 (15.1-36.5)	0.44 (0.31-0.58)	0.46 (0.32-0.66)	2.8 (2.07-3.86)	15.5 (10.8-20.2)
HIGH-CT		2.9 (2.24-3.78)	27.4 (18.3-38.4)	0.47 (0.34-0.60)	0.47 (0.34-0.64)	2.8 (2.09-3.74)	17 (12.4-21.6)
HIGH-RT		3.0 (2.27-4.05)	27.8 (18.2-39.5)	0.50 (0.37-0.63)	0.53 (0.37-0.74)	3.1 (2.27-4.17)	16.8 (12.1-21.3)
OM	F p	35.1 **<0.0001**	8.9 **0.006**	45.0 **<0.0001**	12.0 **0.0002**	18.5 **0.0002**	12.6 **0.001**
Tillage (T)	F p	0.05 0.82	0.02 0.89	6.5 **0.02**	3.9 **0.05**	5.8 **0.02**	1.13 0.29
T X OM	F p	0.16 0.68	0.01 0.93	0.70 0.39	0.22 0.64	1.18 0.28	1.18 0.28

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *TOC* total organic carbon, *LOW* low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage

3.3.3 Effect of tillage and OM addition on the labile carbon stocks across the 10 LTEs

Reduced tillage and high OM input both significantly increased labile carbon stocks expressed in Mg ha^{-1}, i.e. stocks of all the fractions (**Table 3.3**).

POXC and POMC were affected most by the two management factors, as indicated by higher *F* statistics. The TOC stock was less sensitive than the stocks of labile C fractions, being affected neither by organic matter addition nor by tillage.

Table 3.3. Effect of organic matter OM) input (LOW vs. HIGH) and tillage (CT vs. RT) on the labile carbon stocks in the soil layer 0-20 cm expressed in Mg C ha^{-1} for all the trials as analysed with mixed linear effects models (number of observations = 101). In the upper part of the table the estimated means and 95% confidence intervals (in parenthesis) of the labile carbon fractions and TOC in organic matter and tillage management are reported. The lower part of the table shows F statistics and p-values (values <0.05 are given in bold) for the main factors and their interactions.

		Hy-DOC	DOC	POXC	HWEC	POMC	TOC
Layer 0-20 cm		(Mg C ha^{-1})					
LOW-CT		0.009 (0.006-0.012)	0.071 (0.05-0.09)	1.42 (1.05-1.91)	1.77 (1.14-2.39)	11.40 (6.74-16.10)	83.6 (44.03-123.2
LOW-RT		0.012 (0.009-0.015)	0.093 (0.06-0.12)	1.73 (1.24-2.29)	2.05 (1.39-2.76)	13.55 (8.77-18.33)	84.5 (44.80-124.3)
HIGH-CT		0.012 (0.009-0.014)	0.089 (0.07-0.12)	1.70 (1.28-2.33)	2.09 (1.53-2.81)	14.09 (9.38-18.79)	84.4 (44.79-124.0)
HIGH-RT		0.013 (0.010-0.016)	0.103 (0.07-0.13)	1.87 (1.37-2.55)	2.25 (1.61-2.98)	16.09 (11.31-20.87)	82.3 (42.52-122.0)
OM	F p	13.4 **0.0009**	12.7 **0.001**	32.0 **<0.0001**	14.3 **0.0007**	29.4 **<0.0001**	0.28 0.59
Tillage (T)	F p	7.95 **0.008**	5.32 **0.027**	10.7 **0.002**	6.87 **0.01**	12.8 **0.001**	0.17 0.67
T X OM	F p	3.72 0.06	0.84 0.36	2.38 0.13	0.54 0.46	0.01 0.89	0.64 0.43

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *TOC* total organic carbon, *LOW* low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage

3.3.4 Correlation of labile carbon fractions with other soil quality parameters

We tested the bivariate relationships between the labile carbon fractions and soil chemical, physical and biological indicators across both soil layers where applicable. In addition to bivariate correlations, we validated the obtained relationships by carrying out partial correlations where we corrected for the variation caused by the LTE (**Table 3.4**).

Table 3.4. Partial correlation coefficients (ρ) between the labile organic carbon fractions expressed in mg kg^{-1} soil (Hy-DOC, DOC, POXC, HWEC and POMC) and % (TOC) and various soil chemical, physical and biological indicators used as dependent variable, corrected for the long term field experiments (LTEs). The number of samples used in the analyses was 167, but 101 for earthworm number, and earthworm biomass. In the table also the average correlation coefficients for each indicator group (chemical, physical and biological indicators) is reported, in addition to the overall average correlation coefficient (calculated across all the indicators).

	Hy-DOC		DOC		POXC		HWEC		POMC		TOC	
Chemical indicators												
TOC	0.44	***	0.33	***	0.69	***	0.52	***	0.68	***	1	
TON	0.54	***	0.42	***	0.73	***	0.57	***	0.63	***	0.79	***
CEC	0.18	*	0.27	**	0.43	***	0.24	*	0.23	*	0.35	***
C/N	-0.30	***	-0.39	***	-0.54	***	-0.36	***	-0.21	*	-0.26	**
pH	0.06	-	-0.24	*	0.06	-	0.03	-	0.13	-	0.10	-
P	0.24	*	0.08	-	0.29	**	0.27	**	0.27	**	0.36	***
P Olsen	0.18	*	0.15	*	0.22	*	0.29	**	0.28	**	0.33	***
Mg	0.16	*	0.21	*	0.45	***	0.22	*	0.21	*	0.33	***
Ca	0.24	*	-0.003	-	0.19	*	0.15	*	0.26	**	0.27	**
K	0.16	*	0.15	*	0.40	***	0.29	**	0.33	***	0.50	***
Na	0.15	*	0.11	-	0.02	-	-0.05	-	0.01	-	0.01	-
Average chemical	0.24		0.21		0.36		0.27		0.29		0.33	
Physical indicators												
WSA	0.30	**	0.32	***	0.53	***	0.35	***	0.35	***	0.44	***
WHC	0.19	*	0.19	*	0.30	**	0.28	**	0.25	*	0.49	***
BD	-0.10	-	-0.09	-	-0.28	**	-0.25	*	-0.38	***	-0.31	***
Sand	0.01	-	-0.21	*	0.01	-	-0.02	-	0.07	-	-0.01	-
Silt	0.14	-	0.13	-	0.05	-	0.08	-	0.09	-	0.09	-
Clay	-0.03	-	0.04	-	0.04	-	-0.02	-	-0.13	-	0.03	-
WC	0.20	*	0.20	*	0.24	*	0.12	-	0.32	***	0.29	**
Average physical	0.14		0.17		0.21		0.16		0.23		0.24	
Biological indicators												
MBC	0.40	***	0.13	-	0.59	***	0.52	***	0.53	***	0.54	***
MBN	0.28	**	0.16	*	0.47	***	0.41	***	0.38	***	0.32	***
SR	0.28	**	0.05	-	0.46	***	0.44	***	0.48	***	0.24	*
qCO2	-0.07	-	-0.06	-	-0.15	-	-0.08	-	-0.11	-	-0.37	***
qMic	0.20	*	-0.06	-	0.26	**	0.30	***	0.20	*	0.01	-
Earthworm numbers	0.06	-	-0.16	-	0.07	-	0.02	-	-0.0003	-	-0.07	-
Earthworm biomass	0.05	-	-0.10	-	0.04	-	0.07	-	-0.15	-	-0.18	-
Decomposition	-0.12	-	-0.20	*	-0.34	**	-0.34	**	-0.27	*	-0.23	*
Average biological	0.18		0.11		0.30		0.27		0.26		0.24	
Average overall indicators	0.19		0.17		0.30		0.24		0.27		0.28	

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, HWEC hot water extractable carbon, POMC particulate organic matter carbon, TOC total organic carbon, TON total nitrogen, CEC cation exchange capacity, WSA water stable aggregates, BD bulk density, MBC microbial biomass carbon, MBN microbial biomass nitrogen, SR soil respiration, qCO_2 metabolic quotient, qMIC microbial quotient.
*$p \leq 0.01$, **$p \leq 0.001$, ***$p \leq 0.0001$

POXC was the labile C fraction that was most significantly (*p-values*), and strongly (Spearman's correlation coefficients, ρ), correlated with the soil chemical, physical and biological indicators related to nutrient cycling, soil structure and biodiversity both in the bivariate (**Table S9**) and the partial (**Table 3.4**) correlations. Moreover, POXC proved to be highly positively correlated ($p < 0.0001$) with Hy-DOC (ρ=0.59), DOC (ρ= 0.41), HWEC (ρ= 0.60) and POMC (ρ= 0.70) (Table 5 residuals and S10 original data). The other carbon fractions were correlated with each other but not so strongly, with the only exception of strong positive correlations between Hy-DOC and (in addition to POXC) DOC, HWEC, and POMC ($p < 0.0001$, ρ= 0.41; $p < 0.001$, ρ= 0.41; $p < 0.0001$, ρ= 0.50, respectively) (**Table 3.5**).

Table 3.5. Partial correlation coefficients (ρ) between the labile organic carbon fractions expressed in mg kg^{-1} (Hy-DOC, DOC, POXC, HWEC and POMC) and L g C^{-1} m^{-1} (Hy SUVA and DOC SUVA) used as dependent variable, corrected for the long term field experiments (LTEs). In addition, for comparison with the labile carbon fractions, also the correlation with TOC expressed in mg kg^{-1} has been reported. The number of samples used in the analyses was 167.

	Hy-DOC		**DOC**		**POXC**		**HWEC**		**POMC**		**Hy SUVA**		**DOC SUVA**	
Hy-DOC	1													
DOC	0.41	***	1											
POXC	0.59	***	0.41	***	1									
HWEC	0.41	***	0.24	*	0.60	***	1							
POMC	0.50	***	0.29	**	0.70	***	0.58	***	1					
Hy SUVA	-0.44	***	0.31	***	-0.08	-	-0.06	-	-0.06	-	1			
DOC SUVA	-0.37	***	0.25	*	-0.20	*	-0.20	*	-0.25	*	0.35	***	1	
TOC	0.44	***	0.33	***	0.69	***	0.52	***	0.68	***	-0.07	-	-0.16	*

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *Hy SUVA* hydrophilic specific ultraviolet absorbance, *DOC SUVA* dissolved organic carbon specific ultraviolet absorbance.
$*p \leq 0.01$, $**p \leq 0.001$, $***p \leq 0.0001$

3.4 Discussion

The ranges of labile organic C fractions measured in this study were in accordance with those reported previously (Lucas and Weil, 2012; Benbi et al., 2015; Margenot et al., 2017). Hy-DOC accounted for the smallest part of TOC, followed by DOC, POXC, HWEC and POMC. These results demonstrate that the different methodologies extract different parts of the total organic carbon.

3.4.1 Effect of tillage on the six labile carbon fractions

In the analysis across the six tillage trials, POXC, HWEC and POMC in the upper soil layer were higher in RT than in CT (Figure 3). Several studies reported that RT increases the concentration of soil labile carbon compared to CT (Aziz et al., 2013; Liu et al., 2014; Neogi et al., 2014). Tillage disrupts macro- and micro-aggregates, increases soil temperature and aeration and releases soil organic matter which was protected in these physical structures (Six et al., 1999). Soil organic matter can thus become more available to soil organisms, increasing CO_2 emissions and decreasing the labile fractions. This phenomenon is fostered by the greater transfer of residues, mineral fertilizers and organic amendments to deeper soil layers during conventional tillage. In reduced tillage, on the other hand, the labile carbon protected in aggregates can accumulate in the soil (Jastrow et al., 2006). Under these conditions, microbial biomass and activity can be favoured, increasing the production of enzymes which can increase soil labile C fractions (Melero et al., 2011).

Our study corroborates previous findings which also detected HWEC, POXC and POMC as the labile C fractions that are most sensitive to tillage (Chen et al., 2009; Ćirić et al., 2016), in particular POXC and POMC (Culman et al., 2012; Plaza-Bonilla et al., 2014; Prasad et al., 2016). These fractions were more sensitive than TOC, which confirms the early warning capacity of labile carbon in indicating soil quality disruption due to agricultural practices.

Dissolved organic carbon and Hy-DOC were less sensitive to tillage. Dissolved organic carbon (and consequently the most soluble Hy-DOC fraction) is very much dependent on environmental conditions (i.e. temperature and precipitation), and short-term management (Soon et al., 2007; Mouloubou et al., 2016; Federici et al., 2017). Moreover, in spring, which coincided with our sampling time, the level of DOC is the lowest throughout the year (Haynes, 2005a; Schiedung et al., 2017).

3.4.2 Effect of organic matter addition on the labile carbon fractions

High OM addition increased the concentration of the labile carbon fractions compared to low OM addition. This agricultural practice had greater impact on the labile carbon fractions than tillage, indicating the important role of organic matter addition in increasing

C in soil (**Table 3.3**). Permanganate oxidizable C, Hy-DOC and POMC were more sensitive than the other fractions to OM additions (**Table 3.2**), which is in accordance with Mirsky et al. (2008); Ibrahim et al. (2013); Tatzber et al. (2015). Previous studies found that organic input increases the concentration of soil labile carbon (Benbi et al., 2015; Pezzolla et al., 2015; Tatzber et al., 2015; Li et al., 2018b). Apart from direct effects of organic matter input on labile carbon through addition of organic substrates which stimulate microbial biomass and indirect effects through provision of a suitable physical environment, OM addition can introduce external microbial populations which also can contribute to an increase of the labile organic carbon pools (Bastida et al., 2008).

Some studies did not find effects of tillage and fertilization on the labile C fractions (Sequeira and Alley, 2011; Ladoni et al., 2015; Margenot et al., 2017). This can be due to the soil properties, the non-homogeneous distribution of plant and microbial residues, organic matter input type and quantity, environmental conditions and time of sampling. The soil type, for example, can influence the extent to which agricultural management can affect soil organic carbon. In soils with light texture, organic matter additions can have a higher beneficial effect on TOC and labile carbon than conservation tillage (Chivenge et al., 2007). Our approach, based on the selection of LTEs from different pedoclimatic zones and contrasting soil types, permitted us to identify overall trends correcting for these differences in pedoclimatic zones. Even after such corrections, we found tillage and organic matter additions to have an effect on the labile carbon fractions, and in particular on POXC and POMC.

3.4.3 Labile organic carbon as soil quality indicator

All the labile carbon fractions were positively correlated with each other (**Table 3.5** and **S10**) and also with TOC (**Tables 3.4** and **S9**), indicating that TOC is their main determinant in soil (Geraei et al., 2016; Yu et al., 2017). This suggests that dynamics of labile C fractions can be used as a proxy of TOC dynamics in soils under agricultural management. Labile carbon is, in fact, an essential starting point for the formation of more stable soil organic matter (Cotrufo et al., 2013). Of all labile carbon fractions, POXC and POMC were the two fractions that showed the strongest relationship with TOC. Moreover, POXC and POMC were the labile carbon fractions most sensitive to both tillage and organic matter management. This was true for the concentration per kg soil (**Table 3.1**, **Table 3.2** and **Table 3.3**) and, only for the organic matter addition management, for the concentration per unit TOC (**Table S5** and **Table S7**). By expressing POXC and POMC relative to TOC, any possible interferences from structural differences in total soil organic matter are minimized. Moreover, this normalization to TOC emphasizes generic relationships

affecting labile carbon build-up in the soils, which are not directly related with organic matter additions, such as soil structure and chemical recalcitrance.

POXC was strongly correlated with labile carbon fractions that are extracted with either relatively lower (i.e. Hy-DOC, DOC, HWEC), or higher (POMC) extraction intensity (and bioavailability) than POXC.

As indicated above, POXC responded strongly to tillage and organic matter addition, and differences between sites were relatively small (**Table S2**). Hence, our data suggest that POXC is the most representative labile organic carbon indicator and that its dynamics are the best proxy of TOC dynamics. This agrees with findings of Hurisso et al. (2016), who found that POXC reflected soil management that aimed to increase organic matter content and stability, and suggested that POXC can be a useful indicator of C sequestration.

POXC was also the labile carbon fraction most strongly correlated to various other soil chemical, physical and biological quality parameters (**Tables 3.4** and **S9**). The correlations between POXC, TOC and MBC have been attributed to specific characteristics of the extraction methods used to determine the three fractions (Geraei et al., 2016): the oxidation of POXC mimics microbial decomposition of organic matter, which is confirmed by its often positive correlation with basal respiration, substrate-induced respiration, microbial biomass and soluble carbohydrates (Weil et al., 2003a).

The positive correlation between POXC and HWC, WSA and CEC, which are parameters known to be influenced by more complex organic matter (Wander, 2004), can be explained by the fact that the oxidation during the POXC reaction targets labile but also affects more recalcitrant forms of SOM. Specialized microorganisms can make use of more complex compounds (Lehmann and Kleber, 2015), which could explain the relationship between POXC and microbial biomass and activity even if permanganate reacts with more complex compounds also, as recently confirmed by Romero et al. (2018). Hence, POXC strongly relates to TOC, but also a variety of other soil quality parameters underlining its role as a multifunctional soil quality indicator. Moreover, POXC can be measured relatively cheaply and fast (**Table 3.6**).

The different strengths of the correlations between labile carbon fractions and other soil quality indicators, including TOC, suggest that these fractions quantify distinct parts of the TOC with different functional characteristics.

Currently, little is known about the chemical composition and the seasonal dynamics of POXC. However, there is evidence for the sensitivity of POXC to other types of soil management beside tillage and organic matter input such as the use of cover crops, but this should be validated with further studies (Idowu et al., 2008; Culman et al., 2012). POXC was found to be linked with various soil quality indicators related to multiple soil functions, which is a very important characteristic for effective and informative soil quality

Table 3.6. Time and cost analysis for the labile carbon fractions as calculated according to the methodology applied in the current research, and the prices applied in the Chemical and Biological Laboratory of Wageningen University and Research. Relative time and costs refer to the time and money required for processing permanganate oxidizable carbon (POXC) compared to the other labile carbon fractions.

Labile carbon fraction	Relative time compared to POXC analysis	Relative analysis costs compared to POXC analysis
POXC		
DOC	3x higher	2.4x higher
Hy-DOC	3.5x higher	2.7x higher
HWEC	20x higher	2.4x higher
POMC	32x higher	1.4x higher

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon

indicators. In fact, POXC (named as 'Active Carbon') was included in the Comprehensive Assessment of Soil Health (CASH) framework, where it was recognized as a soil quality indicator besides others biological, chemical and physical parameters. The CASH is available since 2008 (Idowu et al., 2008) and is especially targeted at farmers and land managers, and widely used throughout the USA.

Recently, Fine et al. (2017) found that POXC was the best single predictor of overall soil quality measured using CASH scores. Their study included a large number (n= 930) of samples from different sites in the USA covering different pedo-climatic conditions. Still, the quantitative relationships between currently used indicators and soil functions are generally under-investigated. Therefore, establishing those relationships is of high priority and future studies should particularly address these quantitative linkages.

3.5 Conclusions

The labile organic carbon fractions investigated in 10 LTE fields covering a range of pedoclimatic zones within Europe appeared sensitive to soil management, showing in general increased values in reduced tillage and high organic matter input systems. Our results suggest that the different labile carbon fractions represent different soil organic carbon pools, with POXC and POMC representing pools that appear to be highly sensitive to agricultural management and less variable than the other labile carbon fractions. This makes them more suitable as soil quality indicators than the highly labile DOC and Hy-DOC, HWEC and the slowly changing TOC. In addition, concentrations of POXC and POMC are an order of magnitude higher than Hy-DOC and DOC, which strongly facilitates their

measurement. Moreover, POXC is easily measured at low cost, which makes its use feasible in practice.

POXC represents a labile carbon fraction sensitive to soil management that is highly informative about total soil organic matter, nutrients, soil structure, and microbial pools and activity, parameters commonly used as indicators of various soil functions, such as C sequestration, nutrient cycling, soil structure formation and soil as a habitat for biodiversity. Therefore, we suggest measuring POXC as the labile carbon fraction in soil quality assessment schemes in addition to other valuable soil quality indicators.

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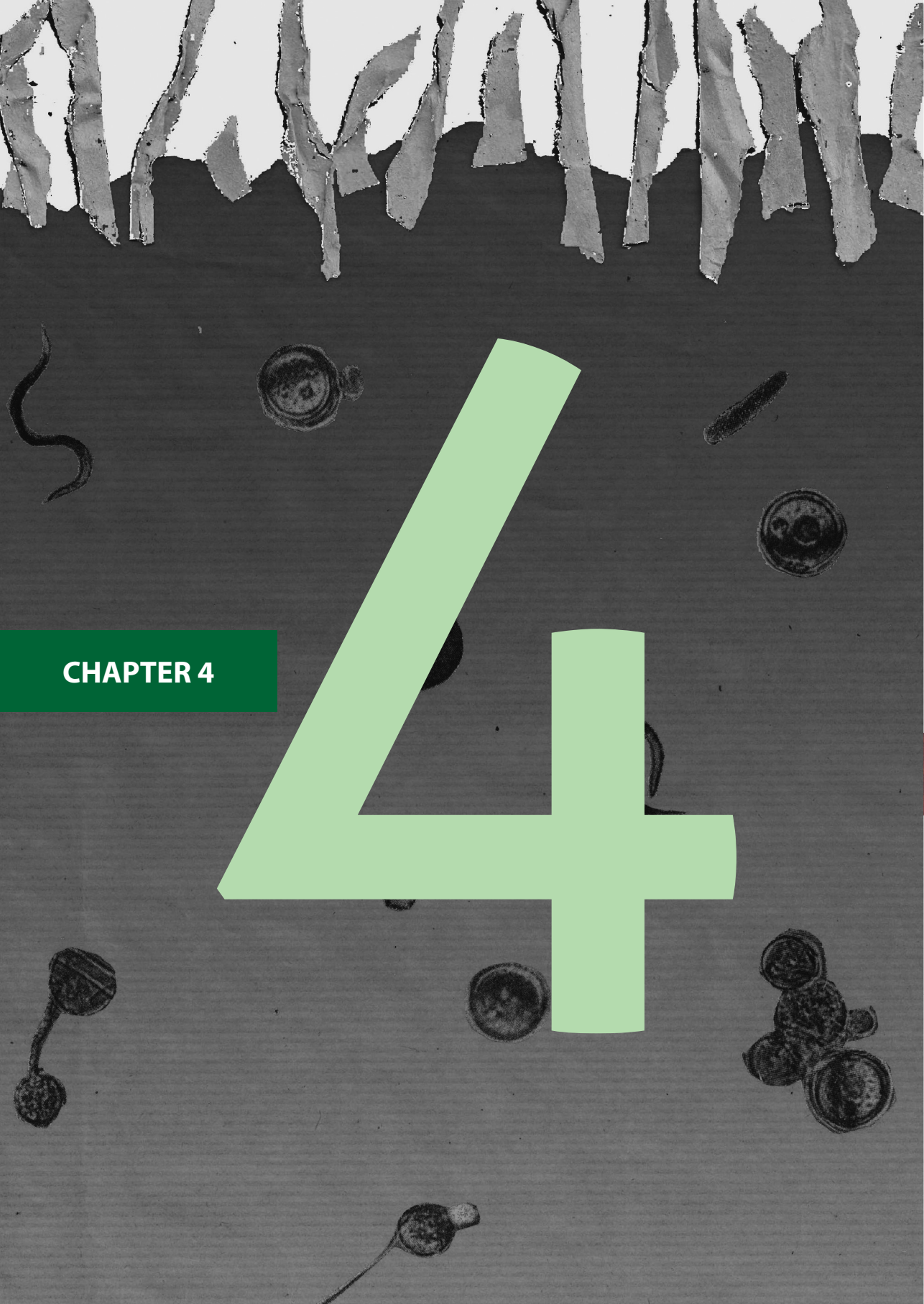
CHAPTER 4
4

Soil suppressiveness to *Pythium ultimum* in ten European long-term field experiments and its relation with soil parameters

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Abstract

Soil suppressiveness to pathogens is defined as the capacity of soil to regulate soil-borne pathogens. It can be managed by agricultural practices, but the effects reported so far remain inconsistent. Soil suppressiveness is difficult to predict and for this reason different soil properties have been linked to it with the aim to find informative indicators, but these relationships are not conclusive. The objectives of this study were i) to test if soil suppressiveness is affected by long-term agricultural management such as tillage and organic matter (OM) addition; ii) to understand the direct and indirect relationships between soil suppressiveness and labile organic carbon fractions; and iii) to understand the relationship between soil suppressiveness and other chemical, physical and biological soil quality indicators. We measured soil suppressiveness with a bioassay using *Pythium ultimum - Lepidium sativum* (cress) as a model system. The bioassay was performed in soils from 10 European long-term field experiments (LTEs) which had as main soil management practices tillage and/or organic matter addition. We found that the site had a stronger influence on soil suppressiveness than agricultural practices. Reduced tillage had a positive effect on the suppressive capacity of the soil across sites using an overall model. Organic farming and mineral fertilization increased soil suppressiveness in some LTEs, but no overall effect of OM was found when aggregating the LTEs. Soil suppressiveness across LTEs was linked mainly to microbial biomass and labile carbon in the soil, but not to total soil organic matter content. From structural equation modelling (SEM) we conclude that labile carbon is important for the maintenance of an abundant and active soil microbial community, which is essential for the expression of soil suppressiveness. However, soil suppressiveness could only partly (25%) be explained by the soil parameters measured, suggesting that other mechanisms contribute to soil suppressiveness such as the presence and the activity of specific bacterial and fungal taxa with high biocontrol activity.

4.1 Introduction

Diseases caused by soil-borne pathogens are among the most important limiting factors for plant growth and productivity in agriculture (Oerke, 2006). Soils can regulate and suppress soil-borne pathogens to a certain extent, a capacity that is highly desirable when developing robust cropping systems that aim to rely less on chemical inputs. This capacity of the soil is known as soil suppressiveness to pathogens or disease suppressiveness of soils (throughout the manuscript we will refer to it as soil suppressiveness) and has been related to chemical, physical and biological soil parameters (Janvier et al., 2007b). The capacity of soils to regulate soil-borne plant pathogens is an essential element of soil quality (Larkin, 2015). Previous investigations have shown evidence that biological, and in particular microbiological, properties play a crucial role in determining soil suppressiveness (Thuerig et al., 2009; Fuchs et al., 2014). General soil suppressiveness to pathogens relates to the activity, biomass and diversity of soil organisms and is based on the collective capacity of non-pathogenic constituents of soil and rhizosphere microbiomes to compete with and be antagonistic to pathogens. Specific soil suppressiveness to pathogens is the result of the presence of specific microbial taxa, such as *Pseudomonas* spp. and *Streptomyces* spp., which act as antagonists through antibiosis, and production of enzymes or siderophores (Schlatter et al., 2017a). Specific suppressiveness is considered less persistent than general suppressiveness (Mazzola, 2002). Soil suppressiveness mechanisms and expression vary according to the pathogen considered. For some pathogens, soil suppressiveness it has often been detected, mainly as one type (e.g. specific soil suppressiveness for *Gaeumannomyces graminis* and *Fusarium* spp.), or as a combined effect of both suppressiveness types, e.g., *Rhizoctonia solani*, *Pythium* spp. (Postma et al., 2008; Cook, 2014; Yadav et al., 2015), while for others it has less often and more recently been observed, e.g., Meloidogyne spp. (Silva et al., 2018). For most soil pathogens the microorganisms and the mechanisms involved in soil suppressiveness are not know. However, soil suppressiveness probably originates from a combined effect of general and specific soil suppressiveness (Postma et al., 2008; Yadav et al., 2015).

Agricultural management can influence soil suppressiveness in the short as well as in the long term through its effects on soil physical, chemical and biological properties (Bailey and Lazarovits, 2003; Sánchez-Moreno and Ferris, 2007). Many studies have shown that compost addition can have a positive short-term effect on soil suppressiveness (Boehm et al., 1993; van Os and van Ginkel, 2001; Pascual et al., 2002; Bonanomi et al., 2007a, c; Chen and Nelson, 2008; Alfano et al., 2011). Fewer studies have addressed the short-term effects of other types of organic matter input such as manure addition (Aryantha et al., 2000; Darby et al., 2006; Tamm et al., 2010), or addition of other organic amendments (Stone et al., 2003) on soil suppressiveness. Although there is less information available

regarding long-term management effects on soil suppressiveness, some studies indicate positive effects of long-term application of practices such as reduction of tillage intensity (Pankhurst et al., 2002; Peters et al., 2003; Campos et al., 2016; van Agtmaal et al., 2018), crop residue retention (Medvecky et al., 2007), crop rotation (Manici et al., 2005) and organic farming (Bonanomi et al., 2018a). Generally, intensive agricultural management (i.e. deep soil cultivation, mineral fertilizers, pesticides, and little organic matter supply) is associated with a decrease in soil biodiversity, including natural enemies and competitors of pathogens, pests and weeds, and consequently a decreased soil suppressiveness is expected (van Elsas et al., 2002; Crowder and Jabbour, 2014). However, the effect of management on soil suppressiveness can be variable, for example the effect of tillage (Yadav et al., 2015) or of organic matter input (Tamm et al., 2010) has been found to be contradictory. Expanding our knowledge on long-term agricultural practices that increase soil suppressiveness could contribute to the development of a more sustainable disease control in agricultural settings.

Soil suppressiveness is difficult to predict due to the interaction of different pathogenic and antagonistic species, heterogeneous distribution of pathogens at field, landscape and regional level, and the incomplete understanding of the mechanisms behind the phenomenon. Since direct measurement of soil suppressiveness using plant-pathogen systems is time-consuming, and requires infrastructure (e.g. growth chambers, clean benches) and trained staff, there is the need of indicators which can help in its assessment. However, the identification of such indicators is one of the main challenges of soil quality assessment in agriculture. Studies that aimed to identify relationships between soil suppressiveness and soil chemical, physical and biological parameters (Höper and Alabouvette, 1996; Darby et al., 2006; Postma et al., 2008) found inconsistent correlations probably depending on the pathogens and antagonists and the system under study (Janvier et al., 2007b). Yet, some studies indicate that the quality of the organic matter may play an important role in soil suppressiveness (Hoitink and Boehm, 1999; Bonanomi et al., 2010; Dignam et al., 2018). Specifically, labile carbon fractions and their characteristics have been associated with soil suppressiveness (Darby et al., 2006; Saadi et al., 2010; van Overbeek et al., 2012; Cao et al., 2016). Labile carbon is a part of the total organic carbon which is available as a source of energy to microorganisms, therefore being correlated to microbial abundance and activity (Haynes, 2005b). Labile carbon has received growing attention recently as a novel soil quality indicator and, in our previous work, it resulted to be linked with various soil quality indicators that have already been linked to soil suppressiveness (Bongiorno et al., 2019b, Chapter 3 (this thesis)). As such, labile carbon might be important in soil suppressiveness because of its positive impact on general microbial activity and on pathogen antagonists' presence and activity. However,

the mechanistic interactions between labile organic carbon, microbial biomass and activity, and soil suppressiveness have not been elucidated yet.

The objectives of the current study were i) to test if soil suppressiveness is affected by long-term agricultural management such as tillage and organic matter (OM) addition; ii) to understand the direct and indirect relationships between soil suppressiveness and labile organic carbon fractions; and iii) to understand the relationship between soil suppressiveness and other soil quality indicators (chemical, physical and biological). To this end, we sampled soils in different long-term field experiments selected from a range of pedoclimatic zones in Europe. We hypothesized that long-term reduced tillage and increased OM addition will result in higher soil suppressiveness, that labile organic carbon, through its positive effect on soil microbial biomass and activity will be an important driver for soil suppressiveness, and that soil suppressiveness will be linked more to soil biological than physical and chemical parameters.

4.2 Materials and Methods

4.2.1 Experimental sites and management

We selected 10 European long-term field experiments (LTEs) with a minimum duration of 5 years and a mean duration of 19 years to investigate the effects of different intensities of tillage and organic matter management on soil suppressiveness (**Figure 4.1, Table S1**). These LTEs were located in five European pedoclimatic zones: Dfb and Dfc (continental climate with cold winters and warm summers without a dry season or with cold winters and temperate summers without a dry season, respectively), Cfb and Csb (temperate climate with warm summer with or without dry season, respectively) and Bsk (arid cold steppe climate) (Köppen, 1918). In addition, the LTEs covered six different soil types (Vertic Cambisol, Haplic Luvisol, Haplic Fluvisol, Gleyic Podzol, Eutric Gleysol, and Eutric Cambisol (WRB, 2014) (Table S1).

Eight LTEs consisted of arable crops, two LTEs of permanent crops (PT1, ES4). All LTEs had individual tillage and fertilization regimes, which were classified in two main treatment factors: tillage (T) and organic matter addition (OM) (**Figure 4.1**). The contrast in tillage was categorised as conventional tillage (CT, ploughing to 20-25 cm depth) versus reduced tillage (RT, tillage to 0-10 cm), the level of OM addition was categorised as low organic matter input (LOW, no organic matter additions or only mineral fertilization) versus high organic matter input (HIGH, organic matter additions or organic matter additions with mineral fertilizer) as in Bongiorno et al. (2019b) and Chapter 3 (this thesis). LTEs had either a complete randomized block design or a split plot design with 3 or 4 replicates per treatment which was taken into account into the statistical models (**Table S1**).

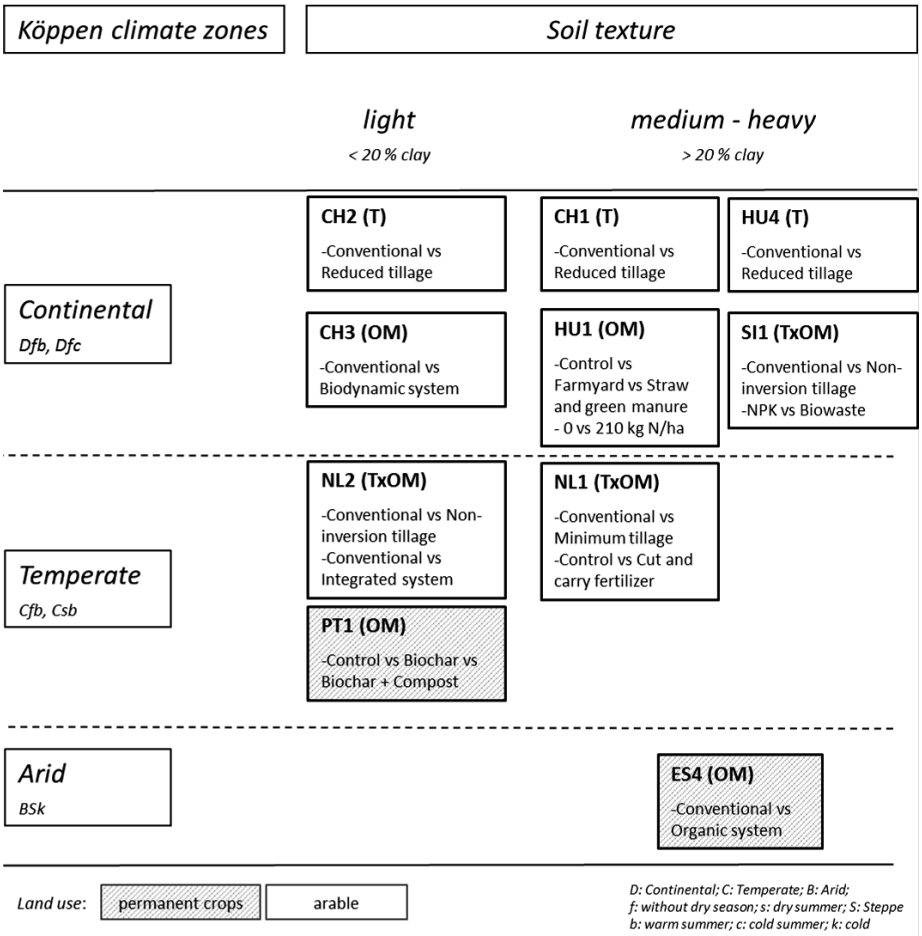

Figure 4.1. Main pedoclimatic characteristics and management practices (i.e. tillage or organic matter input, or a combination of the two practices) of ten European long-term field experiments. T tillage, OM organic matter addition. CH1 Frick trial, CH2 Aesch trial, HU4 Keszthely trial, CH3 DOK trial, HU1 Keszthely trial, SL1 Tillorg trial, NL2 de Peel trial, NL1 BASIS trial, PT1 Vitichar trial, ES4 Pago trial. For detailed information about the experiments see Table S1 in the supplementary materials.

4.2.2 Sampling procedure and sample handling

The soil samples were collected in spring 2016 before any major soil management was applied to the plots. Each sample comprised 20 soil cores, which were randomly collected in the central area of a plot to circumvent border effects. In the trials with tillage included in the management factor (CH1, CH2, NL1, NL2, SL1, HU4, ES4), samples were taken from 0-10 cm and 10-20 cm soil depth with the exception of NL1 experiment, where samples were taken from 0-15 cm and 15-30 cm (Table S1). For these tillage management trials, only the soil samples from the 0-10 cm (0-15 cm for NL1) were used. In the trials with fertilization as the only management factor (CH3, HU1, PT1), samples were taken from

0-20 cm soil depth, and this layer was used for the current study. The total number of samples used in this study was 101. Upon collection, a subsample was air-dried (40 ºC) and another part was stored field moist at 3˚C. Field-moist samples were sent in cooling boxes to Wageningen University (The Netherlands), the Research Institute of Organic Agriculture (FiBL, Frick, Switzerland), and the University Miguel Hernandez (Alicante, Spain), and dry samples were sent to the University of Trier (Germany) shortly after collection. Soil samples were sieved to 5 mm at the sampling location or immediately after shipping and, if field moist, stored at 3˚C. Biological parameters were assessed within 3 months, while chemical, and physical were assessed within 6 months after sampling. The soil suppressiveness bioassays were performed within one year after sampling. The soil suppressiveness measured with the *Pythium*-cress bioassay have shown in previous studies and trials to yield constant results in same soils for a period of two years (see results of the reference natural soil "REC", and the soil "THE" and "STC" in Thuerig et al. (2009) and Tamm et al. (2010).

4.2.3 Chemical, physical and biological parameters

Several chemical, physical and biological soil parameters were measured by various laboratories and details about the methodology used are presented in **Table 4.1**.

4.2.4 Soil suppressiveness bioassays

We used *Pythium ultimum – Lepidium sativum* (cress) as a model pathosystem to test the soil suppressiveness under standardized laboratory conditions. The *P. ultimum* - cress bioassay has been successfully used as a model pathosystem (or indicator) for general disease suppressiveness in previous studies (Thuerig et al., 2009; Tamm et al., 2010).

The bioassay was based on the protocol of Tamm *et al.* (2010). In short, cress was sown on soils which had or had not been inoculated with *P. ultimum* two days before sowing. A *P. ultimum* concentration usually causing distinct disease symptoms but not complete yield losses was selected. The protocol of Tamm et al. (2010) was modified as follows. Ten days before sowing the cress, inoculum of *P. ultimum* (culture code: Py1, 2005) originally isolated from tomato (provided and stored by Biointeraction and Plant Health, Wageningen Plant Research, The Netherlands) was produced on millet (24 g of sterile millet used as a substrate plus 20 ml of demineralized water) and incubated in the dark at 20º C. Nine days before sowing the cress, autoclaved and non-autoclaved soil (see 4.2.4.1 and 4.2.4.2) was taken out of the cold room and incubated at 20º C for one week to acclimatize and permit the reactivation of microorganisms. After eight days of mycelium growth, and two days before sowing the cress, the mycelium/millet culture was chopped and homogenized with a sterilized metal spatula. The homogenized *P. ultimum*/millet

Table 4.1. Overview on methods used to determine chemical, physical, and biological parameters linked with soil functions as measured in the framework of the iSQAPER project, and the methods used to measure labile carbon fractions (Bongiorno et al., 2019b; Chapter 3(this thesis)).

Parameters	Methodology	Unit	Laboratory of analysis
Chemical parameters			
Total organic carbon (TOC)	SIST ISO 10694: Soil quality - Determination of organic and total carbon after dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
Total nitrogen (TN)	SIST ISO 13878:1999: Soil quality - Determination of total nitrogen content by dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
pH	$CaCl_2$ determination- SIST ISO 10390:2006: Soil quality - Determination of pH	-	University of Ljubljana (SL)
Cation exchange capacity (CEC)	ISO 13536:1995 - Soil quality - Determination of the potential cation exchange capacity and exchangeable cations using barium chloride solution buffered at pH = 8,1	mmol 100 g^{-1} soil	University of Ljubljana (SL)
Plant available phosphorus (P_2O_5)	ÖNORM L 1087 - modification: ammonium lactate extraction	mg kg^{-1} soil	University of Ljubljana (SL)
Available phosphorus (P-Olsen)	SIST ISO 11263-1996	mg kg^{-1} soil	University of Ljubljana (SL)
Plant available potassium (K_2O)	ÖNORM L 1087 - modification: ammonium lactate extraction	mg kg^{-1} soil	University of Ljubljana (SL)
Exchangeable magnesium, calcium, sodium, and potassium (Mg^{2+}, Ca^{2+}, Na^+, K^+)	ammonium acetate extraction; Soil survey laboratory methods manual, 1992	mg kg^{-1} soil	University of Ljubljana (SL)
Physical parameters			
Water stable aggregates (WSA)	Wet sieving method modified as in Kandeler (1996)	mg kg^{-1} soil	FiBL (CH)
Bulk density (BD)	Volumetric assessment with ring	g cm^{-3}	Field assessment by LTE owners
Silt, Clay and Sand	SIST ISO 11277:2011: Soil quality - Determination of particle size distribution in mineral soil material - Method by sieving and sedimentation	%	University of Ljubljana (SL)
Penetration resistance	Pressure needed to insert penetrometer in the soil	Mpa	Field assessment by LTE owners
Water holding capacity (WHC)	Calculated with a pedotransfer function using the % clay, silt and total organic carbon (Tóth et al., 2015)	%	Wageningen University & Research (NL)

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culture was then mixed with sand (1:80 (w/w)) to allow for a homogeneous distribution of *P. ultimum* in the soil. Subsequently, 10 g of the *P. ultimum*/millet/sand mixture was mixed per liter of soil to obtain a final concentration of 0.125 g of *P. ultimum/*millet culture per litre of soil. The test soils did not receive any fertilization.

The soil suppressiveness bioassays were run with two types of soil samples: (a) pooled LTE samples (section 4.2.4.1) and (b) management treatment samples (section

Continued

Parameters	Methodology	Unit	Laboratory of analysis
Biological parameters			
Microbial biomass carbon (MBC)	Fumigation extraction method (Vance et al., 1987)	mg kg^{-1} soil	Trier University (DE)
Microbial biomass nitrogen (MBN)	Fumigation extraction method (Vance et al., 1987)	mg kg^{-1} soil	Trier University (DE)
Soil respiration	Incubation of soil at 25ºC for 72 h in thermostat bath	µg h^{-1} g^{-1} soil	University Miguel Hernandez (ES)
Earthworm abundance and biomass	Hand sorting from 30*30*30 cm^3 monolith	Number and fresh weight (g m^{-2})	Field assessment by LTE owners
Tea bag decomposition	Tea bag incubation (tea bag index) (Keuskamp et al., 2013)	g mass loss	Field assessment by LTE owners
Labile carbon fractions			
Dissolved organic carbon (DOC)	Extraction with ultrapure water and filtration at 0.45 µm filters.	mg kg^{-1} soil	Wageningen University (NL)
Hydrophilic dissolved organic carbon (Hy-DOC)	Fractionation of DOC with DAX-8 resin (Van Zomeren and Comans, 2007).	mg kg^{-1} soil	Wageningen University (NL)
Dissolved organic carbon and hydrophilic dissolved organic carbon specific ultraviolet absorbance (DOC SUVA and Hy SUVA)	Analysis of DOC and Hy solution with spectrophotometer at 254 nm (Weishaar et al., 2003; Amery et al., 2008).	L g C^{-1} cm^{-1}	Wageningen University (NL)
Permanganate oxidizable carbon (POXC)	Oxidation with K_2MnO_4 (Weil et al., 2003a).	mg kg^{-1} soil	Wageningen University (NL)
Hot water extractable carbon (HWEC)	Extraction with hot water (80ºC) for 16 hours and filtration at 0.45 µm filters (Ghani et al., 2003b).	mg kg^{-1} soil	Wageningen University (NL)
Particulate organic matter carbon (POMC)	Suspension in NaCl for 15 hours, wet-sieving through a 53 µm sieve and calculation of POM by loss on ignition (Salas et al., 2003).	mg kg^{-1} soil	FiBL (CH)

4.2.4.2.). All the bioassays were run in the laboratory facilities of Unifarm, Wageningen University and Research and executed by the first author.

4.2.4.1 Soil suppressiveness bioassay with pooled LTE samples

To assess the soil status before pathogen inoculation and the soil suppressiveness in the different LTEs, equal parts of soil (approximately 100 ml) were collected from each treatment replicate in a given LTE. These samples from different treatments were pooled and mixed to obtain 1 L of soil for each LTE (further called 'pooled LTE samples'). This

resulted in 10 pooled LTE samples, one for each of the 10 LTEs. To confirm the biological nature of soil suppressiveness, half of each pooled LTE sample (0.5 L) was autoclaved at 121° C for 20 minutes to exclude the majority of the soil microorganisms, including soil pathogens (Trevors, 1996). The other half was not manipulated and both 0.5 L samples were stored for up to two days at 3°C before conducting biosassays. One week before the inoculation, autoclaved and not autoclaved soils were placed in a climate chamber at 20°C to permit stabilization of the microbial communities (soil equilibration).

The experimental setup included 10 autoclaved and 10 non-autoclaved pooled LTE samples, two dosages of *P. ultimum* (0, i.e. no *P. ultimum* added, and 0.125 g L^{-1} of *P. ultimum*/ millet/sand mixture added), and 4 replicates per *P. ultimum* inoculum concentration (a total of 160 pots). The inoculated and non-inoculated soils were placed in plastic polypropylene containers (Ø 133 cm, 0.5 L) perforated at the top and pre-incubated in the dark at 20° C for two days. After this pre-incubation, each soil was used to fill 4 replicate pots (Ø 6 cm, 95 ml). Each pot was sown with 0.5 g untreated biological seeds of *L. sativum* (De Bolster, Epe, The Netherlands). The pots were placed on individual plant saucers to avoid cross-contamination between different soils and treatments. Pots were completely randomized and incubated in a growth chamber at 23°C (day) and 18°C (night) with a day-length of 16 hours and 80% relative humidity (Unifarm, Wageningen University, The Netherlands). For the first two days after sowing, a plastic sheet covered the pots to prevent evaporation and ensure 100% relative humidity for germination. After two days, the plastic sheet was removed and the pots were irrigated from below when needed. Seven days after sowing, shoot fresh weight in each pot was assessed by cutting the shoots with scissors directly above the ground.

4.2.4.2 Soil suppressiveness bioassay with management treatment samples to compare management treatments within individual LTEs

To assess the effect of management treatments on soil suppressiveness, bioassays were run in 10 separate batches, one for each LTE. The procedure was identical to that for the pooled samples, with the exception that no autoclaved soils were included. For each LTE, all soil samples collected in the field (i.e. the number of management treatments X number of treatment replicates, resulting in a total number of 101 samples for all the LTEs) were tested with two dosages of *P. ultimum* (0 and 0.125 g L^{-1}) with four replicate pots per *P. ultimum* inoculum concentration (this resulted in a total of 808 pots across all the bioassays performed with the management treatment samples). Trial CH3 was repeated in order to check the reproducibility of the bioassay (**Fig. S2**, **Table S6**). In the statistical analyses, the mean of the four replicate pots per *P. ultimum* inoculum concentration was used.

4.2.4.3 Calculation of soil health and soil suppressiveness indices

To characterise the soil status before inoculation, a soil health index was calculated for pooled LTE samples as follows:

$$SHI\ (\%) = 100 * (Wn * Wa^{-1})$$

where *Wn* = shoot weight of cress in pots with natural soil not inoculated with *P. ultimum*, and *Wa* = mean cress weight in autoclaved soil not inoculated with *P. ultimum*.

In our study we use the term soil health not as a synonym for soil quality, but we use it taking into account its association with soil biota (Bünemann et al., 2018). We consider a soil as healthy in which disease outbreaks are limited (similarly to Janvier et al. (2007b)). In our case the autoclaved soils showed the possible growth in the absence of pathogens.

As a measure for robustness of soils towards inoculation with *P. ultimum*, soil suppressiveness indices were calculated as follows:

(a) For the non-autoclaved pooled LTE samples and the non-autoclaved management treatment samples,

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

where *Wni* = shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and *Wn* = mean shoot weight in natural soil not inoculated with *P. ultimum*.

(b) For autoclaved pooled LTE samples,

$$SSai\ (\%) = 100 * (Wai * Wa^{-1})$$

where *Wai* = shoot weight of cress in pots with autoclaved soil inoculated with *P. ultimum*, and *Wa* = mean cress weight in autoclaved soil not inoculated with *P. ultimum*.

4.2.5 Statistical analysis

All statistical calculations were performed using R version 3.3.2 (R Development Core Team, 2013). For the linear mixed effects model and the generalized least square model, the packages *nlme*, (Pinheiro et al., 2018) and *emmeans* (Lenth et al., 2018) were used, for the multiple linear regression and the correlation analysis the packages *car* and *stats* were used. For the structural equation model the *lavaan* and *piecewiseSEM* package was used (Rosseel, 2012; Lefcheck, 2018).

For each pooled LTE sample, the effect of the four different soil treatments (natural soil, natural soil with *Pythium,* autoclaved soil, and autoclaved soil with *Pythium*) on the fresh weight of cress was analysed with one-way ANOVA followed by a Tukey's HSD post-hoc test to assess significant differences between treatments.

The effects of the agricultural treatments on the soil suppressiveness (SSni) were assessed by linear mixed effect models (LMEs). The LMEs were run independently for each LTE. Tillage and/or organic matter addition were included as fixed factors while, depending on the trial, block, main plot and subplot were introduced as random factors to take the nested design of the experiments into account. In addition, a model merging all the LTEs, and one merging only the trials were tillage was part of the management factor (CH1, CH2, NL1, NL2, SL1, ES4 and HU4) was run to test the effect of tillage and organic matter addition on soil suppressiveness. In this case tillage and organic matter addition were included as fixed factors while, LTE, main plot and subplot were introduced as random factors. The results were considered statistically significant at $p \leq 0.05$. The effects of tillage and organic matter addition and their interaction on soil suppressiveness (SSni) were assessed by analysis of variance (function *anova*) on the linear mixed effect model. For all the models, normality and homogeneity of variances of the residuals were checked both visually (plotting sample quantiles *versus* theoretical quantiles and residuals *versus* fitted values) and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). For these tests, results were considered statistically significant at $p \leq 0.05$. When the ANOVA indicated a statistically significant effect at $p \leq 0.05$, Tukey's HSD post-hoc test was used to assess significant differences between treatments.

Spearman's rank order correlation was used to examine relationships between soil suppressiveness (SSni) and biological, physical, and chemical soil quality parameters, including labile carbon fractions (bivariate correlations). For the correlation analyses, data from the management treatments samples were used (n= 101). The relationship between soil suppressiveness and other soil parameters was validated using partial correlations, correcting for variation caused by the intrinsic differences of the LTEs (pedoclimatic zones). To rank the relative importance of the variables in predicting soil suppressiveness (SSni), we standardized all the variables by subtracting the mean and dividing the result by the standard deviation. Thereafter we performed linear mixed model regression with SSni as the dependent variable and the chemical, physical and biological parameters as explanatory variables, checking one after the other. To take the nested structure of the experimental design of the LTEs (**Table S1**) into account, we allowed the slope and the intercept to vary depending on the LTE (random slope and intercept model) (Zuur, 2009). The variables that resulted to be significant ($p \leq 0.05$) in explaining variation in SSni were selected and used in multiple mixed model regression, but only after discarding variables which were highly correlated ($\rho > 0.80$). T-values are reported to quantify the contribution of each predictor to the model (Field et al., 2012). We applied manual stepwise regression, and we selected the final model with the *anova* function and the Akaike Information Criterion (AIC) (Field et al., 2012). We used a multiple regression model with only the LTEs

as random intercept, because it appeared that this model did not differ significantly from a model with random slope and intercept. All the models were checked for normality and homogeneity of the residuals.

Piecewise structural equation modelling (SEM) was used to evaluate the direct and the indirect effects of the labile carbon fractions on SSni, taking into account the dependent structure of the data coming from the same LTE (Lefcheck, 2016). For this reason, the LTE was used as random factor in the analysis. We established an *a priori* model including the main physical, chemical and biological variables and labile carbon fractions that appeared to be of importance for SSni according to the results obtained in the correlation and the multiple regression model analyses and according to ecological mechanisms (**Figure S1**). The hypothesised relationships acted as a framework for the optimization of the piecewise SEM. The data matrix was fitted using the log-transformed variables, and SSni was logit transformed. The evaluation of the AIC was used to estimate the robustness of the models and to select the appropriate final model (Shipley, 2013). 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 (Lefcheck, 2016). We calculated and reported the total standardized effects of the predictors on soil suppressiveness (SSni).

4.3 Results

4.3.1 Characterisation of sites (pooled LTE samples)

4.3.1.1 Soil health status

The growth of cress in native and autoclaved pooled LTE samples (without inoculation) was compared (**Fig. 4.2**) to characterize the 'health status' of soils, and a soil health index (SHI) was calculated (relative growth of cress in natural soils compared to the growth of cress in soils after removal of the majority of microorganisms by autoclaving).

Growth of cress on natural pooled LTE samples showed high variability between LTEs. After autoclaving, growth of cress was similarly high in all pooled LTE samples (fresh weight about 3 grams, **Fig. 4.2**), with the exception of CH1, where autoclaving decreased the cress weight compared to the natural soil (-79%, $p < 0.05$) (Fig. 2), mirrored in a soil health index above 100% (Fig. 3A). Cress grew very poorly on natural soils from PT1, ES4 and NL2 (fresh weight below 2 g) (**Fig. 4.2**), and the related soil health indices were all below 50% (**Fig. 4.3A**, **Tab. S2**). On natural soil from SL1, cress showed intermediate growth (average fresh weight 2.2 g) (**Fig. 4.2**) and the related soil health index was 79% (**Fig. 4.3**). In natural soils from CH1, CH2, NL1, HU1 and HU4, cress showed good and similar (**Fig. 4.2**, n compared to a) growth (fresh weight >2.5 g), and soil health indices were between 87% and 107%, with the exception of CH1 (SHI of 180%, see above).

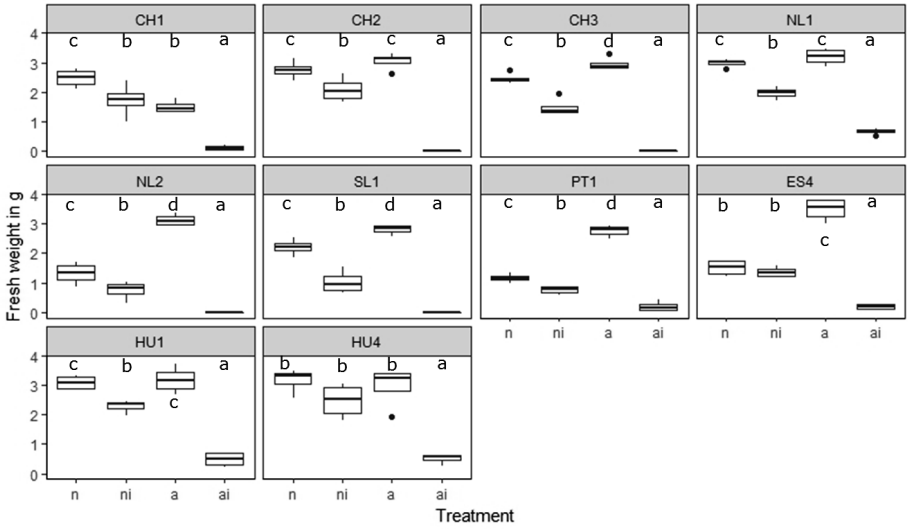

Figure 4.2. Shoot fresh weight of L. sativum grown in natural or autoclaved LTE pooled soil samples not inoculated or inoculated with *P. ultimum*. n = natural soil, ni = natural soil inoculated with P. ultimum, a = autoclaved soil, ai = autoclaved soil inoculated with *P. ultimum*. CH1 Frick trial, CH2 Aesch trial, CH3 DOK trial, NL1 BASIS trial, NL2 De Peel trial, SL1 Tillorg trial, PT1 Vitichar trial, ES4 Pago trial. HU1 Keszthely trial, HU4 Keszthely trial. For a detailed description of the trials we refer to Table S1. The boxes in the graph summarize the results of 4 individual pot replicates and represent the values between the 25th and the 75th percentiles, the horizontal line within a box is the median, and the extending lines represent the minimum and the maximum values. The black dots close to the boxes are observations which are considered outliers. Letters indicate significant differences between treatments in each long-term field experiment at $p \leq 0.05$ tested with ANOVA followed by a Tukey HSD post-hoc test.

4.3.1.2 Soil suppressiveness to Pythium ultimum

In natural pooled LTE samples inoculated with *P. ultimum*, cress reached on average 68% of the biomass of non-inoculated natural soils (mean soil suppressiveness index of natural soils, SSni) (**Fig. 4.2**, n compared to ni, **Fig. 4.3B**). In autoclaved pooled LTE samples inoculated with *P. ultimum*, cress reached between 0 to 20 % of the biomass compared to non-inoculated soils (soil suppressiveness index of autoclaved soils, SSai) (**Fig. 4.2**, a compared to ai, **Fig. 4.3C**).

In natural soils, ES4 and HU4 showed the highest soil suppressivenes indices SSni (90% and 78%, **Fig. 4.3B**). However, the fresh weight of cress showed different situations: in ES4 we observed low fresh weight in non-inoculated soil and comparable low fresh weight in inoculated soils, while in HU4 the cress fresh weight was high in non-inoculated soil and comparably high in inoculated soils (**Fig. 4.2**, n compared to ni). In all other LTEs, cress weight was significantly reduced in natural inoculated compared to non-inoculated soils ($p < 0.05$) (**Fig. 4.2**). Soil suppressiveness indices SSni were lowest in soils from SL1,

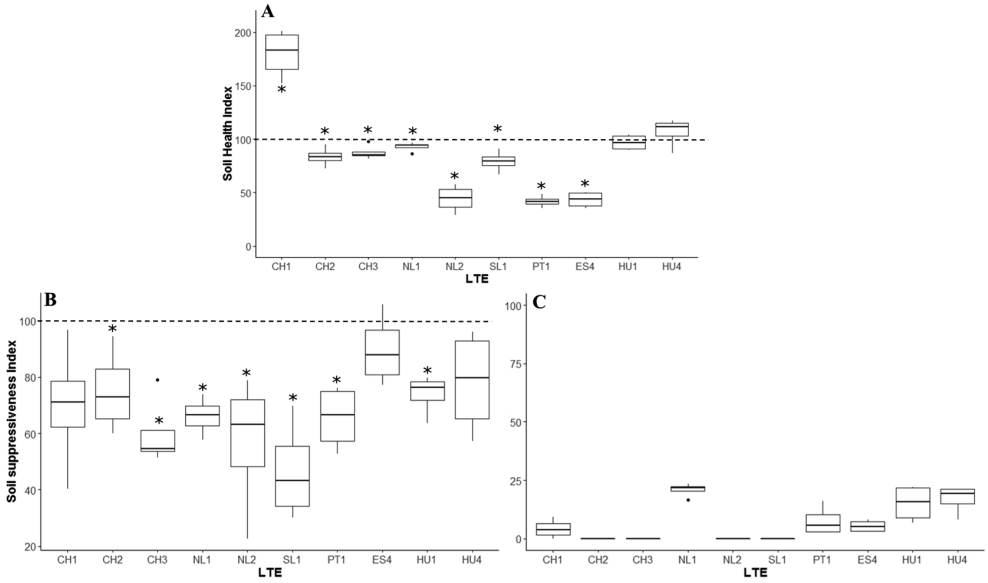

Figure 4.3. Soil Health Index in the pooled LTE samples calculated for A) natural non-inoculated soils relative to autoclaved non-inoculated soils (Soil Health Index, a measure of the natural disease pressure in the soil). Soil suppressiveness index in the pooled LTE samples was calculated for B) natural soils inoculated with *P. ultimum* relative to natural non-inoculated soil (Soil Suppressiveness Index in natural inoculated soil (in the text SSni), a measure of soil suppressiveness upon pathogen addition), and C) inoculated autoclaved soils relative to non-inoculated autoclaved soils (Soil Suppressiveness Index in autoclaved inoculated soil (in the text SSai)). The boxes in the graph depict the results of four individual pots and represent the values between the 25th and the 75th percentiles, the horizontal line within a box is the median, and the extending lines represent the minimum and the maximum values. The black dots close to the boxes are observations that are considered outliers. The asterisks in panel A and C indicate statistical significant difference ($p \leq 0.05$) from the line which is set to 100. *CH1* Frick trial, *CH2* Aesch trial, *CH3* DOK trial, *NL2* De Peel trial, *NL1* BASIS trial, *SL1* Tillorg trial, *PT1* Vitichar trial, *ES4* Pago trial, *HU1* Keszthely trial, *HU4* Keszthely trial.

NL2 and CH3 (**Fig. 4.3B**) (average SSni of 46%, 57%, and 60 %, respectively, see also **Table S2**).

4.3.2 Influence of management treatments on soil suppressiveness

We tested the effect of tillage and organic matter-based additions on soil suppressiveness in each LTE separately. Tillage did neither affect cress fresh weight in non-inoculated soils nor the soil suppressiveness index (SSni) in any of the six LTEs including tillage as a management factor (CH1, CH2, NL1, NL2, SL1 and HU4) separately (**Table 4.2**). However, reduced tillage resulted in higher yield in natural soils and higher SSni than conventional tillage, when testing the effect of tillage in an overall model with all the LTEs included (**Table 4.3**, $p = 0.05$) and a model with only the LTEs including tillage in the management factor (**Table S3**, $p = 0.01$).

Table 4.2. Effect of different tillage (T) and organic matter additions (OM) on cress shoot yield (g) in natural non-inoculated soil and soil suppressiveness index (SSni). Least square means, standard errors (in parentheses) and F and p values for mixed linear effect models are reported for the different type of tillage and fertilization. Mean and standard errors were calculated from the biological (spatial) replicates in each long-term field experiment (LTE). Differences are considered significant at $p \leq 0.05$ (values ≤ 0.05 are given in bold).

Long term field experiment (LTE)	Management		Fresh weight in non-inoculated soil (g)	Soil suppressiveness index (SSni) (%)
CH1	CT		2.72 (0.1)	81 (5.5)
	RT		2.67 (0.1)	78 (5.5)
	Tillage (T)	F *p*	0.13 0.73	0.14 0.72
CH2	CT		3.02 (0.2)	90 (3.3)
	RT		2.98 (0.2)	87 (3.3)
	Tillage (T)	F *p*	0.04 0.84	0.54 0.49
NL1	CT		3.98 (0.1)	51 (5.5)
	CT-Cut and carry fertilizer		4.18 (0.1)	59 (5.5)
	RT		4.25 (0.1)	60 (5.5)
	RT-Cut and carry fertilizer		4.35 (0.1)	68 (5.5)
	Tillage (T) Organic matter (OM)	F *p* F *p*	5.74 0.14 3.22 0.13	1.94 0.30 1.82 0.23
NL2	CT-Conventional		1.80 (0.3)	19 (3.5)
	CT-Integrated		1.76 (0.3)	20 (3.5)
	RT-Conventional		2.43 (0.3)	21 (3.5)
	RT-Integrated		2.07 (0.3)	22 (3.5)
	Tillage (T) Organic matter (OM)	F *p* F *p*	3.06 0.14 0.26 0.63	0.15 0.71 0.02 0.88
SL1	CT-Mineral		2.20 (0.2)	51 (3.5)
	CT-Biowaste		2.26 (0.2)	46 (3.5)
	RT-Mineral		2.67 (0.2)	55 (3.5)
	RT-Biowaste		2.37 (0.2)	50 (3.5)
	Tillage (T) Organic matter (OM)	F *p* F p	1.79 0.31 0.34 0.59	0.82 0.46 4.28 0.09
CH3	Conventional		1.96 (0.2)	64 (7.1)
	Biodynamic		3.16 (0.2)	60 (7.1)
	Farming system	F *p*	20.08 **0.02**	0.27 0.63
ES4	Conventional system		1.21 (0.16)	63 (6.2)
	Organic system		2.63 (0.16)	88 (6.2)
	Farming system	F *p*	114.13 **0.008**	22.80 **0.04**

Continue

Continued

Long term field experiment (LTE)	Management		Fresh weight in non-inoculated soil (g)	Soil suppressiveness index (SSni) (%)
PT1	Control		2.52 (0.15)	60 (14)
	Biochar		2.49 (0.15)	20 (14)
	Biochar + compost		2.07 (0.15)	29 (14)
	Organic fertilization	F *p*	2.66 0.18	6.03 0.06
HU1	Control		2.98 (0.2)	43 (5.2)
	Control + Nitrogen		3.18 (0.2)	61 (5.2)
	Farmyard manure		2.70 (0.2)	43 (5.2)
	Farmyard manure + Nitrogen		3.60 (0.2)	61 (5.2)
	Straw		2.04 (0.2)	50 (5.2)
	Straw +Nitrogen		3.10 (0.2)	68 (5.2)
	Mineral fertilization Organic fertilization	F *p* F *p*	10.67 **0.006** 3.09 0.08	11.90 **0.005** 0.93 0.42
HU4	CT		3.25 (0.19)	62 (6.4)
	RT		3.46 (0.19)	81 (6.4)
	Tillage (T)	F *p*	1.21 0.35	6.80 0.08

CT conventional tillage, *RT* reduced tillage

*Calculated as: $SSni\ (\%) = 100 * (Wni * Wn^{-1})$
where *Wni* = shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and *Wn* = mean shoot weight in natural soil not inoculated with *P. ultimum*.

In two (CH3, ES4) out of three system comparison trials (ES4, CH3 and NL2), significant effects of management were observed. In ES4, soil suppressiveness to *P. ultimum* (SSni) as well as the fresh weight of cress in natural, non-inoculated soils was higher in plots that were managed organically compared to plots that were managed conventionally ($p = 0.04$ and $p=0.008$, respectively). Similar results were found in CH3, with significantly higher weights of cress in soil from the biodynamic than from the conventional treatment (**Table 4.2**). At the same time, however, soil suppressiveness in CH3 was not affected by soil management (**Table 4.2**). In one (HU1) out of four organic matter addition trials (PT1, HU1, NL1, and SL1), significant management effects on performance of cress were found. In HU1, SSni was significantly higher ($p = 0.005$) in plots that had received mineral N fertilization either alone or in combination with organic fertilizers (farmyard manure or straw plus green manure). In NL1 and SL1, the cut and carry fertilizer and the bio-waste application, respectively, did neither affect SSni nor growth of cress on native non-inoculated soils (**Table 4.2**). In PT1, we found a tendency ($p = 0.06$) towards lower SSni when biochar (either alone or in combination with compost) was added to the soil as

Table 4.3. Effect of different tillage (T) and organic matter additions (OM) on cress shoot yield (g) in natural non-inoculated soil and soil suppressiveness index (SSni) for all the trials as analysed with mixed linear effect models (number of observations = 101). Least square means, standard errors (in parentheses) and F and p values for mixed linear effect models are reported for the different types of tillage and organic matter additions. Differences are considered significant at p ≤ 0.05 (values ≤ 0.05 are given in bold).

		Fresh weight in non-inoculated soil (g)	Soil Suppressiveness index (SSni)* (%)
CT- LOW		2.57 (0.22)	57.90 (6.69)
RT- LOW		2.97 (0.24)	65.08 (7.26)
CT- HIGH		2.69 (0.23)	56.83 (6.78)
RT- HIGH		2.97 (0.24)	63.60 (7.26)
Tillage(T)	F *p*	8.05 **0.008**	3.59 **0.05**
OM	F *p*	1.10 0.30	0.05 0.81
T X OM	F *p*	0.41 0.53	0.004 0.94

LOW low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage, *OM* organic matter addition, *T* tillage.

*Calculated as: $SSni\ (\%) = 100 * (Wni * Wn^{-1})$
where *Wni* = shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and *Wn* = mean shoot weight in natural soil not inoculated with *P. ultimum*.

compared to the non-amended control soil (**Table 4.2**). In the overall model taking into account all the LTEs we did not observe an effect of organic matter additions on the fresh weight of plants in natural soil nor on the soil suppressiveness (SSni) (**Table 4.3**).

4.3.3 Correlations of soil suppressiveness with soil parameters

Bivariate correlation analysis showed that soil suppressiveness (SSni) (calculated from the management treatment samples) was positively associated with higher values of various chemical (pH, total N, cation exchange capacity (CEC), Ca and K), physical (water holding capacity (WHC), silt, clay, penetration resistance), microbial parameters (microbial biomass C and N (MBC and MBN), soil respiration (SR), microbial quotient (qMic), tea bag decomposition, earthworm number and biomass, and labile carbon fractions (hydrophilic dissolved organic carbon (Hy-DOC), permanganate oxidizable carbon (POXC) and hot water extractable carbon (HWEC)) (Table S4). In contrast, we found negative correlations with C to N ratio (C/N), bulk density (BD), sand, dissolved organic carbon and hydrophilic organic carbon specific ultraviolet absorbance (DOC SUVA and Hy SUVA). The partial correlation showed that after normalization for structural differences between the LTEs (i.e. for the pedoclimatic characteristics) higher values of total N, MBC, soil respiration,

qMic, earthworm number, Hy SUVA, POXC, HWEC and carbon in the particulate organic matter (POMC) were associated with higher values of SSni, while higher values of C to N ratio, tea bag index and DOC SUVA were associated with lower values of SSni (**Table 4.4**).

Table 4.4. Partial correlation coefficients (ρ) between the soil suppressiveness index (SSni) and chemical, physical and biological parameters used as dependent variables, corrected for the long-term field experiments (LTEs). The number of samples used in the analyses was 101.

Chemical parameters							
TOC	pH	TN	C/N	CEC	Ca	Mg	K
0.06	-0.10	**0.21***	**-0.32***	0.01	-0.08	-0.03	0.02
Physical parameters							
WSA	WHC	BD	Silt	Clay	Sand	Penetration resistance	
0.10	-0.15	0.005	0.06	-0.07	0.14	-	
Biological parameters							
MBC	MBN	Soil respiration	qCO_2	qMic	Earthworm number	Earthworm biomass	Tea bag decomposition
0.26*	0.18	**0.25***	-0.04	**0.27***	**0.35****	**0.16**	**-0.21***
Labile carbon fractions							
Hy	Hy SUVA	DOC	DOC SUVA	POXC	HWEC	POM-C	
0.09	**0.23***	0.04	**-0.32***	**0.27***	**0.26***	**0.21***	

TOC total organic carbon, *TN* total nitrogen, *C/N* carbon to nitrogen ratio, *CEC* cation exchange capacity, *WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, qCO_2 metabolic quotient (soil respiration/MBC), *qMic* microbial quotient (soil respiration/TOC), *Hy* hydrophilic carbon, *Hy SUVA* specific ultraviolet absorbance of hydrophilic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POM-C* particulate organic matter carbon.

* $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$

4.3.4 Multiple regression and structural equation model (SEM) with soil parameters and soil suppressiveness

The mixed linear regression models carried out for each soil parameter revealed that the variables C to N ratio, sand and silt, WHC, MBC and MBN, and HWEC (**Table 4.5**) significantly explained the variation in SSni in the LTEs.

Since sand was highly correlated with silt and WHC (ρ >0.80) and MBC was highly correlated with MBN, only WHC, MBC, the C to N ratio and HWEC were retained for the multiple mixed linear model. The most important variable for explaining SSni resulted to be MBC (**Table 4.6**).

The structural equation model (SEM) fitted to investigate the direct and indirect effects of the labile carbon fractions on the SSni indicated that the HWEC, POXC and water holding capacity (WHC) had an indirect positive effect on SSni through their positive effects

Table 4.5. Simple mixed linear model with random slope and intercept for each LTE determined from soil parameters measured in the 101 soil samples. The dependent variable was the soil suppressiveness index (SSni). The explanatory variables were chemical, physical and biological indicators. In the table estimates, standard error, t-value, p-value and marginal and conditional R^2 (R^2_m and R^2_c respectively) are reported. Differences are considered significant at $p \leq 0.05$ (significant parameters are given in bold).

	Estimate	Std. error	t value	*p*-value	R^2_m	R^2_c
Chemical parameters						
TOC	0.03	0.19	0.2	0.87	0.001	0.75
TN	0.38	0.22	1.7	0.14	0.12	0.75
pH	0.007	0.16	0.04	0.96	<0.0001	<0.0001
CEC	0.13	0.19	0.7	0.50	0.02	0.68
C/N	**-1.58**	**0.52**	**-3.0**	**0.03**	**0.52**	**0.93**
Ca	0.22	0.14	1.6	0.16	0.05	0.59
Mg	0.04	0.26	0.2	0.88	0.001	0.69
K	0.10	0.12	0.8	0.60	0.01	0.68
Physical parameters						
WSA	0.22	0.19	1.11	0.37	0.04	0.71
WHC	**0.72**	**0.11**	**6.3**	**0.002**	**0.49**	**0.64**
BD	-0.07	0.18	-0.39	0.69	0.004	0.68
Clay	0.10	0.27	0.4	0.72	0.01	0.67
Sand	**-0.78**	**0.11**	**-7.2**	**0.003**	**0.52**	**0.68**
Silt	**0.70**	**0.23**	**4.4**	**0.03**	**0.37**	**0.73**
Biological parameters						
MBC	**0.52**	**0.13**	**3.9**	**0.005**	**0.25**	**0.71**
MBN	**0.37**	**0.11**	**2.1**	**0.04**	**0.14**	**0.66**
SR	0.30	0.30	1.0	0.44	0.07	0.75
qCO_2	-0.22	0.18	-1.2	0.50	0.04	0.69
qMic	0.46	0.22	2.0	0.12	0.19	0.73
Earthworm number	0.88	0.56	1.58	0.22	0.20	0.92
Earthworm biomass	0.21	0.13	1.63	0.21	0.05	0.65
Tea bag decomposition	-0.11	0.31	-1.2	0.22	0.01	0.74
Labile carbon parameters						
Hy	0.06	0.11	0.5	0.60	0.004	0.69
Hy SUVA	0.16	0.09	1.7	0.09	0.02	0.78
DOC	-0.05	0.18	-0.3	0.77	0.002	0.81
DOC SUVA	-0.30	0.11	-2.6	0.12	0.08	0.71
POXC	0.24	0.13	1.8	0.09	0.05	0.71
HWEC	**0.34**	**0.13**	**2.6**	**0.05**	**0.11**	**0.68**
POM-C	0.41	0.31	1.3	0.23	0.08	0.86

Table 4.6. Multiple mixed linear model determined from soil parameters measured in the 101 soil samples. The dependent variable was the soil suppressiveness index (SSni). Differences are considered significant at $p \leq 0.05$. The Akaike information criterion (AIC) is an estimator of the quality of the statistical model, the R_m^2 (marginal coefficient of determination) indicates the proportion of the variation explained by the predictor variables and the R_c^2 (conditional coefficient of determination) indicates the variation explained by both the fixed and the random factors.

Dependent variable	Starting model	Final model	Model type	Significant parameters	AIC	R^2_m	R^2_c
Soil suppressiveness (%, SSni)	~WHC_scaled+ MBC_scaled+ HWEC_scaled+ C.N_scaled+ (1|LTE)	~MBC_scaled+ (1|LTE)	Multiple mixed linear model	MBC_sclaled (0.0001; 4)	208	0.25	0.70

WHC water holding capacity, *MBC* microbial biomass carbon, *HWEC* hot water extractable carbon, *C/N* carbon to nitrogen ratio

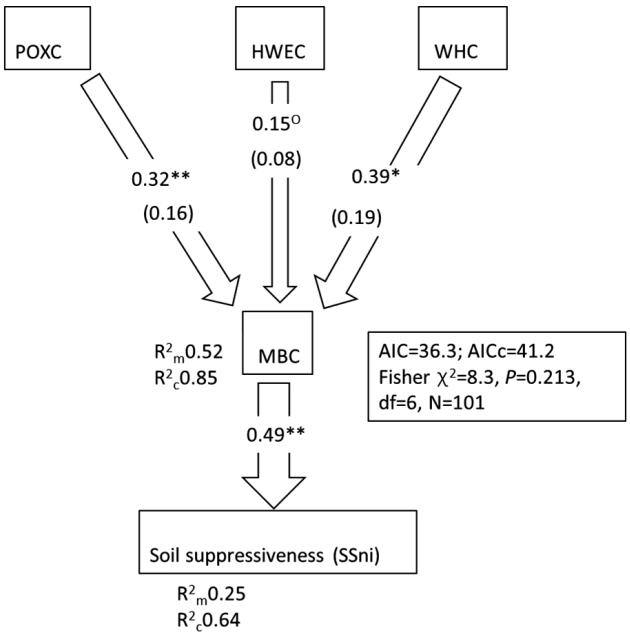

Figure 4.4. Piecewise structural equation model (SEM) of soil quality parameters as predictor of soil suppressiveness (SSni). Boxes represent measured variables and arrows represent the unidirectional relationship between the parameters. Numbers on the side of the arrows indicate standardized effect size (reported as path coefficients) and the width of the arrow is proportional to the strength of the path coefficient. The 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) 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. Variables lacking the R_m^2 and the R_c^2 acted only as predictor. Values in parentheses are the indirect effects strength on SSni. In the box adjacent to the figure the Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AIC_c), Fisher chi-square (Fisher χ^2), *p* value (P) of the test, degrees of freedom (df), and the number of observation used for the analysis (N) are indicated. SEM models with a χ^2 with a $p \geq 0.05$ are considered to be statistically significant. *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *WHC* water holding capacity, *MBC* microbial biomass carbon.
$^{O}p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

on microbial biomass carbon (MBC) (**Figure 4.4**). In particular, within the labile carbon fractions only the POXC revealed a highly significant ($p = 0.0007$) positive effect on the microbial biomass carbon. The piecewise SEM explained 25% of variation in the SSni.

4.4 Discussion

4.4.1 Quality of the bioassay

The quality of the bioassay was considered good as we obtained relatively low variability between replicate plots and highly reproducible results (**Fig. S2** and **Table S6**). This is in line with results from Thuerig et al. (2009). Shoot fresh weight is a good measure for the combined effect of *P. ultimum* on germination and growth of cress. In the short time of the bioassay (7 d) we expect that differences in the level of nutrients have been negligible and did not affect the results of the bioassay.

4.4.2 Soil health and suppressiveness indices in the pooled LTE samples

Cress fresh weight in native non-inoculated soils from pooled LTE samples differed significantly between the LTEs, with low yields in NL2, PT1 and ES4, high yields for CH1, CH2, CH3, NL1, HU1, HU4 and an intermediate yield in SL1. After autoclaving of soils, the fresh weight was high and similar in all - except one (CH1) - LTEs. It is well known that autoclaving (as any other type of sterilization) can make nutrients available by killing organisms (Trevors, 1996). Nevertheless, autoclaving of soils has been used extensively before to assess the effect of living microorganisms and/or pathogens on growth/suppressiveness (van Os and van Ginkel, 2001; Medvecky et al., 2007; Mitsuboshi et al., 2018). Yet, the facts that (i) none of the soils was nutrient-deficient before autoclaving (**Table S5**) (ii) the cress bioassay is very short (6 days in total) and consequently does not require a lot of external nutrients (cress can even been grown on simple filter paper, as done in many germination experiments (Buss and Masek, 2014; Luo et al., 2018)), and (iii) all soils reached similar levels of biomass after autoclaving of soils (**Figure 4.2**, a) indicate that the main growth-limiting factor for cress in native soils is of biological nature.Thus, we hypothesize that the observed yields in natural soils reflect mainly the outcome of the competition between putatively present soil-borne pathogens and beneficial soil microbiota.

CH1 was the only site where the cress yield in autoclaved soils was lower than in natural non- inoculated soils. We speculate that the autoclaving process either released some toxic elements (i.e. manganese, aluminium), ammonium (NH_4-N), nitrite or organic compounds. Autoclaving soils is known to reduce soil organisms, but also nutrients and salts are released, and the soil structure is disrupted (Razavi Darbar and Lakzian, 2007).

The high values of organic matter, total nitrogen and labile organic carbon present in CH1 (**Table S5**) could have facilitated the release of toxic elements or substances during autoclaving (Jager et al., 1968; Sonneveld and Mulder, 1979).

Native soils from pooled LTE samples differed in their capacity to mitigate the impact of inoculation with *P. ultimum*. The suppressiveness index (SSni) ranged between 46% and 90% on natural soils, and these values are in the same range as those found by Thuerig et al. (2009) and Tamm et al. (2010) for the same (CH3) or other natural soils. In the autoclaved soils, soil suppressiveness was dramatically reduced after inoculating the soil with *P. ultimum* as reported before in other studies (van Os and van Ginkel, 2001; Knudsen et al., 2002; Thuerig et al., 2009; Gravel et al., 2014; Löbmann et al., 2016), confirming the biological nature of soil suppressiveness against *P. ultimum*. Soil suppressiveness to *Pythium* spp. has been often reported and ascribed to mechanisms of both general and specific soil suppressiveness (Postma et al., 2005; Adiobo et al., 2007; Alfano et al., 2011; Oberhaensli et al., 2017), and also to abiotic mechanisms such as nutrient availability and physical properties (Adiobo et al., 2007; Löbmann et al., 2016).

Taken together, our results underline that soils from different fields have specific characteristics (chemical, physical but in particular biological) which have a diverse potential to interact with pathogens.

In this study we found that high soil suppressiveness can coincide with high yield (HU1 and HU4), but also with low yield in non-inoculated natural soils (ES4), resulting in large differences in yields in natural inoculated soils. These results emphasize the importance of taking into account both parameters (yield in natural non-inoculated soil together with measures of soil suppressiveness) when assessing suppressiveness of soils. For agriculture, the ideal soil is a soil combining high initial yield and high suppressiveness, as observed for HU1 and HU4.

The calculation and evaluation of soil health and suppressiveness indices from the pooled LTE samples, permitted a rapid and general characterisation of differences in soil health between sites, and for the assessment of the biological nature of the phenomenon of soil suppressiveness.

4.4.3 Effect of soil management practices on soil suppressiveness

We found several significant long-term management effects on yield (fresh weight in non-inoculated soils) and SSni within sites (**Table 4.2**, **Table 4.3**). However, these effects were smaller than the differences between the sites. This result is in accordance with previous studies (Knudsen et al., 2002; Tamm et al., 2010; Löbmann et al., 2016).

We found higher values of cress shoot fresh weight and soil suppressiveness in reduced tillage compared to conventional tillage when combining the trials together in overall

models, which is in accordance with our expectations. Reduced tillage is known to have a positive effect on soil properties (e.g. water stable aggregates, total organic carbon, bulk density) which can create a favourable environment for microorganisms (D'Hose et al., 2018), antagonists of pathogens (Peters et al., 2003) and plant growth. It is well known that soil microbial biomass and total soil organic carbon are enriched in the uppermost soil layer due to vertical stratification effects after reduced tillage, which was demonstrated also for the Frick trial (CH1) (Gadermaier et al., 2012; Krauss et al., 2017). As shown in previous studies and in this study, these factors, and in particular the microbiological properties, can favour soil suppressiveness (Thuerig et al., 2009).

Farming systems (organic versus conventional agriculture) showed a significant impact on soil suppressiveness in one out of three long-term trials (ES4), with a higher SSni in organic than in the conventional system. This agrees with other studies that found higher soil suppressiveness in organic compared to conventional farming systems (Manici et al., 2003; He et al., 2010; Tamm et al., 2010; Bonanomi et al., 2018a). This could be due to the positive effect of organic management on various soil chemical, physical and biological parameters, such as nutrients, organic carbon, water-stable aggregates, microbial biomass and activity (Biswas et al., 2014; Lori et al., 2017) and to the retention of both readily available and complex organic substrates in the organic system. Some complex substrates, for example lignocellulosic substrates, can increase the presence of natural antagonists like other *Pythium* spp. and *Trichoderma* spp., and more readily available substrates can increase general microbial activity (Medvecky et al., 2007). In the present study, in the organic management treatment in ES4, we found a higher concentration of labile carbon, which is positively related to microbial biomass and activity (Bongiorno et al., 2019; Chapter 3 (this thesis)), cation exchange capacity (p = 0.01*)*, water-stable aggregates (p = 0.02*)*, microbial biomass carbon (p = 0.003*)* and soil respiration (p = 0.004*)*. The concentration of lignocellulosic substrates was not measured.

We did not find an effect of organic matter additions on the SSni neither in the individual nor in the overall models. Organic matter additions have been reported to have, in the short-term, positive, negative or neutral effects on soil suppressiveness (Bonanomi et al., 2007b), but studies reporting positive effects predominate (Bailey and Lazarovits, 2003). Variable results could be partly explained by the fact that the chemical composition of the organic matter added to the soil is crucial for soil suppressiveness (Bonanomi et al., 2018b). Organic matter should preferably be decomposed, but not excessively, in order to support soil suppressiveness (Litterick et al., 2004). These observations suggest that changes in the nature of the organic matter (i.e. chemistry, quality and stage of decomposition, time of application, temporal effects) and in the soil environment are central for soil suppressiveness.

Furthermore, the suppressive capacity of organic material added to the soil can disappear some months after its application and it can differ between different batches of the same material and depending on the frequency of application (Litterick et al., 2004; Bonanomi et al., 2018b). For example, Darby et al. (2006) found that the disease severity of root rot of sweet corn increased with time (after 6 months - 1 year) in soil which received organic amendment and slightly decomposed free particulate organic matter (free-POM). Therefore, it is possible that in the present study, organic matter additions had a short-term effect that was lost some months after their application (we sampled in spring, before any agricultural management was applied), or that they lacked readily available substrates which are favourable for antagonistic and competitive microbial activity.

The positive effect of the mineral fertilization found in HU1 can possibly be ascribed to its enhancing effect on plant biomass (**Table 4.2**), which increases also root biomass and in turn can have a stimulatory effect on microbial activity.

All the soil management measures investigated in the current study have been applied at the end of summer or in the autumn. In order to focus on long-term effects of soil management rather than on short-term effects, soil sampling was done in spring. This time lapse might have played a role in the non-significant effect of soil management on soil suppressiveness found in various LTEs. To compare short-term to long-term effects and to study the development of the studied parameters throughout the year, soils should be sampled at several times.

4.4.4 Relationship between soil suppressiveness and soil parameters

Suppression of *Pythium* spp. has often been associated with the biomass and activity of the entire microbial community (van Os and van Ginkel, 2001; Scheuerell et al., 2005; Gravel et al., 2014). In this study, we assessed the relationship between soil suppressiveness and relevant soil biological parameters (microbial biomass, soil respiration, qMic), soil parameters routinely used in soil quality assessment (e.g. TOC, pH, TN, WSA) and in addition labile organic carbon fractions (Hy-DOC, DOC, POXC, HWEC and POM). Only occasionally, soil suppressiveness has been related before with labile organic carbon fractions (Pane et al., 2011; Cao et al., 2016; De Corato et al., 2018).

Using a multiple regression model we found that microbial biomass C was the most important parameter for explaining soil suppressiveness. The importance of biotic factors in this study is also reflected in the positive correlations (bivariate and partial) between soil suppressiveness and the biological parameters measured in the study, i.e. microbial biomass C and N, soil respiration and qMic. These observations suggest that increased microbial populations and activity are associated with a decreased disease severity,

and support the hypothesis that soil biota, and in particular microbial communities, are involved in soil suppressiveness against *P. ultimum*.

In our study, we found correlations of soil suppressiveness with various labile carbon fractions (positive correlations for POMC, HWEC and POXC), but not with TOC. Both organic matter and labile carbon fractions were found to be positively correlated to soil suppressiveness, an effect that is ascribed to their positive impact on the competitive potential of soil microbial communities against pathogens (Mazzola, 2004; Schlatter et al., 2017a). Labile carbon is considered the primary energy source for microorganisms, and probably contains part of the microbial biomass and microbial by-products. Therefore, labile organic carbon can favour soil suppressiveness supporting an active soil microbial community, which will compete for nutrients and space and can thrive on nutrients released by the plant during attack by the pathogen (Pascual et al., 2002; De Corato et al., 2018). This hypothesis is supported by our structural equation model (SEM), where POXC and water holding capacity (WHC) had a significant indirect positive effect on soil suppressiveness through a direct positive effect on microbial biomass. Our results support the hypothesis that the quality of the organic matter (in our case labile carbon fractions) and its effect on soil microorganisms are more important in explaining soil suppressiveness, than just soil organic matter quantity (Hoitink and Boehm, 1999).

We could explain only part of variability in soil suppressiveness with several measured soil parameters. Additional measures of microbial activity, for example fluorescein diacetate hydrolysis (De Corato et al., 2018) or other enzymatic activities (Pane et al., 2011), might add to the model and help in predicting soil suppressiveness. In addition, soil microorganisms are known to contribute to soil suppressiveness with various mechanisms, such as competition for nutrients and space, parasitism, predation, production of specific compounds (e.g. fungistats, siderophores, enzymes), and host mediated resistance (Mazzola, 2002; Charest et al., 2005; Pane et al., 2011; Van Agtmaal et al., 2017). Therefore, the composition of the microbial community and the presence and activity of specific microbial groups or taxa will affect soil suppressiveness (Mazzola, 2002; Trivedi et al., 2017). For example, the presence of *Bacillus* (Erhart et al., 1999; De Corato et al., 2018) and Acidobacteria and Cystobasidiomycetes has been found to be positively associated with *Pythium* suppressiveness (Yu et al., 2015). Therefore, elucidating the composition of soil microbial communities during soil suppressiveness assessment using molecular methods such as next generation sequencing and DNA microarrays, and coupling this with the detection of metabolites or genes that contribute to suppressiveness, and with functional bioassays, might further contribute to the understanding of the role of microorganisms in soil suppressiveness.

4.5 Conclusions

We found clear differences in soil suppressiveness between sites, whereas the effects of long-term agricultural practices on soil suppressiveness were less pronounced. Tillage had a positive effect on suppressiveness of the soil taking into account all the trials together. Organic farming and mineral fertilization increased soil suppressiveness in some LTEs, however the effect of organic matter addition across all the LTEs was not significant.

Soil suppressiveness across LTEs was linked mainly to microbial biomass and soil organic carbon quality (labile carbon, and in particular HWEC and POXC), but not to total soil organic matter content. We conclude that labile carbon is important for the maintenance of an abundant and active soil microbial community, which is essential for the expression of soil suppressiveness.

Soil suppressiveness could only partly (25%) be explained by the soil parameters measured and used in the SEM, suggesting that other mechanisms contribute to soil suppressiveness, such as the presence and activity of specific bacterial and fungal taxa, the activity of specific enzymes, or the presence of specific compounds with a detrimental effect on the pathogen.

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CHAPTER 5
5

Reduced tillage, but not organic matter input, increased nematode diversity and food web stability in European long-term field experiments

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Abstract

Soil nematode communities and food web indices can inform about the complexity, nutrient flows and decomposition pathways of soil food webs, reflecting soil quality. Relative abundance of nematode feeding and life-history groups are used for calculating food web indices, i.e. maturity index (MI), enrichment index (EI), structure index (SI) and channel index (CI). Molecular methods to study nematode communities potentially offer advantages compared to traditional methods in terms of resolution, throughput, cost and time. In spite of such advantages, molecular data have not often been adopted so far to assess the effects of soil management on nematode communities and to calculate these food web indices. Here, we used high-throughput amplicon sequencing to investigate the effects of tillage (conventional *vs* reduced) and organic matter addition (low *vs* high) on nematode communities and food web indices in ten European long-term field experiments and we assessed the relationship between nematode communities and soil parameters. We found that nematode communities were more strongly affected by tillage than by organic matter addition. Compared to conventional tillage, reduced tillage increased nematode diversity (23% higher Shannon diversity index), nematode community stability (12% higher MI), structure (24% higher SI), and the fungal decomposition channel (59% higher CI), and also the number of herbivorous nematodes (70% higher). Total and labile organic carbon, available K and microbial parameters explained nematode community structure. Our findings show that nematode communities are sensitive indicators of soil quality and that molecular profiling of nematode communities has the potential to reveal the effects of soil management on soil quality.

5.1 Introduction

The capacity of soils to perform multiple processes defines and determines soil quality (Bünemann et al., 2018). Soil management can negatively affect soil processes exerting threats (e.g. soil erosion, compaction, acidification and organic matter losses) on chemical, physical and biological properties (Toth et al., 2008). Tillage and fertilization are widespread soil management measures which can have a substantial influence on these soil threats, ultimately affecting soil processes and soil quality.

Soil nematodes are abundant and ubiquitous organisms that have an important role in various processes such as nutrient cycling, decomposition, pest and pathogen population regulation (Ekschmitt et al., 2001; Neher et al., 2012). In soils, nematodes are present at all trophic levels, and can therefore be divided into functional groups based on their feeding preferences (Yeates et al., 1993). Nematodes can also be differentiated according to their life-history strategies reflected in their position on a colonizer-persister (c-p) scale, which goes from group 1 (colonizers= *r* selected species) to group 5 (persisters= *K* selected species) (Bongers, 1990). Colonizers thrive in nutrient-rich habitats, are generally bacterivores, tolerant to stress and pollutants, with short generation times, while persisters poorly react to conditions of high food availability, are bigger omnivorous and/or predatory nematodes sensitive to stress, have longer generation times and generally live in temporally stable habitat. Many species have intermediate characteristics. Relative abundance of nematode feeding and life-history groups are used for calculating food web indices, i.e. the maturity index (MI- measure of environmental disturbance), enrichment index (EI- measure of resource availability), structure index (SI- measure of degree of trophic links and capacity to recover from stress) and channel index (CI- indication of predominantly fungal or bacterial decomposition pathway) (Bongers, 1990; Ferris et al., 2001), which are used to determine soil processes affecting soil quality.

Due to interactions with other soil biota and the influence of chemical and physical abiotic factors (Bongers and Ferris, 1999), changes induced by soil management affect nematode communities (Ferris and Bongers, 2006; Sánchez-Moreno et al., 2009). These changes in the nematode community can be due to modifications in food resources such as plant residues, nutrients, and environmental properties such as pH, oxygen content, porosity and temperature (Yeates and Bongers, 1999; Mekonen et al., 2017). Thus, data on nematode communities integrate information from soil chemical, physical and biological properties (Neher, 2001; Mekonen et al., 2017). This can increase our understanding of the impact of soil management on soil processes and, indeed, on soil quality in general.

Nematode diversity and specific nematode groups (i.e. based on feeding and/or life-history strategies) or taxa (i.e. family, genus, or species) have been shown to respond differently to soil management such as tillage and fertilization (Yeates and Bongers, 1999;

Moura and Franzener, 2017). More in detail, previous studies found higher nematode diversity and higher percentages of fungal feeders, omnivores and predators (slow-growing nematodes of c-p groups 4 and 5) in less disturbed conditions such as systems under reduced tillage or with perennial crops (Niles and Freckman, 1998; Yeates and Bongers, 1999; Liu et al., 2016a). In contrast, fast-growing bacterivorous nematodes (c-p groups 1 and 2) have been associated with eutrophic and mineral fertilized, disturbed systems (De Goede et al., 1993; Darby et al., 2013; Zhao and Neher, 2013; Quist et al., 2016). Also the application of different organic materials such as manure, compost and cattle slurry has been shown to increase the abundance of bacterivorous nematodes (Forge et al., 2005; Leroy et al., 2007), and, in some cases, to decrease the abundance of plant parasitic nematodes (Leroy et al., 2007).

In most publications so far, the response of nematode communities to tillage and fertilization was studied in single field experiments (Zhao and Neher, 2013; Ito et al., 2015; Quist et al., 2016), sometimes yielding contradictory results (Leroy et al., 2007; Ferris et al., 2012; Treonis et al., 2018). One factor hampering the study of management effects across multiple study sites is that traditional microscopy is the most common method to study nematodes, which is time-consuming, requires specialists and is expensive (Ritz et al., 2009b). Molecular methods to assess nematode absolute abundances (qPCR) and diversity (high-throughput amplicon sequencing, DGGE, T-RFLP) are faster, cheaper, and allow higher throughput than visual methods (Ahmed et al., 2016; Geisen et al., 2018). Amplicon sequencing may allow identification of taxa that cannot be distinguished morphologically. One limitation of PCR-based molecular methods is that not actual abundances of the specimen but rather their relative number of DNA copies are assessed (Waite et al., 2003; Porazinska et al., 2009). However, there is recent evidence that molecular methods might give similar ecological patterns as traditional methods (Hamilton et al., 2009; Porazinska et al., 2010; George and Lindo, 2015; Quist et al., 2016; Geisen et al., 2018). Hence, amplicon sequencing has high potential to assess soil management effects on nematode communities across multiple field experiments.

The goal of the present study was to i) assess the effect of tillage and organic matter addition on nematode qPCR counts, alpha- and beta-diversity, and food web indices as measured by amplicon sequencing of the 18S rRNA gene; ii) investigate the relationships between nematode community characteristics and other soil parameters related to soil processes; and iii) identify taxa that could serve as indicator organisms for soil management. We expected that molecular techniques would be sensitive, efficient tool to reveal general patterns of soil management effects on nematode communities in ten long-term field experiments across Europe. We hypothesized that i) reduced tillage will increase nematode qPCR counts, alpha diversity, MI, SI and CI, and will decrease levels

of bacterivorous nematodes with short life-cycles compared to conventional tillage, and that ii) high organic matter addition will increase qPCR counts, alpha diversity, EI, and will alter the nematode communities towards higher populations of bacterivorous nematodes compared to low organic matter input. We also hypothesized that iii) the positive effect of reduced tillage and organic matter addition on total and labile organic matter, available nutrients, water stable aggregates, and microbial biomass and activity will result in a positive relationship between these soil parameters and the nematode communities. Finally, we hypothesized that iv) nematode taxa with long life cycles and sensitive to management (such as predatory and omnivorous nematodes in c-p groups 4 and 5) will be more associated with less disturbed systems, and as such will be sensitive indicator taxa for soil disturbance.

5.2 Material and Methods

5.2.1 Long-term field experiments and management

We selected ten European long-term field experiments with either arable or permanent crops and a minimum duration of five years and a maximum duration of forty-four years (**Figure 5.1**, **Table S1**). Throughout the paper we will refer to these long-term field experiments as 'LTEs'.

This selection covered five different European climatic zones (Köppen, 1918) (**Figure 5.1**, **Table S1**) and six soil textural classes (Table S1) (WRB, 2014).

Each LTE had unique management characteristics and a different experimental design, with three or four replicates per treatment (**Table S1**). However, LTEs were comparable because the main soil management types were tillage (T) and organic matter addition (OM) as described in Bongiorno et al. (2019b) and Chapter 3 (this thesis). The contrast in tillage was classified as conventional tillage (ploughing at 20-25 cm depth, CT) versus reduced tillage (no-tillage or non-inversion tillage at 0-10 cm with different light machinery, RT). The contrast in organic matter addition was classified as low organic matter addition (LOW, no organic matter additions or only mineral fertilization) versus high organic matter addition (HIGH, organic matter additions without or with mineral fertilizer). At some LTEs, both treatment factors (i.e. tillage and organic matter addition) were applied and at others only one of these was present (**5.1**).

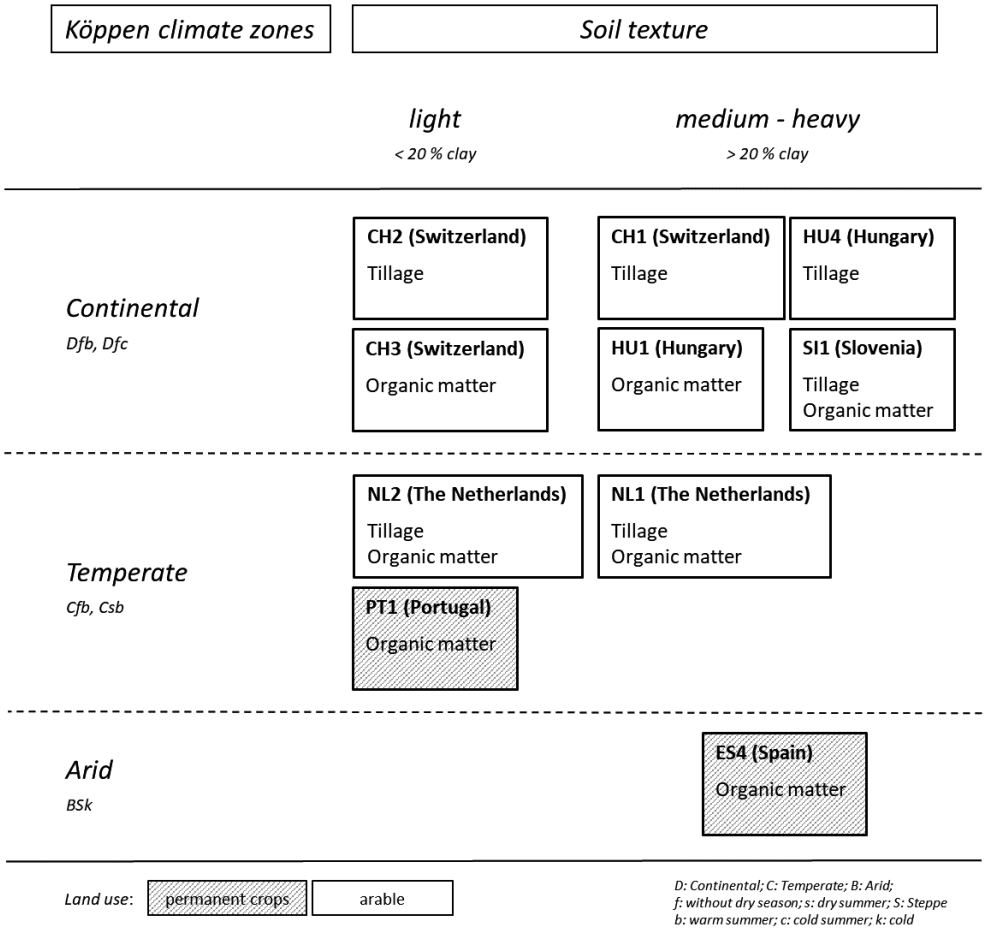

Figure 5.1. Main pedoclimatic characteristics and soil management (tillage, organic matter input, or a combination of the two) of ten long-term field experiments analysed in the current study. *T* tillage, *OM* organic matter addition, *CH1* Frick trial, CH2 Aesch trial, *CH3* DOK trial, *HU4* Keszthely trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* De Peel trial, *NL1* Basis trial, *PT1* Vitichar trial, *ES4* Pago trial. For detailed information about the experiments we refer to Table S1 in the supplementary materials.

5.2.2 Sampling procedure and sample handling

A total of 167 soil samples were collected in spring 2016 before any major soil or crop management was started in the LTEs. Each sample consisted of a composite sample of 20 soil cores randomly collected in the central area of the plot, to avoid border effects, and mixed. In the tilled LTEs, samples were taken from two depths: 0-10 cm and 10-20 cm. In the LTEs with organic matter addition as the only management factor (no tillage factor), samples were taken from the 0-20 cm layer because we did not expect to find a stratification effect due to tillage. After soil sampling, 400 g of the samples were air-dried (40° C) for subsequent chemical analysis. Fresh soil samples were sent to Wageningen

University (The Netherlands), Research Institute of Organic Agriculture (Frick, Switzerland), University of Trier (Germany) and University Miguel Hernandez (Alicante, Spain), and air-dried samples were sent to University of Ljubljana (Slovenia). Upon arrival, the samples were sieved at 5 mm and, when fresh, stored at 3˚C until further processing.

5.2.3 Chemical, physical and biological soil properties

The following soil properties were measured for this study: total organic carbon (TOC; %), pH ($CaCl_2$), total nitrogen (TN; %), cation exchange capacity (CEC; mmol 100 g^{-1} soil), plant available phosphorus (P; mg kg^{-1} soil), plant available potassium (K; mg kg^{-1} soil), exchangeable magnesium, calcium, and sodium (Mg^{2+}, Ca^{2+}, Na^{+}; mg kg^{-1} soil), water-stable aggregates (WSA; mg kg^{-1} soil), water holding capacity (WHC; %), bulk density (BD; g cm^{-3}), percentages of silt, clay, and sand, microbial biomass carbon (MBC; mg kg^{-1} soil), microbial biomass nitrogen (MBN; mg kg^{-1} soil), soil respiration (SR; µg CO_2-C h^{-1} g^{-1} soil), number and biomass of earthworms (number and g m^{-2}), decomposition through tea bag index (% mass loss) and soil suppressiveness to *Pythium ultimum* (%) (Bongiorno et al., 2019c; Chapter 4 (this thesis)). Microbial quotient (qMic) and metabolic quotient (qCO_2) were calculated as the microbial biomass carbon divided by the total organic carbon, and the soil respiration divided by the microbial biomass carbon, respectively. Besides chemical, physical and biological parameters, five different labile carbon fractions were measured: hydrophilic dissolved organic carbon (Hy-DOC; mg kg^{-1} soil), dissolved organic carbon (DOC; mg kg^{-1} soil), permanganate oxidizable carbon (POXC; mg kg^{-1} soil), hot water extractable carbon (HWEC; mg kg^{-1} soil), and particulate organic matter carbon (POMC; mg kg^{-1} soil) (Bongiorno et al., 2019b; Chapter 3 (this thesis)). In addition, the specific ultraviolet absorbance of Hy (Hy SUVA; L g C^{-1} cm^{-1}) and DOC (DOC SUVA; L g C^{-1} cm^{-1}) was measured to assess the recalcitrance of these labile carbon fractions. All analyses were performed within six months after sampling and the details about the methodology and the locations were the analyses took place are presented in **Table S2** (modified from Bongiorno et al. (2019c); Chapter 4 (this thesis)).

5.2.4 Nematode analysis

5.2.4.1 Nematode extraction, DNA extraction and DNA purification

Within two weeks after sampling nematodes were extracted from 100 g field moist subsamples using a modified elutriator (Oostenbrink, 1960). Thereafter nematodes were incubated for 72 hours on a double cotton-wool filter (Hygia milac). A subset of samples from each LTE (a total of 97 samples) was counted microscopically, with 1/10 of each sample counted in duplicate under a dissecting microscope. The number of nematodes was expressed per 100 g of field moist soil. The nematode suspensions were subsequently

concentrated and lysed with a lysis buffer containing proteinase K, β-mercaptoethanol and an internal mammalian standard in order to correct for the loss of DNA during lysis and DNA purification (Holterman et al., 2006; Vervoort et al., 2012). Thereafter, DNA extracts were purified using a glass fibre column-based procedure (Ivanova et al., 2006) and stored at -20°C until further use.

5.2.4.2 Quantitative PCR (qPCR) analysis of total nematode DNA

The purified DNA extracts were used as templates in qPCR using two primer sets to assess total nematode densities (Vervoort et al., 2012; Quist et al., 2017). The first primer set targeted DNA across the phylum Nematoda and the second targeted the mammalian internal standard. After the qPCR reactions, the Ct-values obtained were related to the microscopic counts to obtain a calibration curve at the ^{10}Log scale (see Vervoort et al. (2012)). Thereafter, Ct-values were converted into nematode densities using this linear relationship between the Ct values and the ^{10}Log (number of target nematodes) (**Figure S1A**). The maxima of the negative, first mathematic derivative of the melting curves were checked to confirm the correct nature of the amplicons. The internal control was used to monitor and correct for loss of DNA during the sampling handling. Throughout the manuscript qPCR-based quantification of nematode densities is referred to as 'nematode qPCR counts'.

5.2.4.3 18S rRNA gene amplification and sequencing

Nematode DNA was quantified with Nanodrop® (NanoDrop 2000 Spectrophotometer, Thermo Fischer Scientific) and subsequently sent on dry ice to GenomeQuebec (Montreal, Canada) for 18S rRNA gene amplification and sequencing on the Illumina MiSeq platform. In a first step a targeted PCR amplification with tagged primers for the hypervariable eukaryotic V4 region of the 18S rRNA gene was performed (**Table S3**). We used the universal eukaryotic primers 3NDf (5'-GGCAAGTCTGGTGCCAG-3') in combination with 1132rmod (5'-TCCGTCAATTYCTTTAAGT-3') as used in Geisen et al. (2018). In a next step, Illumina adapters with barcodes sequences were added by PCR to each sample (barcoding step) (**Table S3**). For each sample, the barcoding step was verified with gel electrophoresis. The DNA concentration was quantified with Quant-iT™ PicoGreen® dsDNA Assay kit (Life technologies) and for each sample, an equal amount of DNA was pooled for a sequencing library. After purification with AMPure beads (Beckman Coulter), the pooled DNA library was quantified using the Quant-iT™ PicoGreen® dsDNA Assay kit (Life technologies) and the Kapa Illumina GA Library Quantification kit with revised primers (KAPA SYBR® FAST qPCR Universal kit, Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Sequencing was performed with MiSeq Reagent

kit v3 (600 cycles) from Illumina. After sequencing, the sequences were demultiplexed by GenomeQuebec using the Illumina bcl2fastq Conversion Software version 2.17.1.14.

5.2.4.4 Bioinformatic analysis

The amplicon sequencing data was analyzed by the Genetic Diversity Centre (GDC), ETH Zurich, using the HPC Euler of ETH Zurich. The merging efficiency of the forward (R1) and reverse (R2) reads was relatively low (<11%). For this reason, we restricted the analysis to the forward read only. In a first step, the primer sites were trimmed off the R1 reads and all the reads were trimmed to an equal length of 280 nt using USEARCH (Edgar, 2010). Subsequently, the reads were quality filtered (parameter: GC range 20-80, minimum quality mean 20, no ambiguous nucleotides, and a low complexity filter, dust with threshold 30) using PRINSEQ-lite (version 0.20.4). About 10% of the total sequencing data was lost during primer trimming (7.5%), trimming (<1%), and quality filtering (2.6%). In a next step, UPARSE (Edgar, 2013) was used to cluster the sequences and create a count table. For the annotation of the OTUs SINTAX (Edgar, 2016) and the protist ribosomal reference database (PR2) were used. The OTUs which could not be assigned to a taxonomic group were verified with manual BLAST searches with NCBI nt based references databases (see **Figure S2**).

5.2.4.5 Nematode alpha diversity, trophic groups and food web indices

Alpha diversity is defined as the diversity of organisms within groups (in our case calculated within plots), while beta diversity is defined as the diversity of organisms between groups (Jost, 2010). Alpha diversity is measured through indices of richness, diversity and evenness (Jost, 2010). Nematode OTU or genus richness was calculated as the sum of the OTUs or genera, respectively. Nematode OTU and genus diversity was calculated as the exponential of the Shannon Index (Magurran, 1988):

$$exp^{H} = exp^{\sum_{i=1}^{S} - (Pi * \ln Pi)}$$

where *H* is the Shannon diversity index, *Pi* is the fraction of the entire population made of OTU or genus *i*, *S* is the number of OTU's or genera encountered, and *Σ* is the sum of OTU or genus 1 to OTU or genus *S*. Nematode OTU and genus evenness (Sheldon evenness) was calculated as the exponential of the Shannon diversity divided by the number of OTUs or genera (Heip, 1974).

We calculated the percentages of five trophic groups (bacterivorous, fungivorous, herbivorous, predators and omnivorous nematodes), maturity index (MI), enrichment index (EI), structure index (SI), and channel index (CI), according to the classification of nematode OTUs into functional groups, uploading the count table based on OTU observed

abundance with taxonomic information obtained after the bioinformatic analysis of the nematode sequencing data in the nematode indicator joint analysis (NINJA) program (Sieriebriennikov et al., 2014) (http://sieriebriennikov.shinyapps.io/ninja/ consulted on the 9th January 2019). NINJA was used also to assign nematodes to the colonizer-persister (c-p) scale (from 1 to 5) (Bongers, 1990; Ferris et al., 2001). The absolute abundance of trophic groups and c-p groups was calculated by multiplying the total qPCR counts by the trophic and the c-p groups percentages calculated with NINJA.

5.2.5 Statistical analysis

All statistical calculations were carried out using R version 3.5.1 and RStudio version 1.1.456 (R Development Core Team, 2013; RStudio Team, 2016). The R script is provided as Supplemental information 2, and a workflow of the data analysis steps is given in **Figure S3**. The nematode OTU counts and taxonomy tables were filtered before the analysis to exclude OTUs which were classified as non-nematodes, or whose kingdom or phylum was unassigned. All test results, except for the indicator species analysis, were considered statistically significant at $p \leq 0.05$.

5.2.5.1 Nematode qPCR counts, alpha and beta diversity per LTE

Nematode OTU richness and diversity were calculated after rarefaction (500x) to 10537 seq/sample (the minimum sample sequencing depth) (Bodenhausen et al., 2013).

A general beta diversity analysis was conducted on the nematode communities of all the sites. For this analysis, we filtered the OTU sequence counts retaining only OTUs with a minimum of 5 counts in at least 8 samples. After normalization using the total sum scaling (TSS) with the *decostand* (method="total") function in the *vegan* package (Oksanen et al., 2018), we computed Bray-Curtis dissimilarity matrices on the squared rooted transformed data (Leff et al., 2015). Canonical analysis of proximities (CAP) with *vegan* function *capscale* was performed to visualize and test the relationships between the nematode community and the most important soil chemical, physical and biological parameters measured in the LTEs (Anderson and Willis, 2003). The function *vif.cca* (threshold used vif ≤ 10) was used to retain variables which were not highly correlated ($\rho > 0.80$). The effect of the environmental variables on the nematode communities was assessed with permutation analysis (using the *anova* function in *vegan* by "margin") with 10^4 permutations and correlations between the environmental variables and the first two axes of the CAP to assign their relative importance.

5.2.5.2 Effects of tillage and organic matter additions on nematode qPCR counts, alpha and beta diversity

To test the effects of tillage and organic matter addition on soil nematode communities, two groups of LTEs were created because we expected stratification effects in LTEs with reduced tillage only, as shown in previous analyses (Bongiorno et al., 2019; Chapter 3 (this thesis)) . The following two groups were studied separately in the subsequent analyses:

Group A. The LTEs in which the layers 0-10 cm and 10-20 cm were sampled separately in space: CH1, CH2, NL1, NL2, SL1, HU4 and ES4. In this group, we assessed the effect of tillage, organic matter addition and soil layer.

Group B. The LTEs where the layer 0-20 was sampled: CH3, PT1 and HU1. In this group we only assessed the effect of organic matter addition, since these LTEs were under conventional tillage.

The effect of tillage and/or organic matter addition and, if present, layer on total nematode qPCR counts, OTU and genus richness and diversity, and OTU evenness were assessed in group A and B (using overall models merging the LTEs in the same group) by performing an analysis of variance (standard function *anova*) on fitted linear mixed effect models. Mixed models were used to take into account the possible correlations introduced by the multi-site field experiments and to generalize the effect of the soil management practices across the different LTEs (Bongiorno et al., 2019; Chapter 3 (this thesis)). The tillage and/or the soil organic matter addition and, if present, the layer, their two-way and possibly three-way interactions were used as fixed factors. Random effects for LTEs, blocks, main plots and subplots were introduced in the models to represent the experimental designs of the different LTEs. The effect of the pedoclimatic zone was not included in the fixed part of the model because we were interested in management effects across pedoclimatic zones. The model assumptions of normality and homogeneity of variances of the residuals were checked both visually and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). Total nematode qPCR counts and OTU richness, diversity and evenness were square-root-transformed in order to meet the assumption of normality. All tests were considered statistically significant at $p \leq 0.05$. For the linear mixed effects model, the packages *nlme*, and *emmeans* were used (Pinheiro et al., 2018). The same linear mixed effect models were used to assess differences in relative and absolute abundances of trophic and c-p groups, and in food web indices between soil management.

We then performed multivariate analysis of nematode communities on Bray-Curtis dissimilarities as outlined by Anderson and Willis (2003) using squared-root TSS normalized data. Using a permutational multivariate analysis of variance (PERMANOVA) with 10^4 permutations we tested the effect of tillage and/or organic matter and, if present, the layer on the community dissimilarity. In this analysis, the LTE was specified as random

factor in the *strata* argument which restricts permutations to within LTEs (Anderson, 2001). The function *betadisp* was used to perform permutational analysis of multivariate dispersion (BETADISP) with 10^4 permutations.

We then visualized the effect of soil management with canonical analysis of proximities (CAP) constrained ordination (Anderson and Willis, 2003) using the function *capscale* in the *vegan* package with the LTE as a conditional factor in order to control for the effect of the pedoclimatic zone on the nematode communities. Statistical significance of the CAP was assessed using the *permutest* function in the *vegan* package.

5.2.5.3 Relationships between nematodes and soil parameters

Partial correlations, correcting for the variation caused by the intrinsic differences between the LTEs (pedoclimatic zones), were used to test the relationships between nematode qPCR counts, OTU richness, diversity and evenness and the soil chemical, physical and biological parameters. For the correlation analyses the packages *car, stats* and *ppcor* were used (Kim, 2015).

The relationships between nematode communities and environmental variables shaped by the effect of the soil management practices was visualised using canonical analysis of proximities (CAP) and tested using the *envfit* function in the package *vegan*. The effect of the soil parameters was assessed with permutation analysis with 10^4 permutations.

5.2.5.4 Identification of putative indicator OTUs

Determination of nematode OTUs associated with specific management combinations was done using correlation-based indicator analysis with the function *multipatt* of the R package *indicspecies* (De Caceres, 2016) to calculate the point-biserial correlation coefficient (*r*) of an OTU's positive association to a soil management factor or a combination of factors. The analysis was done with 10^4 permutations and considered a more stringent significance level at $p \leq 0.01$, in order to limit the indicator species to a subgroup of highly sensitive OTUs associated with soil management. In the analysis we restricted the permutation within the blocks and within the LTEs to take into account the nested structure of the design.

5.3 Results

5.3.1 Nematode beta diversity across the long-term field experiments

In the CAP, the community composition showed a clustering of samples according to the long-term field experiments (LTEs) (**Figure 5.2**), and PERMANOVA confirmed that the

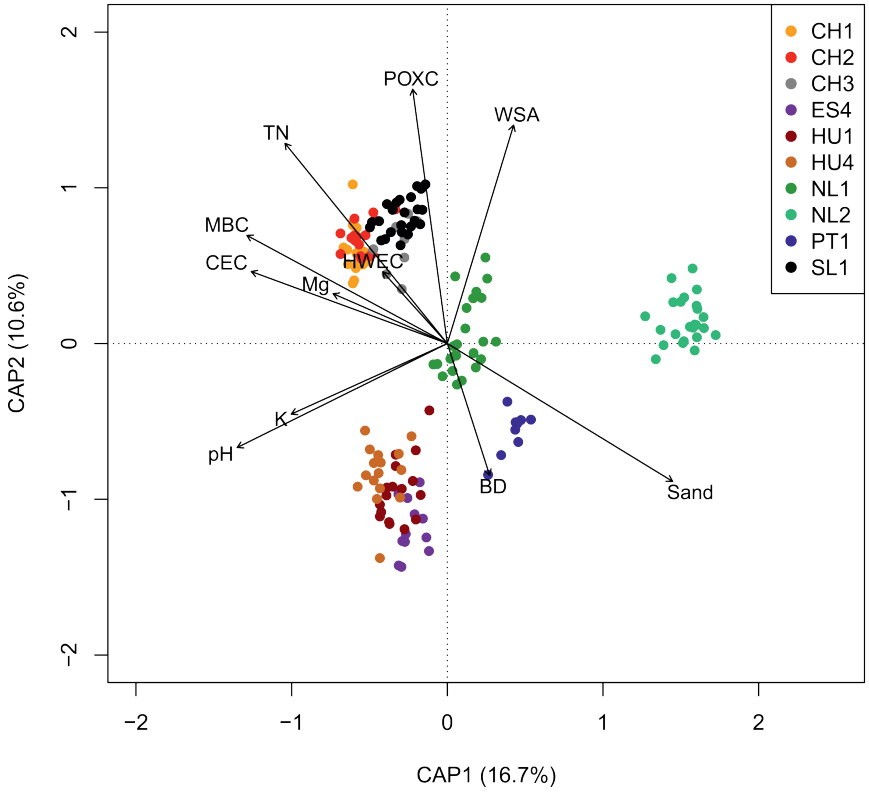

Figure 5.2. Constrained analysis of proximities (CAP) of the nematode communities in the long-term field experiments and the relation with soil parameters. The first axis, CAP1 explains 16.7% and the second axis explains 10.6% of the variation in the beta diversity between the nematode communities in the different sites. *TN* total nitrogen, *CEC* cation exchange capacity, *K* available potassium, *WSA* water stable aggregates, *BD* bulk density, *MBC* microbial biomass carbon, *HWEC* hot water extractable carbon, *POXC* permanganate oxidizable carbon. *CH1* Frick trial, *CH2* Aesch trial, *CH3* DOK trial, *HU4* Keszthely trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* De Peel trial, *NL1* Basis trial, *PT1* Vitichar trial, *ES4* Pago trial.

nematode communities were affected by the LTE ($R^2 = 0.64$; $p = 0.001$). A total of 50% of variation in the nematode beta diversity among the different LTEs was explained by the constraining variables used in the CAP.

According to ANOVA of the constraining variables, all the soil parameters were significantly related to the nematode beta diversity in the LTEs (**Table S4**). The soil parameters that were most important in explaining the variation between the different LTEs (i.e. significant relationship and Pearson correlation coefficient (r) with the canonical axes greater than +0.50 or smaller than -0.50) were for CAP1: sand content, pH, microbial biomass carbon (MBC), cation exchange capacity (CEC), and total nitrogen (TN); for CAP2: permanganate oxidizable carbon (POXC), water stable aggregates (WSA), and total nitrogen (TN).

5.3.2 Effect of soil management on total nematode qPCR counts and alpha diversity

In group A (i.e. LTEs with tillage and organic matter addition as treatments, sampled at two soil depths), nematode qPCR counts were higher in the first layer (0-10 cm) than in the second layer (10-20 cm) (Table 1). We found higher nematode OTU richness, diversity, and evenness and genus diversity and evenness in reduced tillage compared to conventional tillage across the LTEs of group A. In this analysis, OTU richness and diversity, and genus richness had higher values in the upper than in the lower layer, regardless of the tillage treatment (OTU richness and diversity 11% and 18% higher, respectively, and genus richness 9% higher). OTU and genus diversity and evenness were lower (16% and 22% for the OTU and 28% and 28% for genus, respectively) in the high organic matter addition plots.

In group B (i.e. LTEs with organic matter addition only, sampled between 0-20 cm soil depth), we found no significant effects of organic matter addition on total nematode qPCR counts, OTU and genus richness and diversity (**Table 5.1**).

5.3.3 Effect of soil management on beta diversity

PERMANOVA of group A revealed that the largest proportion of the variation in nematode beta diversity was explained by the LTEs (R^2 = 0.628, p = 0.0001). Despite this, tillage (R^2 = 0.012, p = 0.0001), organic matter addition (R^2 = 0.006, p = 0.006), layer (R^2 = 0.014, p = 0.0001) and the interaction between tillage and layer (R^2 = 0.006, p = 0.002) had significant effects on the nematode beta diversity (**Figure 5.3A**, **Table S5**). The significant interaction between tillage and layer indicates that under reduced tillage a significant effect of the layer was found, but not under conventional tillage.

The CAP model of group A explained in total 8 % of the variation in beta diversity related to soil management (tillage, organic matter addition), and the first two axes explained 2.6 % and 2.3 % of variation, respectively. CAP1 axis separated the samples belonging to the lower layer of reduced tillage from the rest, while CAP2 axis, from top to bottom, separated the different tillage treatments.

In group B, PERMANOVA did not reveal effects of organic matter addition (R^2 = 0.013, p = 0.186) on the nematode beta diversity (**Table S5**).

The dispersion tests were not significant, suggesting that differences between management were driven primarily by true biological differences and not by an artefact of the differences of the within-group dispersion (**Table S6**).

Table 5.1. Results of the mixed linear models testing the effect of soil management on total nematode qPCR counts, OTU richness, diversity, and evenness, and genus richness, diversity and evenness. We tested: for group A (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) the effect of tillage, organic matter addition and layer, and for group B (CH3, PT1 and CH3) only the effect of organic matter addition. For each group, in the upper part of the table the estimated means and 95% confidence intervals (in parentheses) are reported. In the lower part of the table, F statistics and *p*-values (values ≤ 0.05 in bold) for the main factors and their interactions are reported. The interactions are not reported because they were all not significant.

			qPCR counts	OTU richness	OTU diversity	OTU evenness	Genus richness	Genus diversity	Genus evenness
				(total OTU number)	(exp^H†)	(exp^H/OTU number)	(total genus number)	(exp^H)	(exp^H/ genus number)
Group A									
0-10 cm	**CT‡- LOW**		6373 (4428-8671)	112 (101-122)	15.8 (12.6-19.4)	0.14 (0.11-0.17)	41 (38-45)	8.3 (6.9-9.6)	0.20 (0.17-0.24)
	RT§- LOW		6640 (4596-9059)	118 (107-130)	19.2 (15.6-23.3)	0.16 (0.13-0.20)	42 (38-45)	9.8 (8.4-11.2)	0.24 (0.20-0.28)
	CT- HIGH		6725 (4574-9289)	117 (105-129)	13.7 (10.5-17.2)	0.11 (0.09-0.15)	42 (38-45)	6.3 (4.9-7.8)	0.15 (0.11-0.19)
	RT- HIGH		6999 (4870-9512)	124 (112-136)	16.8 (13.4-20.6)	0.14 (0.11-0.17)	42 (38-46)	7.9 (6.5-9.3)	0.19 (0.15-0.23)
10-20 cm	**CT- LOW**		4832 (3162-6856)	100 (90-110)	13.4 (10.5-16.7)	0.13 (0.11-0.16)	39 (36-43)	7.5 (6.2-8.8)	0.19 (0.16-0.23)
	RT- LOW		5065 (3304-7201)	106 (96-118)	16.6 (13.2-20.3)	0.16 (0.12-0.19)	40 (36-43)	9.1 (7.7-10.5)	0.23 (0.19-0.27)
	CT- HIGH		5139 (3285-7407)	105 (94-116)	11.4 (8.5-14.7)	0.11 (0.08-0.14)	40 (36-43)	5.6 (4.1-7.1)	0.15 (0.11-0.19)
	RT- HIGH		5379 (3537-7606)	112 (101-123)	14.3 (11.2-17.9)	0.13 (0.10-0.16)	40 (36-44)	7.2 (5.8-8.6)	0.18 (0.14-0.22)
	Tillage (T)	F *p*	0.22 0.64	6.56 **0.02**	10.26 **0.004**	6.45 **0.02**	0.39 0.54	7.31 **0.01**	5.89 **0.02**
	OM¶	F *p*	0.18 0.67	2.70 0.11	3.78 0.05	8.69 **0.007**	0.21 0.65	12.49 **0.002**	11.05 **0.003**
	Layer (L)	F *p*	8.40 **0.005**	38.73 **<0.0001**	7.75 **0.007**	0.75 0.40	10.60 **0.002**	2.47 0.12	0.53 0.47
Group B									
	LOW		4353 (617-11473)	110 (83-140)	17.6 (10.7-26.3)	0.16 (0.10-0.25)	41 (36-46)	9.0 (5.0-14.0)	0.22 (0.12-0.35)
	HIGH		5898 (1393-13521)	117 (91-147)	16.6 (10.9-23.4)	0.14 (0.08-0.21)	43 (38-47)	8.9 (5.4-13.2)	0.21 (0.12-0.32)
	OM	F *p*	3.65 0.08	2.29 0.16	0.22 0.65	1.29 0.28	2.47 0.14	0.01 0.94	0.17 0.69

†*Exp*^{H} exponential of the Shannon diversity index. ‡CT conventional tillage. §RT reduced tillage. ¶OM organic matter.

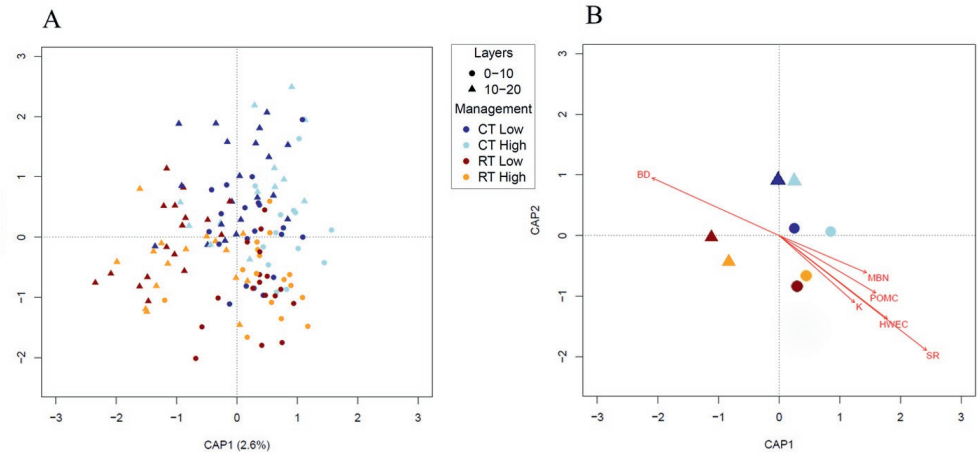

Figure 5.3. Constrained analysis of principal coordinates (CAP) showing in panel A) the effect of management and layer on the nematode beta diversity in group A (CH1, CH2, NL1, NL2, SL1, ES4 and HU4). The CAP model explained in total 8 % of the variation in beta diversity related to soil management (tillage, organic matter addition), and the first two axes explained 2.6 % and 2.3 % of the total variation, respectively. Panel B) shows the relationship between the nematode communities (displayed as centroids) and the soil parameters. Only the significant variables at $p <$ 0.01 are shown. The long-term field experiment (LTE) was used as a random effect (conditioned), and the blocking structure plus tillage, organic matter addition and layer were used as fixed effects. The different colours show the soil management and the different shapes show the different layers.

5.3.4 Effect of soil management on nematode trophic groups and food web indices

Bacterivorous nematodes were the most abundant trophic group, followed by herbivorous, fungivorous, omnivorous and predatory nematodes (**Table 5.2**, **Table S7**). For group A, we found a stratification effect of reduced tillage on relative abundance of bacterivorous nematodes, with lower values in the lower than in the upper layer (24% lower, p=0.0005) (**Figure S4**). The proportion of herbivorous nematodes was higher in the lower layer of reduced tillage (44%) compared to the upper layer of reduced tillage (19%) and both layers of conventional tillage (16% and 19% for higher and lower layer, respectively) (p=0.0004) (**Figure S4**). Its absolute abundance was 70% higher in reduced tillage compared to conventional tilled treatment (p=0.007), both in the 0-10 and 10-20 cm soil layer and regardless of organic matter. There was a 44% higher proportion of fungivorous nematodes in the upper layer of reduced tillage combined with low organic matter addition compared to the lower layer of the same treatment (p=0.009). No effect of soil management was found for relative abundances of omnivorous and predatory nematodes, but the relative abundance of omnivorous nematodes was 68% higher in the upper than in the lower layer across tillage and organic matter treatments.

Table 5.2. Results of the mixed linear models testing the effect of soil management on the percentage of nematode trophic groups (bacterivores, fungivores, herbivores, omnivores and predators). We assessed for group A (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) the effect of tillage, organic matter addition and layer, and for group B (CH3, PT1 and CH3) the effect of organic matter addition. For each group, in the upper part of the table the estimated means and 95% confidence intervals (in parentheses) are reported. In the lower part of the table, F statistics and *p*-values (values ≤ 0.05 in bold) for the main factors and their interactions are reported. Different letters following means (to be read per column) show treatments which are significantly different ($p \leq 0.05$) according to Tukey post-hoc tests for the three way interactions.

			Bacterivores	Fungivores	Herbivores	Omnivores	Predators
			Relative abundance (%)				
Group A							
0-10 cm	**CT†- LOW**		52 (35-68)	12 (6-22) bc	17 (6-39)	1.3 (0.3-4.6)	0.6 (0.2-2.4)
	RT‡- LOW		53 (35-70)	13 (6-25) c	18 (7-41)	2.2 (0.5-8.3)	0.9 (0.2-3.50
	CT- HIGH		65 (46-80)	9 (4-18) abc	16 (5-38)	1.1 (0.2-4.8)	0.4 (0.1-1.5)
	RT- HIGH		56 (38-73)	7 (3-15) abc	20 (7-44)	1.4 (0.3-5.5)	0.8 (0.2-3.0)
10-20 cm	**CT- LOW**		58 (40-73)	10 (5-19) abc	21 (8-45)	0.7 (0.2-2.9)	0.9 (0.2-3.1)
	RT- LOW		40 (25-58)	7 (3-14) a	45 (21-72)	0.5 (0.1-2.3)	0.8 (0.2-3.0)
	CT- HIGH		67 (49-81)	6 (3-13) ab	17 (6-40)	0.3 (0.1-1.5)	0.4 (0.1-1.6)
	RT- HIGH		43 (26-61)	8 (4-17) abc	43 (19-70)	0.4 (0.1-1.6)	0.6 (0.1-2.1)
	Tillage	F p	12.2 **0.002**	0.97 0.33	20.15 **0.0001**	0.09 0.76	1.52 0.23
	OM§	F p	3.7 0.067	5.98 **0.02**	0.20 0.65	1.27 0.27	3.45 0.07
	Layer	F p	3.64 0.06	10.27 0.002	27.43 **<0.0001**	25.35 **<0.0001**	0.02 0.88
	T X OM	F p	2.14 0.15	0.83 0.37	0.52 0.47	0.01 0.92	1.01 0.32
	T X L	F p	13.55 **0.0005**	0.17 0.68	14.49 **0.0004**	1.82 0.18	1.60 0.21
	OM X L	F p	0.13 0.71	3.92 **0.05**	0.39 0.53	0.43 0.51	0.25 0.62
	T X OM X L	F p	0.06 0.79	7.22 **0.009**	0.006 0.94	0.90 0.35	0.005 0.94
Group B							
	LOW-CT		47 (11-86)	9 (4-21)	29 (6-72)	0.7 (0.01-33)	1.7 (0.06-32)
	HIGH-CT		62 (20-92)	11 (4-26)	18 (6-72)	0.9 (0.01-0.36)	1.8 (0.07-32)
	OM	F p	9.82 **0.009**	1.55 0.24	6.65 **0.02**	0.33 0.58	0.05 0.82

†CT conventional tillage. ‡RT reduced tillage. §OM organic matter.

Table 5.3. Results of the mixed linear model testing the effect of soil management on the maturity index, enrichment index, structure index and channel index. We assessed for group A (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) the effect of tillage, organic matter addition and layer, and for group B (CH3, PT1 and CH3) the effect of organic matter addition. In the table F statistics and *p*-values (significance at $p \leq 0.05$ in bold) for the main factors are reported. The interactions are not reported because they were all not significant.

			Maturity index	Enrichment index	Structure index	Channel index
Group A						
0-10 cm	**CT†- LOW**		1.64 (1.44-1.85)	79.4 (65.9-92.9)	32.9 (15.6-50.4)	6.5 (2.0-21.3)
	RT‡- LOW		1.84 (1.63-2.04)	73.4 (59.9-86.9)	40.3 (22.8-57.8)	10.3 (3.1-33.8)
	CT- HIGH		1.56 (1.35-1.77)	80.1 (66.4-93.7)	29.2 (11.5-47.0)	4.9 (1.5-16.4)
	RT- HIGH		1.75 (1.54-1.96)	74.1 (60.5-87.6)	36.5 (18.9-54.1)	7.8 (2.4-25.7)
10-20 cm	**CT- LOW**		1.56 (1.36-1.76)	82.1 (68.6-95.5)	30.2 (12.8-47.6)	5.2 (1.6-16.9)
	RT- LOW		1.75 (1.54-1.96)	76.1 (62.5-89.6)	37.5 (19.9-55.0)	8.1 (2.5-26.7)
	CT- HIGH		1.48 (1.26-1.69)	82.7 (69.1-96.3)	26.4 (8.7-44.2)	3.9 (1.2-12.9)
	RT- HIGH		1.67 (1.46-1.88)	76.7 (63.1-90.2)	33.7 (16.1-51.3)	6.1 (1.9-20.3)
	Tillage	F *p*	13.13 **0.001**	12.56 **0.001**	8.16 **0.008**	8.28 **0.008**
	OM§	F *p*	2.40 0.13	0.12 0.72	1.64 0.21	2.65 0.11
	Layer	F *p*	4.92 **0.03**	4.45 **0.04**	1.56 0.22	3.58 0.06
Group B						
	LOW-CT		2.1 (1.1 - 3.1)	67.2 (42.9 - 91.5)	49.0 (-0.24.8 - 122.9)	20.8 (-1.2 - 42.9)
	HIGH-CT		1.9 (1.0 - 2.9)	74.4 (51.6 - 97.2)	47.5 (-25.9 - 121.0)	11.8 (-9.3 - 33.0)
	OM	F *p*	1.85 0.20	3.10 0.10	0.17 0.69	8.8 **0.01**

†CT conventional tillage. ‡RT reduced tillage. §OM organic matter.

The food web indices, MI, SI and CI were significantly higher in plots where reduced tillage was applied (MI = 1.8, SI = 37.0, CI = 8.0) than in conventional tillage plots (MI = 1.6, SI = 29.8, CI = 5.0), while the EI was significantly higher under conventional tillage (EI = 81.1) than under reduced tillage (EI = 75.1) (**Table 5.3**, **Figure 5.4**). We found significantly higher values of MI in the upper (MI = 1.7) than in the lower layer (MI = 1.6), and significantly higher values of EI in the lower (EI = 79.4) than in the upper layer (EI = 76.8) (**Table 5.3**). Accordingly, we found a 13% higher proportion of c-p 1 (colonizers) and a 32% lower proportion of c-p 4 (persisters) in the lower layer than in the upper layer (Table S8), but in terms of absolute abundance the c-p 1 nematodes were 29% higher in the upper layer (2286 nematodes 100 g field moist soil^{-1}) compared to the lower one (1812 nematodes 100 g field moist soil^{-1}) (**Table S9**).

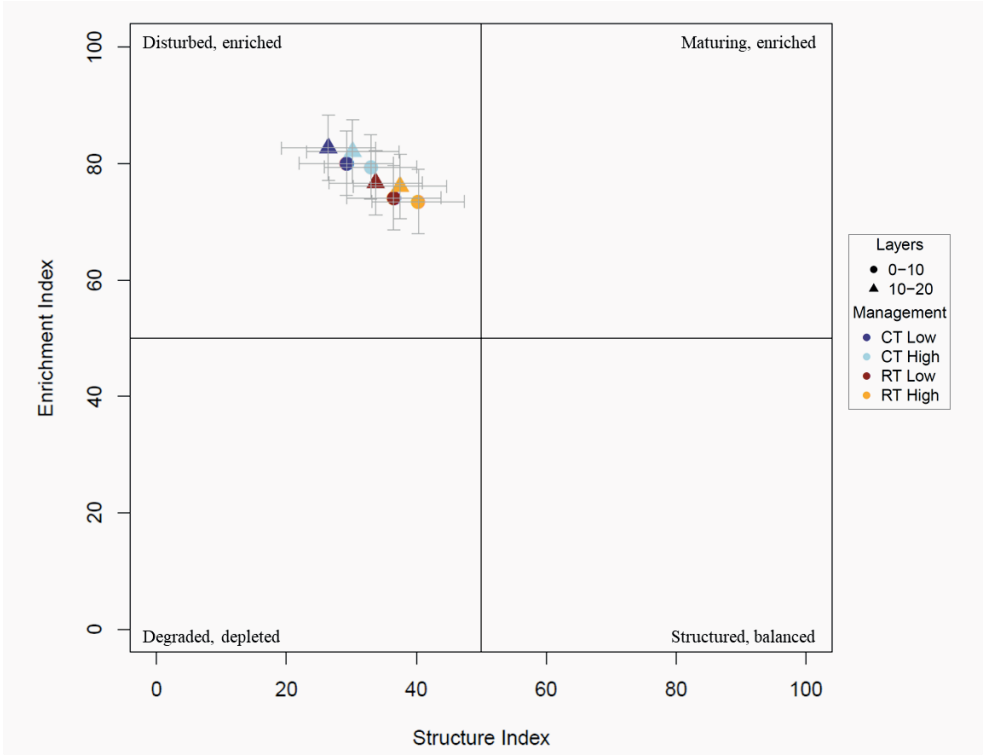

Figure 5.4. Enrichment (y axis) - structure (x axis) diagram for the long-term field experiments (LTEs) of group A (CH1, CH2, NL1, NL2, SL1, ES4, HU4). In panel A) all the four quadrants of the diagram are shown; in panel B) zoom-in of the first quadrant of the diagram is shown. The points and the triangles represent the estimated means from the linear effect mixed models for the respective combination of factors (tillage, organic matter addition) for the first layer and the second layer, respectively. The bars represent the estimated standard errors for the group averages. In the corner of each of the four quadrants we report information relative to structure of the food web and nutrient enrichment, respectively, according to Ferris et al. (2001).

For the LTEs belonging to group B, the proportion of bacterivorous nematodes was significantly increased with high compared to low organic matter addition, while herbivorous nematodes showed the opposite pattern (**Table 5.2**). However, in absolute abundance the herbivorous nematodes did not differ between the two treatments (**Table S7**). We found no effect of organic matter addition on most food web indices. Only the CI was significantly higher in the low than in the high organic matter treatment (**Table 5.3**).

5.3.5 Relationships between soil parameters and nematode communities

Partial correlations between total nematode qPCR counts and soil chemical, physical, and biological parameters are reported in **Table 5.4**. In group A, qPCR counts were positively correlated with many chemical (TN, TOC, available K, Mg), physical (WSA) and biological (SR, MBC, MBN, qMic, soil suppressiveness) parameters, and with four of the labile carbon

Table 5.4. Partial correlation coefficients between total nematode qPCR counts, OTU richness, diversity, and evenness and chemical, physical and biological indicators for the samples belonging to group A (n=132) and group B (n=35).

	Group A								Group B							
	qPCR counts		**OTU richness** (total OTUs number)		**OTU diversity** (exp^H)		**OTU evenness** (exp^H/OTU number)		**qPCR counts**		**OTU richness** (total OTUs number)		**OTU diversity** (exp^H)		**OTU evenness** (exp^H/OTU number)	
Chemical parameters†																
TOC	0.31	**	0.36	***	0.14		-0.0002		0.12		0.003		0.15		0.14	
pH	-0.02		0.06		-0.02		-0.04		0.37		0.06		-0.05		-0.07	
TN	0.34	***	0.34	***	0.18	*	0.04		0.02		-0.22		0.05		0.15	
C/N	-0.35	**	-0.28	*	-0.25	*	-0.15		0.12		0.29		0.17		0.06	
CEC	0.10		0.14		0.33	***	0.30	**	-0.14		-0.39	*	0.19		0.37	
Ca	-0.02		0.04		-0.09		-0.10		0.03		0.07		0.29		0.28	
Mg	0.13		0.18	*	0.24	*	0.18	*	-0.15		-0.27		0.04		0.15	
K	0.21	*	0.39	***	0.25	*	0.14		0.25		0.17		0.11		0.03	
Na	-0.19	*	-0.20	*	-0.10		-0.04		-0.05		0.10		0.11		0.06	
P	0.14		0.25	*	0.08		-0.01		-0.10		0.21		0.15		0.07	
Physical parameters‡																
WSA	0.24	*	0.30	***	0.17	*	0.06		0.10		-0.14		-0.24		-0.17	
WHC	0.06		0.04		0.03		0.01		0.007		-0.03		0.10		0.11	
BD	-0.38	***	-0.38	***	-0.17		-0.03		-0.20		-0.06		0.15		0.17	
Sand	0.04		-0.009		-0.08		-0.08		-0.11		0.48	*	0.28		0.07	
Silt	0.07		0.10		-0.06		-0.11		0.27		0.23		0.02		-0.08	
Clay	-0.05		-0.20	*	-0.04		0.03		-0.51	*	-0.37		0.19		0.36	

Continue

Biological parameters§															
MBC	0.43	***	0.41	***	0.16		0.0007		-0.08		-0.23		-0.06		0.04	
MBN	0.44	***	0.21	*	0.05		-0.04		-0.24		0.13		0.19		0.14	
SR	0.45	***	0.33	***	0.24	*	0.09		0.10		-0.05		-0.15		-0.12	
qMic	0.22	*	0.22	*	0.09		0.009		0.009		-0.22		-0.15		-0.05	
qCO_2	-0.02		-0.02		0.13		0.19	*	0.20	*	0.21		-0.02		-0.11	
Earthworm number	-0.10		-0.09		-0.17		-0.02		0.08		-0.16		-0.10		-0.03	
Earthworm biomass	0.05		-0.04		-0.12		-0.05		0.09		-0.24		-0.16		-0.06	
Tea bag decomposition	-0.49	*	-0.31	*	-0.35	*	-0.27	*	0.002		0.22		-0.12		-0.20	
Soil suppressiveness	0.37	*	0.20		0.13		0.07		-0.16		0.09		0.0008		-0.04	
Labile carbon fractions¶															
Hy SUVA	-0.20	*	0.06		0.07		0.05		-0.10		0.17		-0.11		-0.19	
DOC SUVA	-0.26	*	-0.06		0.07		0.10		0.009		-0.02		0.03		0.04	
Hy-DOC	0.27	*	0.14		-0.06		-0.13		0.08		-0.02		0.05		0.06	
DOC	0.06		0.09		0.08		0.03		0.10		-0.13		-0.09		-0.03	
HWEC	0.48	***	0.35	***	0.19	*	0.05		0.06		-0.16		0.08		0.14	
POXC	0.46	***	0.36	***	0.18	*	0.04		0.17		0.003		0.10		0.09	
POMC	0.49	***	0.46	***	0.12		-0.07		0.24		0.16		0.08		0.01	

†*TOC* total organic carbon, *TON* total nitrogen, *C/N* carbon to nitrogen ratio, *CEC* cation exchange capacity. ‡*WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density. §*MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *qCO2* metabolic quotient, *qMic* microbial quotient. ¶ *Hy* hydrophilic carbon, *Hy SUVA* specific ultraviolet absorbance of hydrophylic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.

*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$

Table 5.5. Indicator species for the combination of tillage, organic matter addition and layer for group A (CH1, CH2, NL1, NL2, SL1, ES4 and HU4). In the table we report the OTU number, taxonomic information at the level of nematode family and genus, feeding group, *c-p* colonizer-persister class, the correlation coefficient and the *p*- value from the analysis, and the relative abundances of the taxa for the combinations of soil management (tillage and organic matter addition). Above the columns with the OTU relative abundance the number of samples belonging to each group is indicated, and in parentheses the number of samples in which that specific OTU was found is given. The analysis has been done for the two layers separately, and only OTUs that had a level of significance $p \leq 0.01$ are reported.

	OTU	Genus	Family	Feeding Group†	c-p	Correlation	p-value	CT-LOW	CT-HIGH	RT-LOW	RT- HIGH
								Relative abundance (%)			
Layer 0-10 cm								N= 20	N=13	N=17	N=16
CT§-LOW	OTU_329	Aphelenchoides	Aphelenchoididae	F	2	0.34	0.007	0.68 (12)	0.06 (6)	0.01 (6)	0.142 (9)
CT-HIGH	OTU_436	NA‡	Tylenchidae	H (1e)	NA	0.38	0.005	0.0001 (2)	0.02 (4)	0.0003 (1)	0 (0)
	OTU_75	Aphelenchoides	Aphelenchoididae	F	2	0.26	0.007	0.35 (15)	0.68 (10)	0.15 (15)	0.035 (9)
RT¶-LOW	OTU_310	Dicelis	Drilonematidae	B	2	0.39	0.006	0.02 (8)	0.004 (9)	0.02 (14)	0.01 (9)
	OTU_44	Oscheius	Rhabditidae	B	1	0.39	0.0001	0.07 (14)	0.21 (13)	0.71 (15)	0.25 (14)
	OTU_84	Aphelenchoides	Aphelenchoididae	F	2	0.37	0.004	0.07 (9)	0.04 (10)	0.28 (11)	0.05 (6)
RT-HIGH	OTU_120	Pratylenchus	Pratylenchidae	H (1b)	3	0.39	0.003	0.002 (2)	0.0001 (1)	0.0001 (1)	0.20 (6)
	OTU_48	NA	NA	NA	NA	0.33	0.0007	0.25 (11)	0.0005 (3)	0.003 (7)	1.41 (12)
	OTU_79	Acrobeloides	Cephalobidae	B	2	0.35	0.005	0.113 (9)	0.047 (3)	0.01 (6)	1.29 (7)
CT-HIGH, RT-LOW, RT-HIGH	OTU_256	NA	NA	NA	NA	0.32	0.004	0.002 (2)	0.01 (5)	0.02 (10)	0.03 (10)
	OTU_82	Aphelenchoides	Aphelenchoididae	F	2	0.43	0.0008	0.06 (16)	0.16 (12)	0.25 (17)	0.23 (16)
	OTU_728	Oscheius	Rhabditidae	B	1	0.33	0.002	0.02 (11)	0.12 (9)	0.21 (14)	0.15 (9)
Layer 10-20 cm											
CT-LOW	OTU_329	Aphelenchoides	Aphelenchoididae	F	2	0.31	0.005	0.71 (11)	0.02 (7)	0.03 (8)	0.06 (7)
CT-HIGH	OTU_75	Aphelenchoides	Aphelenchoididae	F	2	0.37	0.0007	0.38 (12)	1.06 (10)	0.04 (8)	0.02 (12)
RT-HIGH	OTU_218	Nothotylenchus	Anguinidae	F	2	0.24	0.006	0.01 (5)	0.004 (4)	0.01 (3)	0.08 (6)
	OTU_257	Panagrolaimus	Panagrolaimidae	B	1	0.37	0.009	0.00 (2)	0.0002 (1)	0.0003 (2)	0.01 (6)
	OTU_30	Pratylenchus	Pratylenchidae	H (1b)	3	0.38	0.003	0.04 (12)	0.003 (9)	0.007 (11)	4.26 (15)
HIGH	OTU_712	Neopsilenchus	Tylenchidae	H (1e)	2	0.51	0.005	0.06 (13)	0.43 (12)	0.07 (12)	0.82 (13)
RT	OTU_15	NA	Merlinidae	H (1d)	NA	0.25	0.008	0.70 (20)	0.07 (13)	2.91 (17)	1.04 (16)
	OTU_70	NA	Tylenchidae	H (1e)	2	0.28	0.001	0.14 (11)	0.04 (7)	0.47 (14)	0.48 (10)
CT	OTU_2	Rhabditis	Rhabditidae	B	1	0.40	0.002	24.82 (20)	30.31 (13)	14.14 (17)	7.85 (16)
	OTU_39	NA	NA	NA	NA	0.32	0.008	0.88 (17)	2.73 (10)	0.02 (13)	0.11 (11)

†*B* bacterivorous nematode, *F* fungivorous nematode, *H* herbivorous nematode, *1b* herbivorous migratory endoparasitic nematodes, *1d* herbivorous ectoparasitic nematodes, *1e* herbivorous epidermal and root hair feeders. ‡*NA* not assigned to taxon. §CT conventional tillage. ¶RT reduced tillage.

fractions (Hy-DOC, POXC, HWEC, and POMC). Negative correlations were found with the soil C to N ratio, BD, tea bag decomposition, and Hy- and DOC SUVA (**Table 5.4**).

Correlations between OTU richness and soil parameters were similar to those of nematode qPCR counts and soil parameters, although the correlation coefficients were weaker for all the variables except K (**Table 5.4**). In contrast, correlations between OTU diversity or evenness and soil parameters were fewer, and, with the exception of CEC, explained less or the same amount of the variance (**Table 5.4**). For group B we found very few and not very strong significant relationships between soil parameters and nematode communities (**Table 5.4**).

TOC, available K, BD, MBC, MBN, SR, HWEC, POXC, and POMC were significantly associated with nematode community composition (**Table S10**). Of these variables, only the ones with a significance level < 0.01 are reported in **Figure 5.4B** (BD, available K, MBN, POMC, HWEC, SR). With the exception of BD, these parameters, plus TN and Mg, were positively correlated with CAP1 and negatively correlated with CAP2 (**Table S11**), being higher in the upper compared to lower layers (Figure 3B). The contrary was true for the BD, which was higher in the lower layer, in particular under reduced tillage (**Fig. 5.3B**, **Table S11**). In addition, qMic and DOC SUVA were positively and negatively related, respectively, only with CAP1, CEC and WSA were negatively correlated only with CAP2, and C to N ratio was positively correlated only with CAP2 (**Table S11**).

5.3.6 Indicator OTUs for tillage and organic matter addition

Out of 349 OTUs finally used for analysis, 12 OTUs were significantly associated with specific management combinations in the upper layer, and 10 OTUs were significantly associated with the lower layer (group A only, as no differences in nematode communities were found in group B, **Table 5.5**). The indicator OTUs were herbivorous (OTUs assigned as *Pratylenchus*, *Neopsilenchus,* Merlinidae), fungivorous (OTUs assigned as *Aphelenchoides*, *Nothotylenchus*) and bacterivorous (OTUs assigned as *Acrobeloides*, *Panagrolaimus*, *Rhabditis*). Indicator OTUs belonged mainly to c-p groups 1 and 2 and were all present in relative abundance < 0.1 %, apart from OTU_2 (OTU assigned as *Rhabditis*) which was an indicator OTU for conventional tillage in the lower layer. This OTU comprised more than 20% of the relative abundance of all nematode reads.

5.4 Discussion

5.4.1 The largest proportion of variation in nematode communities is explained by site

Measured abiotic and biotic (MBC) differences between the LTEs explained most of the variation in nematode communities, in line with results from Neher et al. (1995) and Thomson et al. (2015). This result is plausible, since the LTEs were selected to maximize inter-site variation and to test if, in spite of large differences in sites across pedoclimatic conditions, effects of agricultural management were yet significant. Indeed, nematode communities were significantly related to all other measured soil parameters when LTE was not used as a random factor.

5.4.2 Reduced tillage increases nematode alpha diversity and alters beta diversity compared to conventional tillage

In accordance with our first hypothesis, nematode OTU richness and, to a larger extent, OTU (and genus) diversity and evenness were increased in reduced compared to conventional tillage across the LTEs of group A, i.e. in LTEs where the 0-10 and 10-20 cm layers were sampled (LTEs: CH1, CH2, SL1, NL1, NL2, ES4, HU4). Previous studies reported positive effects of reduced tillage on nematode abundance, richness, and diversity (Fu et al., 2000; Okada and Harada, 2007; Zhang et al., 2015). Reduced soil disturbance (here very shallow or non-inversion cultivation in the 0-10 cm layer) can exert a positive effect on nematodes through the increase of total organic carbon, soil aggregation and microbial biomass, and a lower physical pressure (Kladivko, 2001). The lower nematode qPCR counts, richness and diversity in the lower soil layer under reduced tillage, where disturbance is lower, could be due to decreased resources present in this layer. Under reduced tillage, soil parameters related to soil organic matter and nutrients have lower values below the plough layer (Franzluebbers, 2002), which can be explained by the retention of crop residues on the soil surface, and the lack of mechanical mixing of soil layers.

In group A, reduced tillage led to a shift in nematode community structures, in agreement with previous studies (Brmež et al., 2006; Okada and Harada, 2007; Griffiths et al., 2012). In this group of LTEs, nematode beta diversity was affected by the organic matter additions, and OTU diversity was lower in the plots with high organic matter additions, which might suggest positive effects of the organic matter added on a few opportunistic nematodes. However, in disagreement with our second hypothesis, we did not find an effect of organic matter additions on nematodes qPCR counts, and alpha and beta diversity in group B, i.e. in LTEs where the 0-20 cm layer was sampled as a whole (LTEs: CH3, PT1, HU1). Also in the literature contradictory results were found, reporting negative

(Wang et al., 2004), neutral (Ito et al., 2015; Quist et al., 2016; Li et al., 2018a) and positive effects of organic matter on nematode numbers (Nahar et al., 2006; Sánchez-Moreno et al., 2009; Ugarte et al., 2013), richness (Sánchez-Moreno et al., 2009) and alpha diversity (van Diepeningen et al., 2006; Okada and Harada, 2007) in systems where organic matter was added.

Organic matter is a food source for microorganisms which in turn are a food source for bacterivorous, fungivorous and omnivorous nematodes; therefore, organic matter, similarly to reduced tillage, can change soil properties favourable to nematodes (food availability, but also water retention and soil aggregation) (Bongers and Ferris, 1999). In the LTEs of group B, we found higher concentrations of total (TOC) and labile (POXC) organic matter ($p = 0.03$ and $p < 0.0001$, respectively) in the high compared to the low organic matter input treatments, but we did not find differences in microbial biomass, cation exchange capacity and water stable aggregates ($p = 0.06$, $p = 0.12$ and $p = 0.51$, respectively). Our contradicting results on the effect of organic matter additions on nematodes could be related to the different types of organic matter used in our LTEs (e.g. compost, biochar, farmyard manure, etc.). The composition and the amount of organic matter applied to the soil is an important factor for its effect on nematodes (Ito et al., 2015; Liu et al., 2016a; Li et al., 2018a). Also, it is possible that the conventional tillage applied to the LTEs of group B neutralized the effect of organic matter additions (Briar et al., 2007). This weak effect of organic matter addition supports previous studies that suggested that tillage has a stronger effect on nematode communities than organic matter addition or other agricultural practices such as organic *vs*. conventional management, irrigation, and cover crops (Neher, 1999; Ito et al., 2015; Zhong et al., 2017; du Preez et al., 2018).

5.4.3 Reduced tillage increases stability and structure of the nematode community compared to conventional tillage

Agricultural management did not have strong effects on the relative abundance of the trophic groups, but it affected the food web indices, indicating effects on rates rather than on structural changes in the food web. This observation supports the suggestion by Neher (1999) that food web indices are less variable and more likely to detect effects of management practices on soil processes than measures based on individual trophic groups.

In accordance with our first hypothesis and in line with previous reports (Habig and Swanepoel, 2015; Zhang et al., 2015; Zhong et al., 2017), reduced tillage resulted in a less disturbed environment than conventional tillage, increasing the stability and the number of food web interactions of the nematode communities (higher MI and SI) in the LTEs of group A. Despite the decreasing level of disturbance in the lower soil layer of reduced

tillage, a lower MI and reduced proportions of omnivorous and stress-tolerant c-p 4 nematodes compared to the upper layer seems to indicate a more stressed environment where opportunistic nematodes can prevail. In our study, reduced tillage increased the channel index (CI), i.e. among the opportunistic microbivorous nematodes there was an increase in the proportion of fungal feeders, confirming previous findings (Sánchez-Moreno et al., 2006; Minoshima et al., 2007; Okada and Harada, 2007). Reduced tillage is known to favour the fungal decomposition pathway (Six et al., 2006), due to less or no disruption of the hyphal network (Minoshima et al., 2007). Since lower values of CI are associated with faster rates of decomposition and nutrient turnover, our results suggest that changes in nematode communities under reduced tillage may contribute to the increased capability of the system to retain nutrients and store carbon (Griffiths et al., 2012). The higher relative and absolute abundance of herbivorous nematodes in reduced tillage compared to conventional tillage is in line with previous studies (Freckman and Ettema, 1993; Fu et al., 2000; Brmež et al., 2006; Treonis et al., 2010; Treonis et al., 2018), and can be explained by a higher incidence of roots in the field, stimulating this nematode group (Minton, 1986; You et al., 2017) Our results indicate a possible trade-off in reduced tillage systems in terms of soil processes, and that in these types of systems care must be taken regarding the assessment and control of herbivorous nematodes. However, the higher alpha diversity, MI and SI found in reduced tillage could indicate that the activity of herbivorous populations might be controlled by a more stable and structured food web.

In agreement with our second hypothesis, high organic matter addition plots resulted in higher percentages of bacterivorous nematodes than low organic matter addition plots, and they showed a statistically lower CI and a tendency towards lower SI, MI, and higher EI. High EI (Berkelmans et al., 2003; Forge et al., 2005; Sánchez-Moreno et al., 2009), low MI (Neher and Olson, 1999; Forge et al., 2005; Wang et al., 2006) and low SI (Villenave et al., 2010; Pan et al., 2015) have been previously reported in systems with organic matter addition. Such changes in MI and CI can be explained by an increase in opportunistic bacterivores (Ferris and Bongers, 2006), and a stimulation of the bacterivore decomposition channel (Wang et al., 2004; Pan et al., 2010). Altogether, these results indicate higher nutrient cycling, N mineralization and fertility in soils with high organic matter additions (Ferris and Matute, 2003). By contrast, the addition of organic matter decreased the proportion of herbivorous nematodes, but this did not coincide with an absolute decrease as this relative decrease resulted from the absolute increase of bacterivorous nematodes.

5.4.4 Nematode communities are mainly related to soil organic carbon and biological parameters

Total and labile organic carbon and microbial parameters were most strongly and positively related to nematode qPCR counts and richness, partly confirming our third hypothesis. Abundance (Sánchez-Moreno et al., 2006), richness (van Diepeningen et al., 2006), but also diversity (Zhang et al., 2017) of soil nematodes have previously been positively linked with the levels of total and labile organic carbon fractions. Higher total and labile carbon are linked to higher microbial biomass, soil respiration, water retention, soil structure and lower bulk density (Bongiorno et al., 2019; Chapter 3 (this thesis)). Increased levels in these soil parameters can optimize the habitat conditions for nematodes, and facilitate their movement through the soil pore water (Nielsen et al., 2014).

Some of the properties that correlated most with nematode qPCR counts and richness (total organic and labile carbon, available K, bulk density, microbial biomass and activity) proved important in explaining differences between nematode communities caused by reduced *vs.* conventional tillage. This suggests that reduced tillage affects nematode communities through its positive effects on these soil properties, either directly through absence of soil inversion, i.e. lower soil disturbance, or indirectly through retention of crop residues at the soil surface, which can increase water retention and infiltration, soil organic carbon, and organism biomass and activity (Mloza-Banda et al., 2016; Ranaivoson et al., 2017).

5.4.5 Only r selected taxa were found to be indicator OTUs for tillage and organic matter addition

Indicator OTU analysis based on group A revealed OTUs that were significantly associated with tillage and organic matter management. Most of the indicator OTUs had a very low relative abundance. These taxa belonged mainly to the c-p 2 group, and to bacterivorous, fungivorous and herbivorous nematode trophic groups. Therefore, contrary to our fourth hypothesis none of the predatory and omnivorous nematodes, or nematodes belonging to c-p groups 4 and 5 were detected as indicator taxa. This can be due to the fact that in these intensively managed European arable systems, relative and absolute abundances of highly sensitive nematode taxa were underrepresented and too variable (i.e. not present in all samples).

5.4.6 Advantages and limitations of studying nematode communities with amplicon sequencing

Our molecular analyses revealed that, despite the big influence of the pedoclimatic characteristics, agricultural soil management resulted in changes in nematode communities

and nematode food web structure in line with previous findings from microscopic analysis and general knowledge of agricultural systems. In addition, nematode molecular analyses provided advantages in terms of costs and number of samples analyzed at the same time, and did not require expert skills for morphological characterisation.

A limitation of current amplicon sequences approaches is that previous studies found that the relative read abundance obtained do not perfectly match absolute abundance data determined microscopically. Possibly, the number of ribosomal DNA copies differ depending on the taxon, the organism's body size, the developmental stage, and PCR primer bias (Darby et al., 2013; Geisen et al., 2018). This has to be considered and standardized in future efforts to allow direct comparisons between morphological and molecular approaches in determining nematode communities.

In our study, a relatively large group of OTUs could not be classified at all. This underlines the problems in reliably assigning OTUs to their correct taxonomic group. Such taxa could belong to not yet studied nematode species, but most likely could indicate lack of information in the data bases. In addition, our methodology used to assign taxonomy, using only forward reads, could have had negative consequences for annotation (resolution power) and error correction which can be applied during read merging.
All in all, future studies should work towards an optimization of molecular methods for assessing relative and total nematode abundance, nematode taxonomy and the definition of standardized protocols and the amelioration of data bases in order to guarantee a more confident application of nematode communities studied with molecular methods in soil quality assessments.

5.5 Conclusion

Molecular nematode community analyses effectively differentiate soil management across ten different European long-term field experiments. In particular, reduced tillage had a stronger effect on nematode communities than organic matter addition, increasing nematode taxon richness, diversity and evenness. Reduced tillage also affected the nematode food web indices, stimulating more mature and fungal-based nematode communities, indicating a more stable food web with higher nutrient retention capability, but also increasing the number of herbivorous nematodes. These results are in line with previous findings based on microscopic analysis and general knowledge on nematode community dynamics in agricultural systems.

The relationships found between soil nematode communities and total and labile organic carbon, total nitrogen, available K, and microbial biomass and activity, underline the relationship between nematode communities and biological soil quality achieved by

reduced tillage, and indicate that nematode communities are equally sensitive indicators of soil quality as these parameters.

Our findings indicate that molecular methods are promising in the assessment of biological soil quality based on nematode community structure and indices, especially if future research will work toward an optimization and standardization of the methods.

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CHAPTER 6
6

Soil management intensity steers microbial catabolic profiles across European long-term field experiments

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Abstract

Assessing soil microbial functionality has the potential to reveal meaningful effects of soil management on soil processes influencing soil quality. We used MicroResp™ to assess microbial respiration upon the addition of six carbon substrates (glucose, alanine, aminobutyric acid, N-acetyl glucosamine, alpha-ketoglutaric acid, and lignin). From this, we calculated the multiple substrate induced respiration (MSIR), the microbial catabolic profile expressed as absolute and relative utilization rate, and the Shannon microbial functional diversity index (H'). We tested the effect of tillage (*reduced* vs. *conventional*) and organic matter addition (*high* vs. *low*) on these parameters in 10 European long-term field experiments (LTEs), and investigated their relationships with various soil parameters linked to soil functions. Reduced tillage and high organic matter input, especially when applied in combination, increased MSIR compared to conventional tillage and low organic matter input, which we consider more intensive soil management. In addition, reduced tillage resulted in a slightly higher functional diversity compared to conventional tillage. An increase in soil management intensity was associated with lower utilization of all the substrates expressed as absolute utilization rate, and a proportionately higher utilization of alpha-ketoglutaric acid compared to the other substrates. These two differences coincided with lower soil quality in the more intensive management systems, as measured by various soil parameters, in particular total and labile organic carbon, total nitrogen, basal respiration, and microbial biomass nitrogen. Labile organic carbon had a direct key role in promoting microbial functional diversity. Our results show that reduced tillage and increased organic matter addition created a more favourable habitat for soil microbial diversity, increasing the capacity of the microbial community to utilize different carbon substrates and, thereby, the potential for nutrient cycling. MicroResp™ was found to be a suitable method for soil quality assessment, and future studies should focus on how to include it in soil quality assessment schemes.

6.1 Introduction

Soil microbial communities have a primary role in various soil processes such as nutrient cycling, decomposition, carbon sequestration, soil structure development, water cycling and retention, and control of pest and pathogen populations (Barrios, 2007; Murphy et al., 2007). Since soil microorganisms and the processes they perform are sensitive to chemical and physical changes in their environment, they can be used to monitor the effects of soil management on soil functioning (Bünemann et al., 2006). Ultimately, changes in soil microbial properties can inform about soil quality, defined as the capacity of soil to perform multiple functions (Bünemann et al., 2018).

Microbial parameters have been included in soil quality assessment schemes since many years. However, they are less frequently used than chemical and physical indicators (Bünemann et al., 2018; Schloter et al., 2018). This is probably related to the fact that microbial dynamics can be very strong, the establishment of standardized procedures for their assessment is challenging, and the interpretation of the results obtained is complicated due to the difficulty of establishing optimal ranges (Lemanceau et al., 2014; Samaritani et al., 2017).

The microbial parameters most often used in soil quality assessments are microbial biomass and basal soil respiration (Bünemann et al., 2018). For soil quality assessment, measures of microbial functionality can be more informative than the assessment of microbial biomass, the presence of individual or groups of organisms and/or community composition based on purely taxonomic information (Zak et al., 1994; Krause et al., 2014; Wood et al., 2015b). For example, taxonomic knowledge about microbial communities is often not powerful enough to obtain concrete information about processes, which is essential in the assessment of soil functions (Barberan et al., 2012). There is evidence that the rate at which ecosystem processes occur is, in fact, governed by functional diversity (Heemsbergen et al., 2004; Mokany et al., 2008). Several studies found that functional and taxonomic diversity were not correlated, and that changes in functional diversity could more successfully be used to understand effects of land use on soil microbial communities and related soil processes than changes in taxonomic diversity (Wood et al., 2015a; Manoharan et al., 2017; Bei et al., 2018; Cheng-yu et al., 2018; Li et al., 2019). This fits very well in the more general observation that functional diversity is a better predictor of processes and ecosystem stability than phylogenetic diversity (Pankhurst et al., 1997a; Degens et al., 2001; Mokany et al., 2008; Fanin and Bertrand, 2016).

For the study of microbial functional diversity, community level physiological profiling (CLPP), also called catabolic profiling, is often used. The most widely applied CLPP systems are BIOLOG™ and the MicroResp™ (Garland and Mills, 1991; Campbell et al., 2003). These systems quantify the functional diversity of the microbial community by

monitoring its potential to decompose a selection of carbon substrates with contrasting chemical characteristics, i.e. carbohydrates, amines, amino-acids, carboxylic acids and more recalcitrant compounds such as polymers or phenolic compounds (Garland and Mills, 1991; Campbell et al., 2003). MicroResp™ has been used in previous studies for determining differences in microbial functional diversity due to land use (Brackin et al., 2013; Murugan et al., 2014; Creamer et al., 2016b; Moscatelli et al., 2018) and to soil management practices such as mineral fertilization and organic matter addition (Ge et al., 2013; Murugan et al., 2014; Hupfauf et al., 2016; Pan et al., 2016; Martínez-García et al., 2018; van der Bom et al., 2018) and, to a lower extent, tillage (Rincon-Florez et al., 2016; Zhang et al., 2018). Soil conservation management practices such as organic matter addition and reduced tillage are known to have a positive effect on various chemical, physical and biological properties in soil (White et al., 2012; Li et al., 2015; D'Hose et al., 2018). In particular, organic matter addition can increase microbial biomass and alter the microbial community composition, steering it towards higher abundance and activity especially of those organisms that can degrade a wide variety of substrates (Ge et al., 2013; Martínez-García et al., 2018; van der Bom et al., 2018) and recalcitrant substances (Hartmann et al., 2014; Francioli et al., 2016), thereby enhancing microbial functional diversity (Gomez et al., 2006; Govaerts et al., 2007; Nair and Ngouajio, 2012; Reilly et al., 2013; Murugan et al., 2014). Also reduced tillage generally affects microbial community composition and increases microbial activity and metabolic capacity in the topsoil (Mbuthia et al., 2015; Hao et al., 2019), although no effect on microbial communities has also been reported (Cheng-yu et al., 2018; Li et al., 2019). However, we are not aware about studies assessing the combined long-term effects of tillage and organic matter addition on microbial catabolic profiles. Moreover, knowledge is lacking about the general suitability of microbial catabolic profiles as soil quality indicators in arable fields across different pedoclimatic zones, and their relation with soil parameters. Our study is the first one to address these knowledge gaps, trying to expand our understanding of the drivers of microbial catabolic profiles.

The objectives of our study were to i) determine if tillage (reduced *vs.* conventional) and organic matter addition (high *vs.* low) affect basal respiration, multiple substrate induced respiration, the catabolic profiles, and microbial functional diversity; ii) identify how the utilization of carbon substrates discriminates between the different soil management practices, and which are the most important substrates contributing to this separation; iii) investigate the relation between the catabolic profiles, microbial functional diversity and soil chemical, physical and biological properties; and iv) assess which are the most important parameters driving microbial functional diversity. To address our objectives, we measured the CLPP with the MicroResp™ system in soil samples from ten European long-term field experiments located in different pedoclimatic zones. Thereafter, we related

the CLPP results with an extensive set of soil quality parameters that were measured in the same samples to understand the consequences of agricultural management on soil functioning and to improve our mechanistic understanding of these relationships.

We hypothesised i) that reduced tillage and increased organic matter addition, considered as less intensive soil management, will stimulate microbial activity, and ii) that such soil management will result in consistent differences in catabolic profiles coinciding with increased microbial functional diversity; iii) that the differences in response of more labile and more recalcitrant carbon substrates will allow discriminating soil management effects, such that reduced tillage and high organic matter addition will show higher utilization of more recalcitrant substrates; and iv) that the structure of the catabolic profiles and the functional diversity under less intensive soil management will be positively correlated with widely applied soil quality parameters such as total and labile organic carbon fractions, total nitrogen and microbial biomass.

6.2 Materials and methods

6.2.1 Long-term field experiments, management and soil sampling

Ten European long-term field experiments (LTEs) with different pedoclimatic characteristics were sampled in spring 2016 before any soil management was applied (**Table S1**). Each LTE had unique management characteristics and a different experimental design, with three or four replicates per treatment. Despite their uniqueness, the main soil management types (i.e. tillage and organic matter addition) were in common between the LTEs, making them comparable (Bongiorno et al., 2019a, b, c; Chapter 3, 4 and 5 (this thesis)). The contrast in tillage was classified as conventional tillage (ploughing at 20-25 cm depth, CT) versus reduced tillage (no-tillage or non-inversion tillage at 0-10 cm, RT). The contrast in organic matter addition was classified as low organic matter addition (Low, no organic matter addition or only mineral fertilization) versus high organic matter addition (High, organic matter additions with or without mineral fertilizer). In some LTEs, both treatment factors were applied, while at others only one of these was present.

In spring 2016 a total of 167 samples were collected from the fields before any major soil or crop management was applied in the LTEs. Each sample was composed of 20 soil cores randomly collected in the central area of the plot, to avoid border effects, and mixed. In seven trials (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) two layers (0-10 cm and 10-20 cm) were sampled because tillage was part of the soil management. In three trials (CH3, PT1 and HU1) only one layer (0-20 cm) was sampled because the only management factor was organic matter addition and we did not expect a stratification effect due to tillage. In the current study we used the samples from the 0-10 cm layer for CH1, CH2, NL1, NL2, SL1,

HU4, ES4 and the 0-20 cm layer samples for CH3, PT1 and HU1, for a total of 101 samples (the same samples as used in Bongiorno et al., 2019c; Chapter 4 (this thesis)). Fresh soil samples were sent to Wageningen University (The Netherlands), Research Institute of Organic Agriculture (Frick, Switzerland), University of Trier (Germany) and University Miguel Hernandez (Alicante, Spain), and air-dried samples were sent to University of Ljubljana (Slovenia). Upon arrival, the samples were sieved at 5 mm and, when fresh, stored at 3˚C until further processing.

6.2.2 Chemical, physical and biological soil parameters

Various soil properties were measured for the current study: total organic carbon (TOC; %), pH ($CaCl_2$), total nitrogen (TN; %), cation exchange capacity (CEC; mmol 100 g^{-1} soil), extractable phosphorus by the Olsen method (P; mg kg^{-1} soil), plant available potassium (K; mg kg^{-1} soil), exchangeable magnesium, calcium and sodium (Mg^{2+}, Ca^{2+}, Na^{+}; mg kg^{-1} soil), water stable aggregates (WSA; mg kg^{-1} soil), water holding capacity (WHC; %), bulk density (BD; g cm^{-3}), percentages of silt, clay and sand, microbial biomass carbon (MBC; mg kg^{-1} soil), microbial biomass nitrogen (MBN; mg kg^{-1} soil), number and biomass of earthworms (number and g m^{-2}), decomposition through tea bag index (% mass loss), soil suppressiveness to *Pythium ultimum* (%) (Bongiorno et al., 2019c; Chapter 4 (this thesis)), nematode abundance (DNA counts 100 g^{-1} soil), and nematode OTU richness and diversity (Bongiorno et al., 2019a; Chapter 5 (this thesis)). Five labile carbon fractions were measured as explained in Bongiorno et al. (2019b) and Chapter 3 (this thesis): hydrophilic dissolved organic carbon (Hy-DOC; mg kg^{-1} soil), dissolved organic carbon (DOC; mg kg^{-1} soil), permanganate oxidizable carbon (POXC; mg kg^{-1} soil), hot water extractable carbon (HWEC; mg kg^{-1} soil), and particulate organic matter carbon (POMC; mg kg^{-1} soil). The recalcitrance of Hy-DOC and DOC was assessed measuring their specific ultraviolet absorbance (Hy SUVA and DOC SUVA; L g C^{-1} cm^{-1}). For details about the methodology for assessing these parameters we refer to previous works (Bongiorno et al., 2019a, b, c; Chapter 3, 4 and 5 (this thesis)) and to **Table S2**.

6.2.3 Community level physiological profiling

For the community level physiological profiling (catabolic profiling) we used the MicroResp™ system. The Microresp™ was done according to Campbell et al. (2003) and Creamer et al. (2016b). The 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 mM KCL and 2.5 m M $NaHCO_3$. An amount consisting in 125 µl of indicator dye was transferred into each well of the detection plate, and the plates were stored in a desiccator with soda lime and a beaker of water and covered with parafilm to avoid desiccation. The soil samples were sieved at

2 mm and water content was adjusted to between 30% and 60 % of the water holding capacity. About 0.3 g of soil was added into each of the wells in the deep-well plates using the MicroResp™ filling device and the plates were stored in an incubator for 6 days at 25°C. This was done to reduce the effect of soil disturbance induced by sampling and sieving on the microbial community. After the incubation, 25 µl of substrate was dispensed into each well of the deep well plate containing the soil. The substrates were prepared to deliver 30 mg/g of C to the soil. We used eight substrates: deionized water as control, glucose (G; as simple sugar), alanine and gamma-amino butyric acid (A and AMA; as amino acids), n-acetyl-glucosamine (NAC; as amide), oxalic acid and alpha-ketoglutaric acid (OA and AKA; as carboxylic acids) and lignin (L; as polymer). These substrates were selected because of their biological relevance in agriculture being components of root exudates, microbes or end products of plants and microbes (Murugan et al., 2014). Moreover, they represented a range of chemical recalcitrance and nutrient content. Previous studies showed that this limited number of substrates was enough to discriminate between different land uses and agricultural management (Stevenson et al., 2004; Gomez and Garland, 2012; Creamer et al., 2016b). After substrate addition, the plates were left open for 30 minutes to allow the release of CO_2 as a result of carbonates present in the soil or induced by the addition of acid substrates (Creamer et al., 2016b). Despite this measure, the respiration rate of oxalic acid resulted to have quite a strong positive relationship with the pH across our samples (partial correlation r=0.53, Figure S1), and for this reason we decided to remove it from the analysis. The initial colorimetric values of the detection plates were read at 570 nm to obtain initial absorbance values (T_0). After 30 minutes standing, the deep well plates were sealed airtight by mounting the detection plates and were put in an incubator at 25°C for 6 hours. Thereafter, the colorimetric values of the detection plates were read again (T_1) and these final absorbance values were normalised using the initial absorbance values at T_0. Absorbance data were converted to CO_2 concentration using a calibration curve: $\%CO_2 = 0.02*A_{570}^{-3.11}$ ($R^2 = 0.93$), where $\%CO_2$ is the concentration in the headspace after incubation and A_{570} is the normalized absorbance (Brolsma et al., 2015). The $\%CO_2$ concentrations were then converted to respiration rates (µg CO_2-C g^{-1} dry soil h^{-1}) using the formula provided in the MicroResp™ procedure. Thereafter, we corrected for the average respiration rate for soil to which the deionized water was added. MicroResp™ uses a 'whole soil' approach and a short incubation time (6 h), trying as much as possible to approach *in situ* conditions (Campbell et al., 2003; Chapman et al., 2007). We used the standard soil respiration measured in the framework of the iSQAPER project as used for previous publications (Bongiorno et al., 2019b; Bongiorno et al., 2019c) as a measure of the soil basal respiration. Multiple substrate induced respiration (MSIR) was calculated as the sum of all the respired substrates per sample, and represents the total microbial

functional capacity (Moscatelli et al., 2018). The absolute utilization of each substrate was converted into relative utilization by dividing it by the MSIR. This standardization removed the influence of differences in microbial biomass due to soil management (Yu et al., 2016). Shannon functional diversity index (H') was used to assess the microbial functional diversity (Kennedy and Smith, 1995) and was determined using the formula:

$$H' = -\sum P_i \, LN \, (P_i)$$

Where *Pi* represents the respiration induced by the *i*th substrate expressed as a proportion of the sum of all respiration rates. The Shannon index is used to assess the evenness of substrate utilization.

6.2.4 Statistical analysis

We merged data from 0-10 cm and 0-20 cm sampling depth because differences in respiration between the two groups were not different (**Table S3**), and all samples were analysed together. Statistical calculations were carried out using R version 3.6.0 and RStudio version 1.2.1335 (R Development Core Team, 2013; RStudio Team, 2016), and results were considered significant at $p \leq 0.05$.

The effect of soil management practices and their possible interaction on basal respiration, MSIR and H' was tested by analysis of variance (function *anova*) on linear mixed effect models (LMEs) assessed using the function *lme* from the *lme4* package (Pinheiro et al., 2018). Tillage and organic matter addition were included as fixed factors while trial, block and subplot were introduced as random factors to take the nested design of the study into account (Bongiorno et al., 2019b). Normality and homogeneity of variances of the residuals from the models were checked both visually and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). Basal respiration and MSIR were log transformed, and the H' was elevated to power in order to meet the ANOVA assumptions.

To test the effect of the tillage and the organic matter addition on the microbial catabolic profiles we performed a permutational analysis of variance (PERMANOVA) with 10^4 permutations using Euclidean distances, using the function *adonis* from the *vegan* package (Oksanen et al., 2018). For this analysis, the absolute substrate utilization was log transformed. The LTEs were added as a random factor in the *strata* argument of the *adonis* function (Anderson, 2001). The function *betadisp* was used to perform permutational analysis of multivariate dispersion (BETADISP) with 10^4 permutations. LMEs were used to analyse the effect of soil management on the utilization of each substrate expressed as absolute and relative utilization rate, and to calculate the estimated means used for graphical representations.

The effect of soil management on catabolic profiles was visualized with redundancy analysis (RDA) using the function *rda* in the *vegan* package, and with the LTEs as a conditioning factor. 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. Thereafter, we correlated the soil quality parameters with the first two RDA axes to check their association with the agricultural management. The relationships between substrate utilization and environmental variables as shaped by soil management practices was visualised using RDA and tested using the *envfit* function in the package *vegan* with 10^4 permutations.

We tested the correlation between basal respiration, MSIR, H' and substrate utilization expressed in absolute and relative utilization rate with the soil quality indicators, performing partial correlation that used the LTE as covariate, correcting for the intrinsic differences between the LTEs. For the correlation analyses the packages *car, stats* and *ppcor* were used (Kim, 2015).

To understand which were the most important variables in explaining H', we performed multiple linear mixed model regressions with H' as the dependent variable and four broad groups of indicators (i.e. chemical, physical, biological and labile organic fractions) as explanatory variables. For each starting model, we assessed and selected the significant variables using the stepwise procedure in r (function *step*, direction "both"). To avoid problems related to multi-collinearity, for each model we selected explanatory variables with *vif* not higher than 3, and not highly correlated ($r > 0.80$). We then ran the multiple regression models for each group with the significant variables to assess the Akaike Information Criterion (AIC), the R_m^2 (marginal coefficient of determination), which indicates the proportion of the variation explained by the predictor variables, and the R_c^2 (conditional coefficient of determination), which indicates the variation explained by both the fixed and the random factors. In addition, we calculated the R^2_{mAdj} to give a measure of the accuracy of the model across different samples (Field et al., 2012). The models were also used to assess the t and the *p*-values, which quantify the contribution of each predictor to the model (Field et al., 2012) and the significance, respectively, of the explanatory variables. We then used the parameters that resulted to be significant ($p \leq 0.05$) from these four regressions for a final multiple mixed model regression. To take the nested structure of the experimental design of the LTEs into account, we allowed the intercept to vary depending on the LTE (Zuur, 2009). Normality and homogeneity of variances of the residuals from the models were checked both visually and with the Shapiro-Wilk and Levene's tests (Zuur, 2009).

Finally, we performed piecewise structural equation modelling (SEM) to assess the direct and indirect effects of the most important parameters from the multiple regressions on H', taking into account the dependent structure of the data coming from the same LTE (Lefcheck, 2016) using the package *piecewiseSEM*. An *a priori* model was established according to previous results and ecological mechanisms (Figure S2) and was used as a framework for the optimization of the piecewise SEM. The data matrix was fitted using log transformed variables, with the exception of H' which 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 (Shipley, 2013). 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 (Lefcheck, 2016). We calculated and reported the total standardized effects of the predictors on soil functional diversity (H'').

6.3 Results

6.3.1 Effect of soil management on microbial respiration and catabolic profiles

The basal respiration was on average 0.38 µg CO_2-C g^{-1} h^{-1}, and was 51% higher in reduced tillage compared to conventional tillage (**Table 6.1**). Reduced tillage and high organic matter addition increased the multiple substrate induced respiration (MSIR) by 37% and 32%, respectively, compared to conventional tillage and low organic matter addition.

The absolute utilization rate for five out of six substrates was enhanced by reduced tillage and high organic matter addition (**Figure 6.1A** and **Table S4**). Only the utilization of amino-butyric acid was found to be slightly enhanced by reduced tillage and not affected by organic matter addition. The most utilized substrate in all treatments in terms of absolute and relative respiration rate was alpha-ketoglutaric acid (**Figure 6.1** and **Table S4**).

Relative utilization rates of the different substrates were more similar between management classes than absolute utilization rates (**Figure 6.1B**). Nevertheless, the relative utilization rate of the substrates was enhanced by reduced tillage compared to conventional tillage, except for amino-butyric acid and lignin, which were not affected by tillage, and for alpha-ketoglutaric acid, which was enhanced by conventional compared to reduced tillage (**Figure 6.1B** and **Table S4**). Lignin was the only substrate whose utilization was enhanced by the high compared to the low organic matter addition treatment. As a result, the relative substrate use levels of the two reduced tillage treatments were more similar and more balanced than in the two conventional tillage treatments, in particular the conventional tillage with low organic matter addition.

Table 6.1. Basal respiration, multiple substrate induced respiration (MSIR) and Shannon functional diversity index (H'), as affected by tillage and organic matter addition across 10 long-term field experiments, analysed with linear mixed effect models (n = 101). Least square means, confidence intervals (in brackets) and F and p values are reported for each combination of tillage and organic matter addition. Significant differences ($p \leq 0.05$) are given in bold.

		Basal respiration	MSIR[††]	H'[‡‡]
		(μg CO_2-C g^{-1} h^{-1})	(μg CO_2-C g^{-1} h^{-1})	
CT[†]- Low[§]		0.30 (0.21-0.41)	4.3 (1.9-9.7)	1.27 (1.10-1.41)
RT[‡]- Low		0.45 (0.32-0.64)	5.9 (2.6-13.5)	1.35 (1.19-1.50)
CT- High[#]		0.31 (0.23-0.44)	5.7 (2.5-12.9)	1.32 (1.15-1.46)
RT- High		0.47 (0.33-0.67)	7.8 (3.4-17.8)	1.40 (1.24-1.54)
Tillage (T)	F *p*	24.93 **<0.0001**	11.94 **0.002**	7.03 **0.01**
Organic matter (OM)	F *p*	0.81 0.37	13.62 **0.001**	2.13 0.15
T X OM	F *p*	1.34 0.25	1.37 0.25	0.51 0.48

[†]*CT,* conventional tillage; [‡]*RT,* reduced tillage; [§]*Low,* low organic matter input; [#]*High,* high organic matter input; [††]*MSIR,* multiple substrate induced respiration; [‡‡]*H',* Shannon functional diversity index.

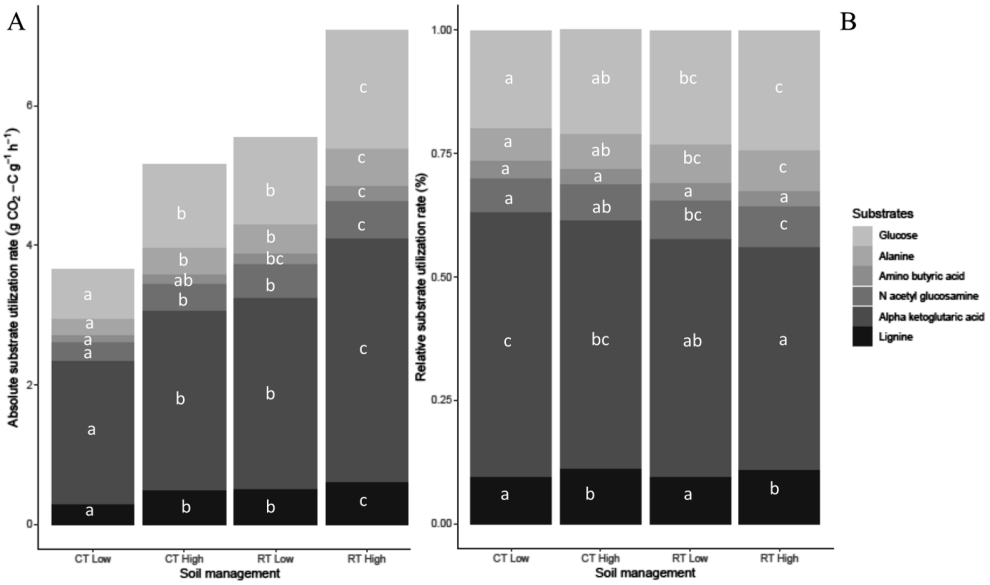

Figure 6.1. Mean utilization of six substrates expressed in A) as absolute utilization rate (μg CO_2-C g^{-1} h^{-1}) and in B) as relative utilization rate (%) for each combination of tillage and organic matter addition across ten European long-term field experiments (n=101). Different letters indicate significant differences in substrate utilization between treatments. *CT*, conventional tillage; *RT*, reduced tillage; *High*, high organic matter addition, *Low*, low organic matter addition.

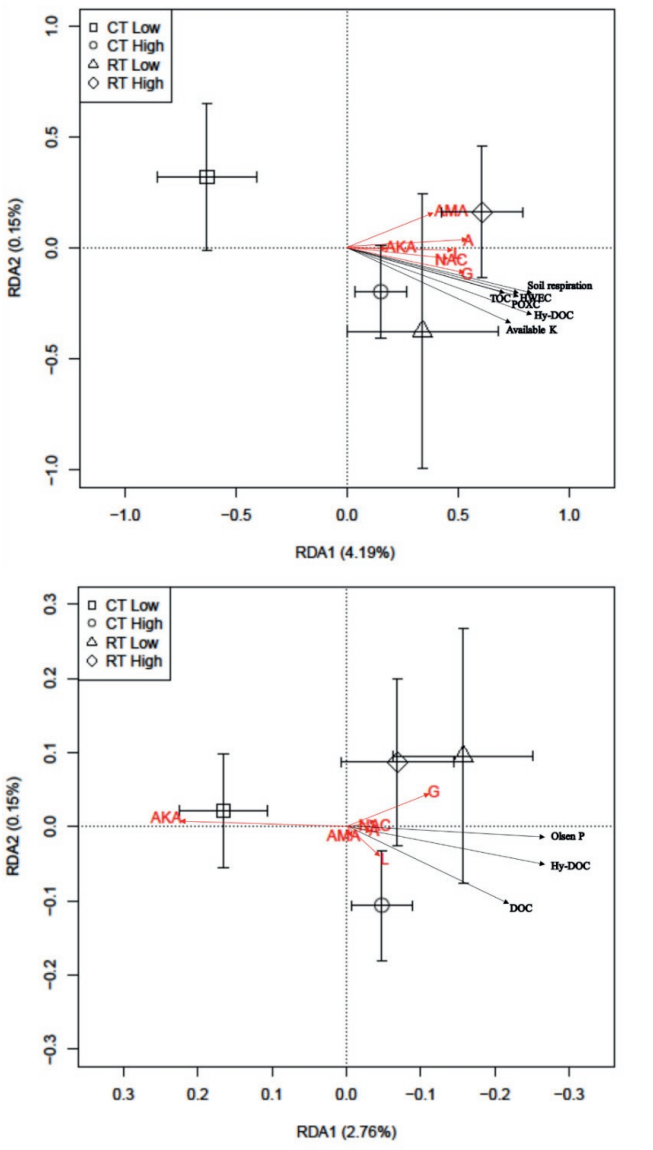

Figure 6.2. Redundancy analysis (RDA) showing the effects of soil management (tillage and organic matter addition), displayed as centroids with standard error bars, on the catabolic profiles of six substrates. In A) substrate utilization refers to absolute utilization rate (µg CO_2-C g^{-1} h^{-1}) and in B) to relative utilization rate (%). In both panels, the substrates are shown with red arrows and the relationship between the substrate utilization profiles and various soil parameters are shown with black arrows (only the significant ones at $p \leq 0.05$). 'Long-term field experiment' was used as a random effect (conditioned), and the blocking structure plus tillage and organic matter addition were used as fixed effects. *CT*, conventional tillage; *RT*, reduced tillage; *High*, high organic matter addition; *Low*, low organic matter addition; *G*, glucose; *A*, alanine; *NAC*, N-acetyl glucosamine; *AMA*, aminobutyric acid; *L*, lignin; *TOC*, total organic carbon; *POXC*, permanganate oxidizable carbon; *HWEC*, hot water extractable carbon; *Hy-DOC*, hydrophilic dissolved organic carbon; *DOC*, dissolved organic carbon.

For both ways to express substrate utilization (i.e. absolute and relative utilization rate), PERMANOVA showed that the conditional variable (LTE) explained approximately 80% of the variation (**Table S5**). Nevertheless, the PERMANOVA for the absolute substrate utilization rate also showed an effect of both tillage ($p = 0.002$) and organic matter addition ($p = 0.003$) (**Table S5**). In the RDA plot of the substrate utilization, expressed as absolute utilization rate, the two most extreme management practices (CT-Low and RT-High) are separated along axis 1, and the two intermediate management practices (CT-High and RT-Low) are both situated in the middle, but still closer to RT-High than to CT-Low (**Figure 6.2A**). All the substrates were positively correlated with RDA axis 1 (average correlation 0.89, Table S6), indicating that they were all more utilized in conventional tillage with high organic matter, in reduced tillage with low organic matter and, most of all, in reduced tillage with high organic matter addition (**Figure 6.2A**).

All the carbon substrates, except alpha-ketoglutaric acid, were equally important (the score on the RDA axis 1 was 0.24 for alpha-ketoglutaric acid and about 0.50 for all the other substrates) in explaining the differences in the catabolic profiles expressed as absolute utilization rate between the different treatments on RDA axis 1 (**Table S6**). None of the substrates had a high score on RDA axis 2.

Regarding the utilization of the substrates expressed as relative utilization rate, PERMANOVA showed significant main effects of tillage ($p = 0.006$) and organic matter addition ($p = 0.03$), and a significant interaction between tillage and organic matter addition ($p = 0.04$) (**Table S5**). The RDA plot shows that the catabolic profile in conventional tillage differed from that in reduced tillage, regardless of the organic matter addition level (**Figure 6.2B**). Organic matter addition only had an effect on the utilization profiles in conventional tillage. Moreover, the most intensive soil management combination (CT-Low) was clearly separated from the others on RDA axis 1, similar to the RDA plot of the absolute utilization rate (**Figure 6.2A**). The position of alpha-ketoglutaric was unambiguously orientated in the direction of CT-Low, (score = 0.24 and correlation coefficient $r = -0.99$ with $p < 0.0001$ on the RDA axis 1, **Table S6**).

6.3.2 Effect of soil management on microbial functional diversity

The Shannon functional diversity index (H') was slightly, but significantly, higher (6%) in reduced tillage compared to conventional tillage, but it was not significantly affected by organic matter addition (**Table 6.1**). The contribution of alpha-ketoglutaric acid in diminishing the functional diversity in conventional tillage, especially with low OM addition, is evident in **Figure 6.1B**. In addition, the utilization of alpha-ketogularic acid expressed in absolute utilization rate was not correlated with H' ($r = 0.13$), and the one

expressed in relative utilization rate was negatively correlated with H' ($r = -0.89$, $p < 0.0001$) (**Table S7**).

6.3.3 Relationships between catabolic profiles and soil properties

Several soil parameters were positively correlated with RDA axis 1 in **Figure 6.2A** and therefore, with less intensive soil management combinations, in particular reduced tillage with high OM (**Table 6.2**). Total organic carbon (TOC), total nitrogen (TN), available Mg, basal respiration and the labile organic carbon fractions were particularly strong in their positive association with RDA1 ($r > 0.30$). From the envfit analysis, we found that TOC, available K, basal respiration, Hy-DOC, POXC and HWEC were significantly associated with RDA axis 1 (**Figure 6.2A**; **Table S8**).

We found similar trends in the profiles, expressed as relative utilization rates. In particular, total organic carbon, Olsen P, microbial biomass nitrogen and the labile carbon fractions had a strong positive correlation with RDA axis 1 ($r > 0.30$) (**Table 6.2**). In addition, Olsen P, basal respiration, Hy-DOC, and DOC were significantly positively associated with RDA axis 1, according to the envfit analysis (**Figure 6.2B**; **Table S8**).

We found that all the absolute utilization rates of all the substrates, especially lignin, alanine, N-acetyl glucosamine, and glucose were positively associated with various soil parameters, and that these relationships were well reflected by MSIR (**Table 6.3**).

Using substrate utilization expressed as relative utilization rate, we found that in particular alanine and N-acetyl glucosamine were positively correlated with total organic carbon, total nitrogen, P Olsen, microbial biomass nitrogen, basal respiration, and the labile carbon fractions (**Table 6.4**). Also Shannon functional diversity (H') correlated with these same soil parameters ($r > 0.30$) (**Table 6.4**). In contrast, alpha-ketoglutaric acid had a pattern opposite to the other substrates. This confirmed the results of the RDA, where alpha-ketoglutaric acid was located away from all the other substrates (**Figure 6.2B**).

6.3.4 Variables explaining microbial functional diversity

POMC and pH explained the variation in microbial functional diversity (H') that remained after using LTE as a co-variate (Model 5 in **Table 6.5**; the models used to arrive at this combined model can be found in **Table S9**).

Based on our *a priori* SEM model (**Fig. S2**) and the results of the multiple regression analyses (**Table 6.5**), we constructed the mechanistic relationships between H' and soil parameters using piecewise structural equation modelling (**Figure 6.3**).

POMC had a direct positive effect on H', while pH had a direct negative effect on H'. POMC stimulated microbial biomass nitrogen and basal respiration, but these two

Table 6.2. Partial Pearson correlation coefficients (ρ) between the first two RDA axes of the catabolic profiles expressed as absolute and relative utilization rate and the chemical, physical and biological parameters measured (n = 101).

	Absolute utilization rate				Relative utilization rate			
	(μg CO_2-C g^{-1} h^{-1})				(%)			
	RDA1		RDA2		RDA1		RDA2	
Chemical indicators†								
TOC	0.42	***	-0.02		0.31	*	-0.11	
TN	0.41	***	-0.01		0.28	*	-0.09	
CEC	0.22	*	-0.15		0.08		0.06	
C/N	-0.22	*	-0.01		0.002		0.05	
pH	0.003		-0.10		-0.02		0.10	
P Olsen	0.24	*	0.01		0.40	***	-0.08	
Mg	0.38	***	-0.13		0.15		0.03	
Ca	-0.06		-0.03		0.03		0.06	
K	0.27	*	-0.12		0.25	*	0.005	
Na	-0.05		-0.11		0.09		0.05	
Physical indicators‡								
WSA	0.25	*	0.09		0.24	*	-0.17	
WHC	0.17		-0.20	*	0.18		0.11	
BD	-0.16		-0.02		0.004		0.007	
Sand	0.01		0.11		-0.02		-0.16	
Silt	-0.03		-0.15		0.17		0.09	
Clay	0.17		0.07		-0.02		-0.12	
Biological indicators§								
MBC	0.19		-0.07		0.22	*	0.11	
MBN	0.29	*	0.02		0.32	*	0.01	
Basal respiration	0.42	***	0.01		0.23	*	0.18	
Earthworm number	0.11		0.10		0.11		0.02	
Earthworm biomass	0.18		0.09		0.17		0.06	
Nematode abundance	0.20		0.06		0.17		0.09	
Nematode richness	0.08		0.04		0.12		0.002	
Nematode diversity	0.002		-0.06		-0.05		0.20	*
Decomposition	-0.15		-0.005		-0.07		-0.008	
Soil suppressiveness	0.09		0.16		0.15		-0.15	
Labile organic carbon#								
Hy-DOC	0.34	**	-0.09		0.37	**	-0.16	
Hy SUVA	-0.02		0.07		0.005		-0.17	
DOC	0.29	*	0.02		0.35	**	-0.27	*
DOC SUVA	-0.002		-0.06		0.07		0.06	
POXC	0.53	***	-0.001		0.40	***	0.005	
HWEC	0.44	***	-0.01		0.36	**	0.07	
POMC	0.37	**	-0.002		0.33	**	0.04	

†*TOC* total organic carbon, *TN* total nitrogen, *CEC* cation exchange capacity, *C/N* carbon to nitrogen ratio, ‡*WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density, §*MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, #*Hy-DOC* hydrophilic dissolved organic carbon, *Hy SUVA* specific ultraviolet absorbance of dissolved organic hydrophilic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.
* $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$

Table 6.3. Partial Pearson correlations (ρ) between basal respiration, utilization of the six substrates (expressed as absolute utilization rate (μg CO_2-C g^{-1} h^{-1})) and multiple substrate induced respiration (MSIR) with various soil chemical, physical and biological indicators (number of observations= 101), corrected for site (LTE).

	Basal respiration		Glucose		Alanine		Amino-butyric acid		N-acetyl glucosa-mine		Lignin		A-keto-glutaric acid		MSIR	
	(μg CO_2-C g^{-1} h^{-1})															
Chemical indicators†																
TOC	0.57	***	0.41	***	0.44	***	0.27	*	0.44	***	0.36	**	0.30	*	0.41	***
TN	0.62	***	0.38	***	0.43	***	0.26	*	0.43	***	0.40	***	0.32	**	0.41	***
CEC	0.38	***	0.25	*	0.22	*	0.08		0.23	*	0.20	*	0.24	*	0.27	*
C/N	-0.57	***	-0.17		-0.22	*	-0.14		-0.24	*	-0.22	*	-0.28	*	-0.27	*
pH	0.11		0.10		0.14		-0.06		-0.03		-0.19		0.06		0.06	
P Olsen	0.27	*	0.20		0.24	*	0.17		0.27	*	0.29	*	-0.01		0.12	
Mg	0.27	*	0.37	**	0.29	*	0.22	*	0.42	***	0.49	***	0.37	**	0.42	***
Ca	0.11		-0.02		0.07		-0.08		-0.07		-0.21	*	-0.07		-0.03	
K	0.27	*	0.28	*	0.31	*	0.12		0.26	*	0.33	*	0.18		0.25	*
Na	-0.06		-0.10		-0.05		-0.10		0.05		-0.008		-0.06		-0.07	
Physical indicators‡																
WSA	0.62	***	0.26	*	0.36	**	0.19		0.20	*	0.14		0.14		0.22	*
WHC	0.02		0.22	*	0.10		0.06		0.26	*	0.21	*	0.11		0.15	
BD	-0.07		-0.14		-0.18		-0.11		-0.16		-0.15		-0.18		-0.17	
Sand	-0.08		-0.01		0.06		0.03		-0.01		-0.01		0.04		0.05	
Silt	0.08		0.02		-0.05		-0.07		-0.03		0.02		-0.18		-0.04	
Clay	0.002		0.14		0.15		0.16		0.23	*	0.09		0.25	*	0.18	
Biological indicators§																
MBC	0.63	***	0.23	*	0.24	*	0.09		0.24	*	0.04		0.12		0.21	*
MBN	0.51	***	0.27	*	0.32	*	0.21	*	0.36	**	0.22	*	0.09		0.21	*
Basal respiration	1		0.42	***	0.46	***	0.29	*	0.45	***	0.28	*	0.38	***	0.44	***
Earthworm numbers	-0.009		0.09		0.13		0.12		0.09		0.09		0.02		0.06	
Earthworm biomass	0.07		0.18		0.21	*	0.16		0.15		0.15		0.09		0.15	
Nematode abundance	0.52	***	0.20		0.21	*	0.17		0.23	*	0.10		0.14		0.19	
Nematode richness	0.25	*	0.10		0.13		0.07		0.04		0.06		0.01		0.09	
Nematode diversity	0.15		-0.001		0.005		-0.05		-0.02		-0.05		-0.007		0.0001	
Tea bag decomposition	-0.39	**	-0.07		-0.11		-0.10		-0.25	*	-0.19		-0.09		-0.15	
Soil suppressiveness	0.15		0.08		0.09		0.16		0.12		0.07		0.01		0.07	
Labile carbon fractions#																
Hy-DOC	0.46	***	0.33	*	0.36	**	0.20		0.40	***	0.38	**	0.14		0.28	*
Hy SUVA	-0.10		-0.09		-0.05		0.0004		-0.04		0.07		-0.04		-0.03	
DOC	0.40	***	0.20	*	0.30	*	0.17		0.27	*	0.34	**	0.03		0.16	
DOC SUVA	-0.17		0.04		0.04		-0.03		-0.09		0.01		-0.04		-0.02	
POXC	0.76	***	0.50	***	0.54	***	0.36	**	0.56	***	0.48	***	0.36	**	0.50	***
HWEC	0.63	***	0.42	***	0.43	***	0.29	*	0.45	***	0.40	***	0.28	*	0.38	**
POMC	0.66	***	0.34	**	0.39	***	0.25	*	0.41	***	0.31	*	0.23	*	0.36	**

†*TOC* total organic carbon, *TN* total nitrogen, *CEC* cation exchange capacity, *C/N* carbon to nitrogen ratio, ‡*WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density, §*MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, #*Hy-DOC* hydrophilic dissolved organic carbon, *Hy SUVA* specific ultraviolet absorbance of dissolved organic hydrophilic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.
* $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.000$

Table 6.4. Partial Pearson correlation coefficients (ρ) between substrate utilization (expressed as relative utilization rate (%)) and various soil chemical, physical and biological indicators, corrected for site (LTE) (n = 101).

	Glucose		Alanine		Amino-butyric acid		N-acetyl glucosa-mine		A-ketoglu-taric acid		Lignin		Shannon index (H')	
	(%)													
Chemical indicators†														
TOC	0.24	*	0.38	**	0.03		0.37	**	-0.31	*	0.04		0.30	*
TON	0.17		0.34	**	0.01		0.36	**	-0.28	*	0.09		0.30	*
CEC	0.11	*	0.11		-0.11		-0.12		0.01		0.002		0.07	
C/N	-0.01		-0.13		-0.002		-0.03		0.04		-0.01		-0.06	
pH	0.15		0.11		-0.13		-0.05		0.03		-0.22	*	-0.06	
P Olsen	0.32	*	0.29	*	0.08		0.34	**	-0.40	***	0.22	*	0.36	**
Mg	0.11		0.07		-0.04		0.20		-0.15		0.11		0.14	
Ca	0.09		0.14		-0.12		0.01		-0.02		-0.08		-0.08	
K	0.22	*	0.27	*	-0.08		0.29	*	-0.24	*	0.08		0.21	*
Na	-0.11		0.03		-0.09		0.22	*	-0.01		0.08		0.04	
Physical indicators‡														
WSA	0.17		0.36	**	0.09		0.17		-0.25	*	0.05		0.22	*
WHC	0.24	*	-0.01		-0.01		0.29	*	-0.17		0.03		0.15	
BD	-0.04		-0.10		-0.05		-0.05		-0.01		-0.07		-0.08	
Sand	-0.09		0.10		0.14		-0.07		0.001		0.005		0.001	
Silt	0.11		-0.09		-0.10		-0.03		-0.16		0.40	***	-0.08	
Clay	0.05		0.07		0.14		0.23	*	0.01		-0.34	**	0.16	
Biological indicators§														
MBC	0.22	*	0.26	*	0.03		0.28	*	-0.21	*	-0.07		0.15	
MBN	0.25	*	0.31	*	0.08		0.42	***	-0.32	*	0.03		0.33	*
Basal respiration	0.24	*	0.35	**	0.03		0.37	**	-0.22	*	-0.16		0.27	*
Earthworm numbers	0.10		0.11		0.07		0.05		-0.10		0.006		0.12	
Earthworm biomass	0.18		0.17		0.09		0.09		-0.17		-0.004		0.17	
Nematode abundance	0.17		0.20		0.06		0.26	*	-0.16		-0.09		0.16	
Nematode richness	0.16		0.13		0.04		-0.01		-0.09		-0.05		0.06	
Nematode diversity	0.11		0.11		-0.05		0.06		0.06		-0.06		-0.01	
Tea bag decomposition	0.03		-0.11		0.02		-0.25	*	0.08		-0.06		-0.05	
Soil suppressiveness	0.11		0.12		0.19		0.16		-0.15		-0.01		0.17	
Labile carbon fractions#														
Hy-DOC	0.23	*	0.33	*	-0.06		0.44	**	-0.37	**	0.24	*	0.33	*
Hy SUVA	-0.08		-0.01		0.09		-0.03		0.02		0.12		0.04	
DOC	0.14		0.36	***	0.06		0.35	*	-0.36	*	0.28	*	0.35	**
DOC SUVA	0.10		-0.01		-0.01		-0.14		-0.01		0.13		0.05	
POXC	0.31	*	0.44	***	0.06		0.49	***	-0.40	***	0.05		0.40	***
HWEC	0.34	***	0.32	*	0.03		0.41	**	-0.35	*	0.05		0.35	*
POMC	0.28	**	0.35	**	0.006		0.43	***	-0.33	*	0.03		0.29	*

†*TOC* total organic carbon, *TON* total nitrogen, *CEC* cation exchange capacity, *C/N* carbon to nitrogen ratio, ‡*WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density, §*MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, #*Hy-DOC* hydrophilic carbon, *Hy SUVA* specific ultraviolet absorbance of hydrophylic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.

* $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.000$

Table 6.5. Combined mixed linear model derived from 101 soil samples from 10 LTEs, as determined from significant soil parameters ($p \leq 0.05$) as explanatory variables, and the Shannon functional diversity index (H') as dependent variable. The Akaike information criterion (AIC) is an estimator of the quality of the statistical model, the R_m^2 (marginal coefficient of determination) indicates the proportion of the variation explained by the predictor variables, and the R_c^2 (conditional coefficient of determination) indicates the variation explained by both the fixed and the random factors. The R^2_{mAdj} and the R^2_{cAdj} provide a measure of the accuracy of the model across different samples.

Indicator group	Starting model	Parameters selected for the final model	AIC	R^2_m	R^2_{mAdj}	R^2_c	R^2_{cAdj}
		(t-value; p-value)					
Combined parameters	Exp_H'† ~ log_pH + log_C/N‡ + log_P+ log_WSA§+ log_MBN# + log_POMC†† + sqrt_DOC‡‡+ (1|LTE)	log_POMC (5.75;< 0.0001) log_pH (-3.53; < 0.0001) log_C/N (1.47; 0.15)	112	0.55	0.52	0.67	0.66

†*H'* Shannon functional diversity index, ‡*C/N* carbon to nitrogen ratio, §*WSA* water stable aggregates, #*MBN* microbial biomass nitrogen, ††*POMC* particulate organic matter carbon, ‡‡*DOC* dissolved organic carbon.

microbial parameters did not significantly affect H'. The variables selected from the multiple regression explained 56% of the variation in H' (**Figure 6.3**).

6.4. Discussion

6.4.1 Soil management affects microbial respiration and catabolic profiles

In accordance with our first hypothesis, reduced tillage and high organic matter addition increased the multiple substrate induced respiration (MSIR), compared to conventional tillage and low organic matter addition. The same result was found for the absolute utilization rate of most substrates separately. It follows that measuring more substrates does not give more information than measuring only one (e.g. glucose utilization, which is also used as a proxy for soil microbial biomass (Anderson and Domsch, 1978)). Reduced tillage and high organic matter addition had a positive effect on important soil properties in the 0-10 cm soil layer, such as total and labile organic carbon, soil nutrients, and microbial biomass, that can sustain higher microbial activity (Bongiorno et al., 2019b). Changes in these soil properties might explain the higher capacity of such systems to process organic matter, which, in turn, may increase their nutrient cycling capacity (Whitford and Ludwig Wade, 2002).

Although soil management stimulated microbial activity for all the substrates, the strength of such stimulation differed per substrate. Such substrate-specific differences in the stimulation of the microbial community could be visualized by expressing the

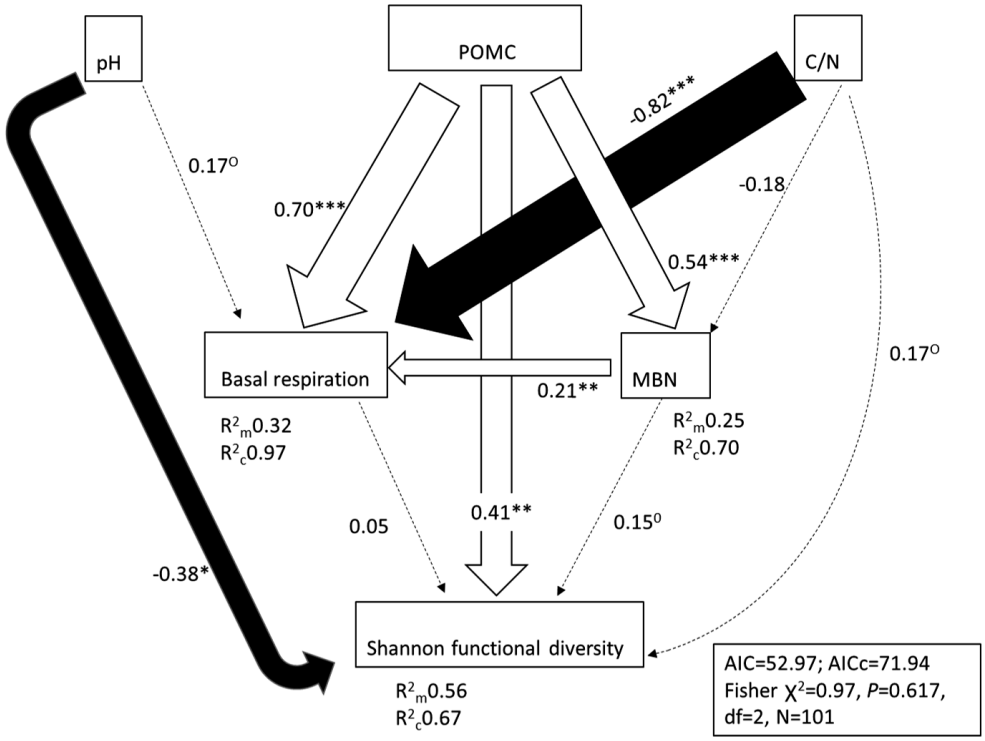

Figure 6.3. Piecewise structural equation model (SEM) of soil quality parameters as predictor of Shannon functional diversity (H'). Boxes represent measured variables and arrows represent the unidirectional relationships between the parameters. Numbers on the side of the arrows indicate standardized effect sizes (reported as path coefficients) and the width of the arrow is proportional to the strength of the path coefficient. White arrows indicate positive relationships, black arrows indicate negative relationships, and dashed arrows indicate not statistically significant relationships. The 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), 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. Variables lacking the R_m^2 and the R_c^2 acted only as predictor. In the box adjacent to the figure the Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AIC_c), Fisher chi-square (Fisher χ^2), *p* value (P) of the test, degrees of freedom (df), and the number of observations used for the analysis (N) are indicated. SEM models with a χ^2 with a $p \geq 0.05$ are considered to be statistically significant. *POMC* particulate organic matter carbon, *MBN* microbial biomass nitrogen.
$^0 p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

substrate utilization as relative utilization rate, which also controlled for the influence of differences in microbial biomass. Investigating both ways of expressing utilization rate can give a more complete picture of microbial functionality and helps to understand the effects of management on soil functioning. Reduced tillage, but not organic matter addition, increased the utilization rate of glucose, alanine and N-acetyl glucosamine. Aminobutyric acid utilization was not a sensitive indicator for soil management, as already found by Sradnick et al. (2013), while lignin utilization rate was related to high organic matter input. Lignin and other aromatic compound concentrations can be increased by long-term organic matter addition (Liu et al., 2010; Chen et al., 2018), resulting in a microbial community more capable of degrading these compounds (Bünemann et al., 2004; Bugg et al., 2011). Alpha-ketoglutaric acid utilization, on the other hand, was higher in conventional compared to reduced tillage, but only when expressed as relative utilization rate. This could indicate a higher presence of organisms with high reproduction rates and fast metabolism (i.e. r-strategists) (Romaniuk et al., 2011). In according with this hypothesis, Bongiorno et al. (2019a) found that conventional tillage had a higher enrichment index (EI), a nematode food web indicator of higher substrate availability, compared to reduced tillage. Such opposite response of the microbial community to carboxylic acids compared to other carbon substrates was also found by Yu et al. (2016), who considered relative utilization rate, and Murugan et al. (2014), Sradnick et al. (2013) and Bending et al. (2000), who considered absolute utilization rate. Alpha-ketoglutaric acid was the most utilized carbon substrate in our study, when expressed both as absolute and relative utilization rate, which corroborates findings of other studies in arable fields (Romaniuk et al., 2011; Sradnick et al., 2013; Pan et al., 2016; van der Bom et al., 2018). The unique role of alpha-ketoglutaric acid in our study will be discussed more in detail in section 6.4.3.

In accordance with our second hypothesis, different combinations of tillage and organic matter addition resulted in different microbial catabolic profiles, both when expressed as absolute and relative utilization rate. An effect of management was found despite that the biggest part of the variation was caused by the pedoclimatic zone. This corroborates previous studies in which shifts in catabolic profiles following organic matter addition (Bucher and Lanyon, 2005; Gomez et al., 2006; Frąc et al., 2012; Bei et al., 2018) and reduced tillage (Govaerts et al., 2007) were found when compared to conventional practices. The changes in catabolic profiles expressed as absolute utilization rate strongly reflected the changes in MSIR, indicating that decreasing agricultural intensity increases soil microbial activity, in accordance with the literature (Zuber and Villamil, 2016; Glodowska and Wozniak, 2019). The shifts in relative substrate utilization indicate changes in substrate utilization preference in the different tillage systems, and in case of

conventional tillage also in the different organic matter application systems. This could be explained by a higher capacity of soils under reduced tillage to counteract negative effects of low organic matter input by increased levels of total and labile organic carbon which will stimulate the microbial community (Bongiorno et al., 2019b).

6.4.2 Reduced tillage, but not organic matter addition, increases microbial functional diversity

In accordance with our second hypothesis, and with the observed shifts in catabolic profiles due to management, reduced tillage slightly but significantly increased the microbial functional diversity (H') compared to conventional tillage. This result is in line with findings of Lupwayi et al. (1998), Mijangos et al. (2006), Legrand et al. (2018) and Hao et al. (2019). Tillage can reduce microbial biomass and activity through direct effects (e.g. destruction of fungal mycelium) and destruction of specific microsites with higher fungal and bacterial activity (e.g. macroaggregates) (Gupta and Germida, 2015). Since macroaggregates are normally dominated by fungi, conventional tillage practices can have negative effects in particular on fungi and, therefore, on the capacity of a soil to degrade recalcitrant organic compounds (Frey, 2005). Moreover, the increased concentration of total and labile organic fractions in reduced tillage systems can serve as an additional energy source for microbial activity (Bongiorno et al., 2019b). Ultimately, a higher functional diversity might contribute to a higher capacity of the microbial community to perform different ecological processes and resist stress or disturbances (Degens et al., 2001; Mokany et al., 2008; Song et al., 2014).

In contrast with our second hypothesis, long-term organic matter addition did not significantly affect H', in line with other studies that did not find an effect of organic addition on functional diversity (Calbrix et al., 2007; Tao et al., 2015; Zhu et al., 2017). In our case, a lack of effect might be due to the heterogeneous nature of the organic matter applied to the different LTEs, which could, depending on their chemical compositions, differ in their suitability to sustain microbial functional diversity and microbial processes such as organic matter decomposition and soil disease suppressiveness (Bongiorno et al., 2019c). Moreover, since the MicroResp™ system monitors in particular the activity of the more copiotrophic bacteria, it cannot be excluded that a possible shift towards more oligotrophic bacteria in case of high compared to low levels of organic matter addition will not be captured (Degens et al., 2000). This hypothesis is supported by the observation that the relative lignin utilization rate was slightly increased by the high organic matter input management. However, our results are in accordance with Bongiorno et al. (2019a) who did not observe an effect of organic matter input on the nematode-based food web indices structure index (SI), a measure of the degree of trophic links and capacity to recover from stress, and enrichment index (EI).

Even though present, the effect of soil management on functional diversity was not very strong. However, the promotion of decomposition of various organic carbon substrates in absolute terms by reduced tillage and high organic matter addition, and the subsequent enhancement of the capacity of the soil systems to cycling nutrients through organism activity, support their adoption in replacing more intensive management.

6.4.3 Carbon substrate utilization discriminates between soil management practices

In accordance with our third hypothesis, the six selected carbon substrates differentially contributed to the discriminating ability of the catabolic profiles. When expressed as absolute utilization rates, the simple sugar (glucose), amino acids (alanine, gamma-amino butyric acid), amides (n-acetyl-glucosamine) and polymer (lignin) substrates, and less so the carboxylic acid (alpha-ketoglutaric acid), contributed to differentiating between the soil management treatments. Similarly, Romaniuk et al. (2011) and Sradnick et al. (2013) found that sugars and amino-acids contributed most to the discrimination between different management practices in arable fields. When expressed as relative utilization rate, the microbial response to alpha-ketoglutaric acid strongly contrasted with that to the other carbon substrates. Alpha-ketoglutaric acid stimulated in particular the microbial community in the conventional tillage - low organic matter addition systems, i.e. in the most intensive soil management system. Our results confirm previous studies that showed higher utilization of carboxylic acids in more intensive soil ecosystems, i.e. arable fields compared to forest and grassland (Creamer et al., 2016b; Rutgers et al., 2016), and higher utilization of amino acids, amines and carbohydrates (relative to carboxylic acids) in conservation agriculture, i.e. systems with increased crop rotation, use of cover crops and mulching (Schutter et al., 2001; Huang et al., 2008; D'Acunto et al., 2018). In addition, Murugan et al. (2014) found a negative relationship between the utilization of carboxylic acids and microbial functional diversity, similar to our findings. However, these studies mainly looked at absolute utilization profiles. Our results suggest that with increasing levels of agricultural intensity in arable systems, the microbial communities are better adapted to degrade organic acids. Organic acids represent one of the key metabolites present in root exudates, and it is known that they are released in the soil by plants (and also microbes) in stressed environments for nutrient acquisition, toxicity defence and attraction of beneficial organisms (Jones, 1998; Canarini et al., 2019), whereas they are less associated with the decomposition of organic matter (Sharma et al., 1998; Schutter et al., 2001). Wu et al. (2017) reported higher levels of organic acids in monoculture systems, which were found to stimulate the growth of pathogenic fungi and the production of toxic compounds. Therefore, they could indicate environments where microorganisms use

labile substrates from the rhizosphere more than from plant residues. It can be expected that under conventional tillage management with low organic matter addition the main organic compounds are derived from root exudates and microbial residues. On the other hand, in less intensive systems the microbes might utilize more amino acids and amines, because of the larger input of such compounds with the addition of organic matter and the higher demand for nitrogen due to the higher C to N ratio of crop residues and organic matter added (Lagomarsino et al., 2012). We therefore suggest that the utilization of carboxylic acids (e.g. alpha-ketoglutaric acid) relative to other substrates could be used as an indicator for stress in agricultural systems.

6.4.4 Carbon substrate utilization profiles, functional diversity and soil properties

In accordance with our fourth hypothesis, total carbon, macronutrients (N, P, K), microbial characteristics (basal respiration and microbial biomass N), and especially the labile organic carbon fractions were strongly correlated with the catabolic profiles and microbial functional diversity (H'). These soil parameters were highest in the less intensive soil management systems. These relationships can be explained by the general loss of more easily decomposable organic carbon fractions in more intensive management practices and the consequent loss in catabolic diversity (Graham and Haynes, 2005).This suggests that the capacity of the microbial communities to decompose different carbon substrates was largely linked to, and probably affected by, the positive influence of conservation agriculture practices on these major soil properties.

Carbon and macronutrients such as N and P have an important role in fostering microbial activity, and previous studies noted that higher TOC levels correspond to higher overall microbial catabolic diversity (Degens et al., 2000; Nsabimana et al., 2004) and substrate utilization efficiency (Zhong and Cai, 2007; Creamer et al., 2016b; Francioli et al., 2016). However, not only the quantity but also the quality of organic matter is crucial for microbial activity, diversity and the relative abundance of different microbial functional groups (Degens et al., 2000; Bending et al., 2002; Huang et al., 2008; Lagomarsino et al., 2012; Gupta and Germida, 2015). Previous studies reported a positive relationship between labile carbon fractions and microbial functional diversity (Huang et al., 2008; Tian et al., 2015). The relationship between labile carbon and the microbial community was clearly demonstrated in our structural equation model (**Figure 6.3**). In line with our *a priori* model, labile carbon expressed as POMC directly increased microbial functional diversity, but not through its positive effect on basal respiration or microbial biomass. Higher levels of labile organic carbon increase the level of food availability for microorganisms and can promote bacterial taxonomic diversity (Murugan et al., 2014; Yu et al., 2016). Microbial community composition and diversity, in turn, have been previously shown to correlate

with organic matter decomposition (Juarez et al., 2013; Bonner et al., 2018; Maron et al., 2018). The link between the microbial biomass and catabolic profile has been questioned before by Kemmitt et al. (2008), and Birge et al. (2015) found that available organic matter, rather than microbial biomass and enzymes limit soil respiration in long-term incubations. Probably microbial biomass, as well as soil respiration, remain the 'black box' which does not give the detailed information necessary to capture changes in microbial communities that link to functionality that we need for understanding of the soil microbial community in an ecological context (Ritz et al., 2009b; Schmidt et al., 2015).

Besides the strong effects of labile carbon on microbial functional diversity, also a strong effect of the soil pH was found (**Figure 6.3**). pH is considered a primary driver of microbial taxonomic and functional diversity (Wakelin et al., 2008; Creamer et al., 2016b; Delgado-Baquerizo et al., 2017b; Moscatelli et al., 2018), usually positively linked to microbial activity and functionality (D'Acunto et al., 2018; van der Bom et al., 2018). Contrary to our expectation, pH had a negative direct effect on functional diversity, a finding also reported by Zhu et al. (2017). Higher pH could have changed microbial composition, having a favourable effect on bacteria at the expenses of fungi or more oligotrophic bacteria, therefore decreasing the fungi to bacteria ratio, eventually resulting in a lower microbial functional diversity. This hypothesis is supported by the negative relationship between relative utilization rate of lignin and pH.

In summary, increasing the availability of labile carbon sources by management practices appears to be an important requirement for sustaining microbial activity and functionality, and fostering stable microbial decomposition (Degens et al., 2000; Bending et al., 2002; Bucher and Lanyon, 2005). Microbial decomposition is a required step prior to microbial assimilation of organic matter and subsequent stabilization in organo-mineral complexes (Degens, 1998; Schmidt et al., 2011; Cotrufo et al., 2013). This means that organic matter losses and the resulting reduction of functional diversity could develop in a reduced capacity of the microbial community to decompose organic matter but also to sequester carbon. Future studies should be focused on clarifying possible trade-offs between organic matter decomposition and storage caused by microbial activity in agricultural soil systems (Wood et al., 2015a).

6.5 Conclusion

The adoption of reduced tillage and higher organic matter application were found to be effective measures for increasing the capacity of the soil microbial community to decompose various carbon substrates. This has important consequences for soil functions, such as the enhancement of nutrient cycling, but potentially also carbon sequestration, presenting once more the dilemma posed by the trade-off between these two microbial

functions. Reduced tillage also slightly promoted microbial functional diversity compared to conventional tillage. Increased system intensity was associated with higher relative utilization of alpha-ketoglutaric acid and with lower soil quality as measured by common soil parameters, in particular labile organic carbon fractions. The latter were found to play a key role in promoting microbial functional diversity, probably increasing food availability for microorganisms and affecting their community structure and diversity. To better value the usefulness of carboxylic acid as an indicator for soil under stress, the mechanisms behind the differences between carboxylic acid utilization and the utilization of other substrates requires further study.

MicroResp™ effectively distinguished the activity and the functional capacity of the soil microbial community between different agricultural management systems, despite the large differences (climate conditions, soil properties, specific agricultural management implementation) between our 10 long-term field experiments. This makes MicroResp™ a promising biological soil quality indicator. To make use of the full potential of the method, establishment of optimal ranges for substrate utilization and microbial functional diversity measured with MicroResp™ is needed to improve the interpretation of the results and, hence, its application in monitoring efforts at various scales (field, landscape, national) in different systems (arable, grassland, forest).

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Please see the supplementary information for Chapter 6 in the following pages.

Table S1. Characteristics of the ten European long-term field experiments used in the current study.

LTE[†]	Location	Coordinates	Year	Climate[§]	Soil type (WRB[‡])	Land use **and treatment factor**	Sampling depth (cm)	Experimental design and no. of reps.	Reference
Frick experiment CH1	Switzerland	47° 30′ 40″ N 8° 01′ 26″ E	2002	MAT: 10.3 °C MAP: 1130 mm (Dfb)	Vertic Cambisol	Arable Tillage (Conventional or Reduced)	0-10 & 10-20	Split plot with 4 reps.	(Krauss et al., 2010)
Aesch experiment CH2	Switzerland	47° 28′ 54″ N 7° 34′ 46″ E	2010	MAT: 10.6 °C MAP: 992 mm (Dfb)	Haplic Luvisol	Arable Tillage (Conventional or Reduced)	0-10 & 0-20	Split plot with 4 reps.	(Messmer et al., 2010)
DOK experiment CH3	Switzerland	47° 30′ 08″ N 7° 32′ 25″ E	1978	MAT: 11.2 °C MAP: 700 mm (Dfb)	Haplic Luvisol with Löss	Arable Organic matter input (Mineral or Biodynamic system)	0-20	Randomized block with 4 reps.	(Mäder et al., 2002; Fließbach et al., 2007)
BASIS experiment NL1	The Netherlands	52° 27′ N 05° 31′ E	2008	MAT: 9.5 C MAP: 750 mm (Cfb)	Haplic Fluvisol	Arable Tillage (Conventional or Minimum) and Organic matter input (None or Cut-and-carry).	0-15 & 15-30	Split plot block with 3 reps.	(Crittenden et al., 2014)
Vredepeel experiment NL2	The Netherlands	51° 32′ 27″ N 5° 52′ 05″ E	2001	MAT: 10.5 MAP: 775 mm (Cfb)	Gleyic Podzol	Arable Tillage (Conventional or Non-Inversion) and Organic matter input (Integrated or Conventional).	0-10 & 10-20	Split plot block with 3 reps.	None
Vitichar experiment PT1	Portugal	40° 26′ 26″ N 8° 26′ 23″ W	2013	MAT: 15 °C MAP: 1000-1200 mm (Csb)	Cambisol	Permanent crop (grapevine) Organic matter input (Control, Biochar-compost or 40 Mg/ha Biochar)	0-20	Randomized block with 3 reps.	None
PAGO experiment ES4	Spain	38° 49′ 20″ N 0° 48′ 32″ W	2005	MAT: 16.3 ° MAP: 420 mm (Bsk)	Cambisol	Permanent crop (grapevine) Organic matter input (Organic+ leguminous cover crops and no tillage or Conventional plus tillage)	0-10 & 10-20	Randomized block with 3 reps.	(García-Orenes et al., 2016)
Tillorg experiment SI1	Slovenia	46° 02′ 56″ N 14° 28′ 16″ E	1999	MAT: 11.3 °C MAP: 1380 mm (Dfc)	Eutric Gleysol	Arable Tillage (Conventional or Non-Inversion) and Organic matter input (NPK or Bio-waste).	0-10 & 10-20	Split plot with 3 reps.	(Kaurin et al., 2015)
Keszthely fertilization experiment HU1	Hungary	46° 43′ 60″ N 17° 13′ 49″ E	1984	MAT: 10.5 °C MAP: 683 mm (Dfb)	Eutric Cambisol	Arable Organic matter input (Farmyard or St-raw-green or Inorganic fertilizer 210 kg N/ha)	0-20	Split plot with 3 reps.	(Kismányoky and Tóth, 2013)
Keszthely tillage experiment HU4	Hungary	46° 44′ 5″ N 17° 13′ 47″ E	1972	MAT: 10.5 °C MAP: 683 mm (Dfb)	Eutric Cambisol	Arable Tillage (Conventional or Shallow disking).	0-10 & 10-20	Randomized block with 4 reps.	(Tóth et al., 2012)

[†]*LTE* long-term field experiment; [‡]*WRB* World Reference Base for Soil Resources; [§]*Dfb* and *Dfc* continental climate with cold winters and warm summers without a dry season or with cold winters and temperate summers without a dry season, respectively; *Cfb* and *Csb* temperate climate with warm summer with or without dry season, respectively; *Bsk* arid cold steppe climate.

Table S2. Overview of methods used to determine chemical, physical, and biological parameters linked with soil processes and the methods used to measure labile carbon fractions and soil suppressiveness (Bongiorno et al., 2019a; Bongiorno et al., 2019b).

Parameters	Methodology	Unit	Laboratory of analysis
Chemical parameters			
Total organic carbon (TOC)	SIST ISO 10694: Soil quality - Determination of organic and total carbon after dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
Total nitrogen (TN)	SIST ISO 13878:1999: Soil quality - Determination of total nitrogen content by dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
pH	$CaCl_2$ determination- SIST ISO 10390:2006: Soil quality - Determination of pH	-	University of Ljubljana (SL)
Cation exchange capacity (CEC)	ISO 13536:1995: Soil quality - Determination of the potential cation exchange capacity and exchangeable cations using barium chloride solution buffered at pH = 8,1	mmol 100 g^{-1} soil	University of Ljubljana (SL)
Plant available phosphorus (P_2O_5)	ÖNORM L 1087 - modification: ammonium lactate extraction	mg kg^{-1} soil	University of Ljubljana (SL)
Plant available potassium (K_2O)	ÖNORM L 1087 - modification: ammonium lactate extraction	mg kg^{-1} soil	University of Ljubljana (SL)
Exchangeable magnesium, calcium, and sodium (Mg^{2+}, Ca^{2+}, Na^{+})	ammonium acetate extraction; Soil survey laboratory methods manual, 1992	mg kg^{-1} soil	University of Ljubljana (SL)
Physical parameters			
Water stable aggregates (WSA)	Wet sieving method modified as in Kandeler (1996)	mg kg^{-1} soil	FiBL (CH)
Bulk density (BD)	Volumetric assessment with ring	g cm^{-3}	Field assessment by LTE owners
Silt, Clay and Sand	SIST ISO 11277:2011: Soil quality - Determination of particle size distribution in mineral soil material - Method by sieving and sedimentation	%	University of Ljubljana (SL)
Water holding capacity (WHC)	Calculated with a pedotransfer function using the % clay, silt and total organic carbon (Tóth et al., 2015)	%	Wageningen University & Research (NL)
Biological parameters			
Microbial biomass carbon (MBC)	Fumigation extraction method (Vance et al., 1987)	mg kg^{-1} soil	Trier University (DE)
Microbial biomass nitrogen (MBN)	Fumigation extraction method (Vance et al., 1987)	mg kg^{-1} soil	Trier University (DE)
Soil respiration	Incubation of soil at 25ºC for 72 h in thermostat bath	µg CO_2 –C h^{-1} g^{-1} soil	University Miguel Hernandez (ES)
Earthworms abundance and biomass	Hand sorting from 30*30*30 cm^3 monolith	Number and fresh weight (g m^{-2})	Field assessment by LTE owners
Tea bag decomposition	Tea bag incubation (tea bag index) (Keuskamp et al., 2013)	% mass loss	Field assessment by LTE owners
Soil suppressiveness to *Pythium ultimum*	Pythium ultimum-cress bioassay (Tamm et al., 2010)	Soil suppressiveness	Wageningen University & Research (NL)

Continue

Table S2 continued

Parameters	Methodology	Unit	Laboratory of analysis
Labile carbon fractions			
Dissolved organic carbon (DOC)	Extraction with ultrapure water and filtration at 0.45 µm filters.	mg kg^{-1} soil	Wageningen University & Research (NL)
Hydrophilic dissolved organic carbon (Hy-DOC)	Fractionation of DOC with DAX-8 resin (Van Zomeren and Comans, 2007).	mg kg^{-1} soil	Wageningen University & Research (NL)
Dissolved organic carbon and hydrophilic dissolved organic carbon specific ultraviolet absorbance (DOC SUVA and Hy SUVA)	Analysis of DOC and Hy solution with spectrophotometer at 254 nm (Weishaar et al., 2003; Amery et al., 2008).	L g C^{-1} cm^{-1}	Wageningen University& Research (NL)
Permanganate oxidizable carbon (POXC)	Oxidation with K_2MnO_4 (Weil et al., 2003).	mg kg^{-1} soil	Wageningen University & Research (NL)
Hot water extractable carbon (HWEC)	Extraction with hot water (80°C) for 16 hours and filtration at 0.45 µm filters (Ghani et al., 2003).	mg kg^{-1} soil	Wageningen University & Research (NL)
Particulate organic matter carbon (POMC)	Suspension in NaCl for 15 hours, wet-sieving through a 53 µm sieve and calculation of POM by loss on ignition (Salas et al., 2003).	mg kg^{-1} soil	FiBL (CH)

Table S3. Results of the linear mixed effect model testing the effect of sampling depth (0-10 cm vs. 0-20 cm layer) on substrate-induced respiration (µg CO_2-C g^{-1} h^{-1}).

Dependent variable	F-value	p-value
MSIR†	0.002	0.96
Glucose	0.14	0.71
Alanine	0.22	0.65
Amino butyric acid	0.03	0.85
N-acetyl glucosamine	0.05	0.82
Alpha-ketoglutaric acid	0.15	0.70
Lignin	0.06	0.81

†*MSIR* multiple substrate induced respiration.

Table S4. Utilization of six substrates expressed as absolute (μg CO_2-C g^{-1} h^{-1}) and relative (%) utilization rate, as analysed across 10 long-term field experiments. Least square means, confidence intervals (in brackets) and F and p values from linear mixed effect models (n = 101) are reported for each combination of tillage and organic matter addition. Significant differences ($p \leq 0.05$) are given in bold.

		Glucose	Alanine	Amino butyric acid	N-acetyl glucosamine	Alpha keto-glutaric acid	Lignine
		μg CO_2-C g^{-1} h^{-1}					
CT[†]- Low[§]		0.72 (0.28-1.85)	0.23 (0.09-0.61)	0.11 (0.04-0.28)	0.26 (0.11-0.60)	2.06 (0.77-5.48)	0.29 (0.16-0.52)
RT[‡]- Low		1.15 (0.44-3.02)	0.42 (0.16-1.14)	0.15 (0.06-0.41)	0.48 (0.20-1.14)	2.75 (1.02-7.45)	0.50 (0.27-0.93)
CT- High[#]		1.14 (0.44-2.94)	0.39 (0.15-1.02)	0.14 (0.05-0.36)	0.39 (0.17-0.89)	2.56 (0.96-6.85)	0.49 (0.28-0.88)
RT- High		1.82 (0.70-4.75)	0.54 (0.20-1.47)	0.21 (0.08-0.58)	0.53 (0.22-1.26)	3.50 (1.29-9.45)	0.61 (0.33-1.12)
Tillage (T)	F *p*	15.74 **0.0004**	11.65 **0.002**	4.11 **0.05**	11.03 **0.002**	10.60 **0.003**	12.21 **0.001**
Organic matter (OM)	F *p*	17.31 **0.0002**	12.10 **0.001**	2.41 0.13	5.71 **0.02**	9.58 **0.004**	14.86 **0.0005**
T X OM	F *p*	0.99 0.33	1.10 0.30	0.08 0.78	1.33 0.25	0.01 0.90	2.46 0.13
		%					
CT- Low		0.20 (0.13-0.27)	0.06 (0.04-0.08)	0.03 (0.02-0.05)	0.07 (0.05-0.08)	0.54 (0.42-0.67)	0.10 (0.03-0.16)
RT- Low		0.23 (0.16-0.30)	0.08 (0.06-0.10)	0.03 (0.01-0.05)	0.08 (0.06-0.10)	0.46 (0.33-0.59)	0.09 (0.03-0.16)
CT- High		0.21 (0.14-0.28)	0.07 (0.05-0.09)	0.03 (0.01-0.05)	0.07 (0.05-0.09)	0.49 (0.36-0.62)	0.11 (0.04-0.18)
RT- High		0.24 (0.17-0.31)	0.08 (0.06-0.10)	0.03 (0.02-0.05)	0.08 (0.06-0.10)	0.47 (0.34-0.61)	0.11 (0.04-0.18)
Tillage (T)	F *p*	11.03 **0.002**	6.82 **0.01**	0.07 0.79	6.46 **0.02**	7.44 **0.01**	0.0006 0.98
Organic matter (OM)	F *p*	2.15 0.15	1.00 0.32	0.81 0.37	1.06 0.31	2.85 0.10	4.15 **0.05**
T X OM	F *p*	2.54 0.12	0.40 0.53	1.30 0.26	2.57 0.12	3.04 0.09	3.70 0.06

[†]*CT,* conventional tillage; [‡]*RT,* reduced tillage; [§]*Low,* low organic matter input; [#]*High,* high organic matter input.

Table S5. Permutational analysis of multivariate variance (PERMANOVA) testing the effect of tillage and organic matter addition on absolute (µg CO_2-C g^{-1} h^{-1}) and relative (%) substrate utilization across 10 long-term field experiments (LTEs) (n=101). In the table also the effect of site (LTE), block, main plot and subplot are reported, which were included as fixed factors in the analysis.

	Absolute utilization rate		**Relative utilization rate**	
	(µg CO_2-C g^{-1} h^{-1})		(%)	
	R^2	p	**R^2**	p
LTE†	0.79	**0.001**	0.82	**0.007**
Block	0.01	0.336	0.007	0.26
Mainplot	0.001	0.268	0.002	0.27
Subplot	0.001	0.300	0.001	0.56
Tillage (T)	0.02	**0.002**	0.013	**0.006**
Organic matter (OM)	0.01	**0.003**	0.005	**0.03**
T x OM	0.002	0.354	0.007	**0.04**

†*LTE,* long-term field experiment.

Table S6. Scores, Pearson correlation coefficients (r) and related *p*-values of the six carbon substrates used for the MicroResp™ trials on the first two RDA axes for the catabolic profiles expressed as absolute (µg CO_2-C g^{-1} h^{-1}) and relative (%) utilization rate (n = 101).

	Absolute utilization rate						**Relative utilization rate**					
	(µg CO_2-C g^{-1} h^{-1})						(%)					
	RDA1			**RDA2**			**RDA1**			**RDA2**		
	score	**r**	p	**score**	**r**	p	**score**	**r**	p	**score**	**r**	p
Glucose	0.54	0.94	***	-0.12	0.08		0.12	0.82	***	0.05	0.39	*
Alanine	0.55	0.95	***	0.04	0.42	***	0.04	0.74	***	-0.006	-0.07	
Amino-butyric acid	0.47	0.90	***	0.17	0.73	***	-0.004	0.57	***	-0.01	-0.12	
N-acetyl glucosamine	0.47	0.95	***	-0.05	0.28	*	0.04	0.67	***	0.001	-0.005	
Alpha-ketoglutaric acid	0.24	0.72	***	0.004	0.08		-0.24	-0.99	***	0.01	0.19	
Lignin	0.50	0.86	***	-0.02	0.13		0.05	0.38	**	-0.04	-0.83	***

Table S7. Partial correlations (ρ) between the utilization of six substrates, expressed as absolute (μg CO_2-C g^{-1} h^{-1}) and relative utilization rate (%) with the Shannon functional diversity index (H') (number of observations= 101), corrected for site (LTE).

	H'†			
	Absolute utilization rate		Relative utilization rate	
	(μg CO_2-C g^{-1} h^{-1})		(%)	
Glucose	0.64	***	0.69	***
Alanine	0.73	***	0.82	***
Amino-butyric acid	0.78	***	0.69	***
N-acetyl glucosamine	0.71	***	0.78	***
Alpha-ketoglutaric acid	0.13		-0.89	***
Lignin	0.59	***	0.08	

† *H'* Shannon functional diversity index.

Table S8. Coordinate of the head of the fitted vectors scaled by correlation coefficient, R^2 and *p*-values of the first two RDA axes (RDA1, RDA2) for the variables used in the *envfit* function in vegan to investigate their relation with the utilization of carbon substrates as shaped by soil management (tillage and organic matter addition).

	Absolute utilization rate				**Relative utilization rate**			
	($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)				(%)			
	RDA1	RDA2	R^2	*p-value*	RDA1	RDA2	R^2	*p-value*
Chemical parameters†								
TOC	0.23	-0.06	0.06	**0.05**	0.16	0.02	0.03	0.26
pH	0.02	-0.04	0.002	0.89	0.01	0.13	0.02	0.43
TN	0.17	-0.05	0.03	0.19	0.11	-0.008	0.01	0.53
C/N	-0.08	0.02	0.007	0.69	0.01	0.04	0.002	0.92
CEC	0.13	-0.07	0.02	0.33	0.06	0.06	0.007	0.70
P Olsen	0.16	-0.04	0.03	0.24	0.27	-0.02	0.07	**0.02**
Ca	-0.02	-0.002	0.0004	0.98	0.02	0.04	0.002	0.91
Mg	0.14	-0.06	0.02	0.30	0.05	-0.01	0.002	0.88
K	0.26	-0.12	0.08	**0.02**	0.16	0.02	0.02	0.35
Na	-0.01	-0.07	0.006	0.76	-0.01	-0.09	0.009	0.62
Physical parameters‡								
WSA	0.10	-0.002	0.01	0.61	0.10	-0.01	0.009	0.63
WHC	0.04	-0.03	0.002	0.88	0.05	-0.03	0.001	0.94
BD	-0.04	0.01	0.002	0.90	-0.04	0.001	0.0001	0.99
Sand	-0.01	0.02	0.0004	0.98	-0.03	0.001	0.0004	0.98
Silt	0.01	-0.02	0.0006	0.97	0.03	-0.003	0.002	0.90
Clay	0.05	0.003	0.002	0.89	-0.005	-0.01	0.005	0.78
Biological parameters§								
MBC	0.09	-0.04	0.01	0.60	0.10	0.04	0.01	0.56
MBN	0.19	-0.04	0.04	0.15	0.20	0.02	0.04	0.13
Basal respiration	0.28	0.001	0.09	**0.01**	0.18	0.10	0.04	0.11
Decomposition	-0.08	0.02	0.008	0.67	-0.004	0.05	0.005	0.78
Earthworm numbers	0.04	0.02	0.002	0.90	0.04	0.009	0.002	0.92
Earthworm biomass	0.08	0.006	0.006	0.73	0.08	0.02	0.007	0.72
qPCR counts	0.17	-0.02	0.03	0.23	0.16	0.08	0.03	0.21
Nematode OTU diversity	0.03	-0.05	0.004	0.83	-0.03	0.18	0.03	0.18
Nematode OTU richness	0.07	0.006	0.005	0.77	0.01	0.01	0.01	0.60
Soil disease suppressiveness	0.03	0.09	0.009	0.65	0.08	0.007	0.007	0.71
Labile carbon parameters#								
Hy SUVA	-0.03	0.03	0.002	0.90	-0.01	-0.09	0.009	0.65
DOC SUVA	0.02	-0.04	0.002	0.90	0.05	0.03	0.004	0.82
Hy-DOC	0.28	-0.11	0.09	**0.009**	0.27	-0.05	0.08	**0.02**
DOC	0.19	-0.04	0.04	0.15	0.23	-0.11	0.06	**0.04**
HWEC	0.25	-0.07	0.07	**0.03**	0.20	0.01	0.04	0.11
POXC	0.29	-0.07	0.09	**0.01**	0.21	0.02	0.04	0.11
POMC	0.17	-0.03	0.03	0.19	0.15	0.03	0.02	0.30

†*TOC* total organic carbon, TN total nitrogen, C/N carbon to nitrogen ratio, *CEC* cation exchange capacity, K available potassium, ‡*WSA* water stable aggregates, WHC water holding capacity, *BD* bulk density, §*MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *SR* soil respiration, *Hy SUVA* specific ultraviolet absorbance of hydrophylic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, #*Hy* hydrophilic carbon, *DOC* dissolved organic carbon, *HWEC* hot water extractable carbon, *POXC* permanganate oxidizable carbon, *POM* particulate organic matter.

Table S9. Mixed linear models derived from 101 soil samples from 10 LTEs, as determined from significant soil parameters ($p \leq 0.05$), with the Shannon functional diversity index (H') as dependent variable. The Akaike information criterion (AIC) is an estimator of the quality of the statistical model, the R_m^2 (marginal coefficient of determination) indicates the proportion of the variation explained by the predictor variables, and the R_c^2 (conditional coefficient of determination) indicates the variation explained by both the fixed and the random factors. The R^2_{mAdj} and the R^2_{cAdj} provides a measure of the accuracy of the model across different samples.

Indicator group	Starting model	Parameters selected for the final model	AIC	R^2_m	R^2_{mAdj}	R^2_c	R^2_{cAdj}
		(t-value; p-value)					
Chemical parameters[†] (Model 1)	Exp_H'[††] ~ log_TOC + log_CEC+ log_C/N + log_pH + log_P+ log_Mg + log_K + sqrt_Na+ (1|LTE)	log_TOC (3.73; 0.0004) log_pH (-2.40; 0.02) log_C/N (2.05; 0.05) log_P (2.0; 0.05)	116	0.30	0.23	0.65	0.63
Physical parameters[‡] (Model 2)	Exp_H' ~ log_WSA + log_WHC + log_BD + (1|LTE)	log_WSA (2.44; 0.02)	124	0.09	0.07	0.68	0.66
Biological parameters[§] (Model 3)	Exp_H' ~ log_MBN + log_SR + log_EN + log_GT + sqrt_Nematode + log_nema_diversity + log_nema_richness+ logit_SS+ (1|LTE)	log_MBN (3.22; 0.002)	121	0.07	0.04	0.72	0.70
Labile carbon fractions[#] (Model 4)	Exp_H' ~ log_POMC + sqrt_DOC + log_Hy-DOC + log_HWEC + log_DOC_SUVA + log_Hy_SUVA + (1|LTE)	log_POMC (2.87; 0.005) sqrt_DOC (2.59; 0.01)	115	0.28	0.24	0.65	0.63

[††]*H'* Shannon functional diversity index, [†]*TOC* total organic carbon, *CEC* cation exchange capacity, *C/N* carbon to nitrogen ratio, [‡]*WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density, [§]*MBN* microbial biomass nitrogen, *SR* soil respiration, *EN* earthworms number, *GT* decomposition from tea bag, *SS* soil suppressiveness, [#]*Hy-DOC* hydrophilic dissolved organic carbon, *Hy SUVA* specific ultraviolet absorbance of hydrophilic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.

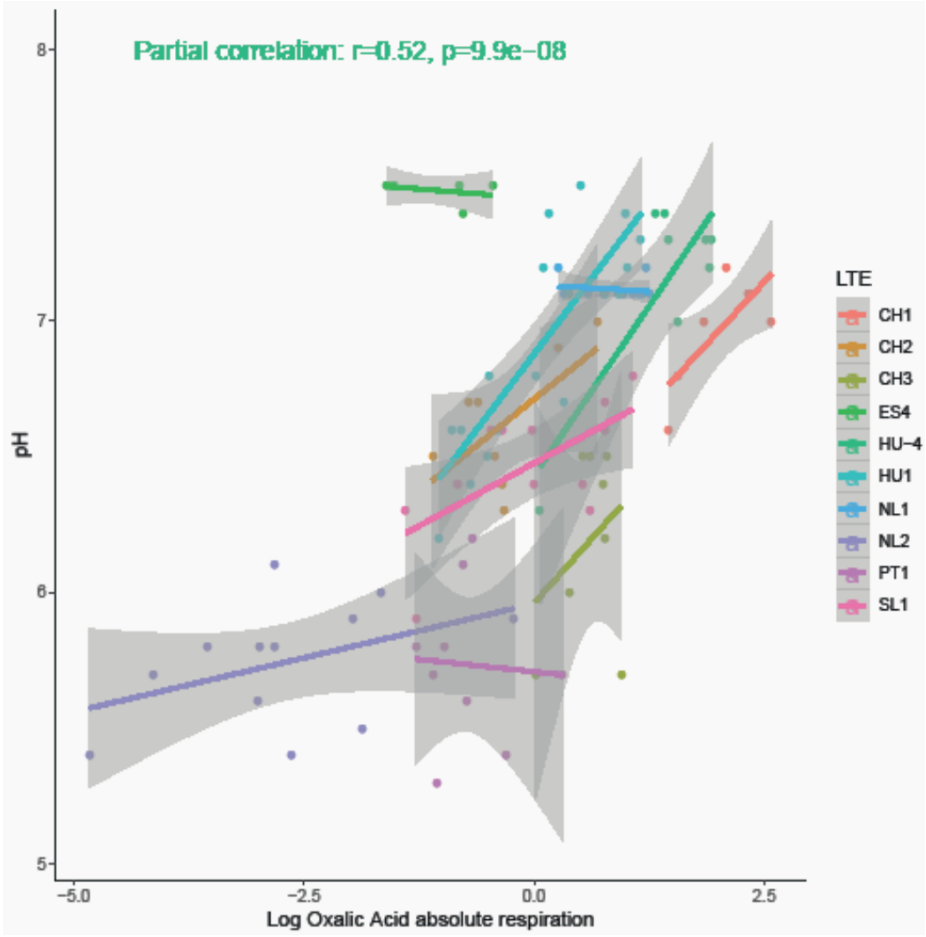


Figure S1. Correlation between absolute utilization (μg CO_2-C g^{-1} h^{-1}) of oxalic acid (log transformed) and the pH visualized for each long-term field experiment. On the top of the graph, the partial correlation between the two variables across all long-term field experiments (LTEs) is shown with relative p value. *LTE* long-term field experiment, *CH1* Frick trial, *CH2* Aesch trial, *CH3* DOK trial, *NL2* De Peel trial, *NL1* BASIS trial, *SL1* Tillorg trial, *PT1* Vitichar trial, *ES4* Pago trial, *HU1* Keszthely trial, *HU4* Keszthely trial.

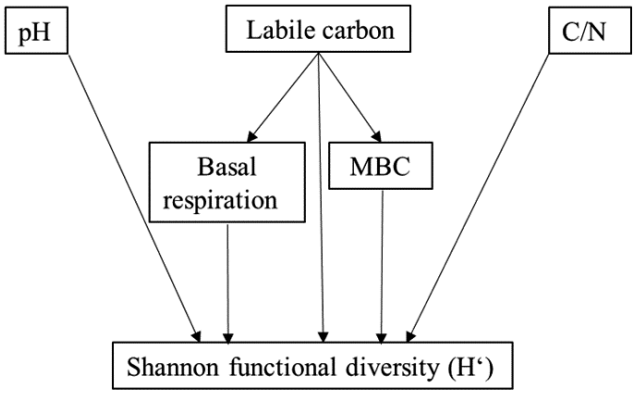


Figure S2. *A priori* structural equation model (SEM). White arrows indicate a positive relationship between the parameters in the boxes. *C/N* carbon to nitrogen ratio, *MBC* microbial biomass nitrogen.

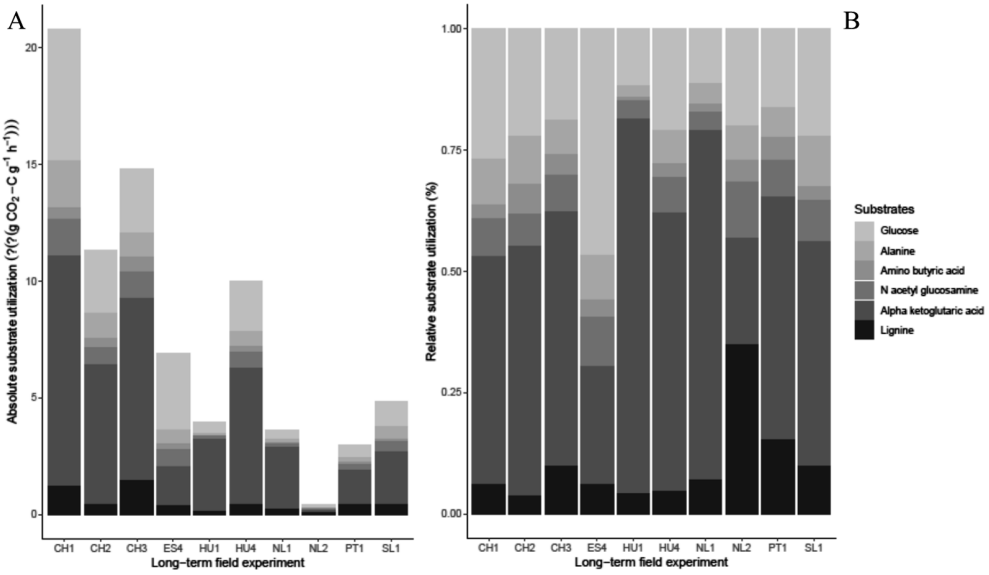


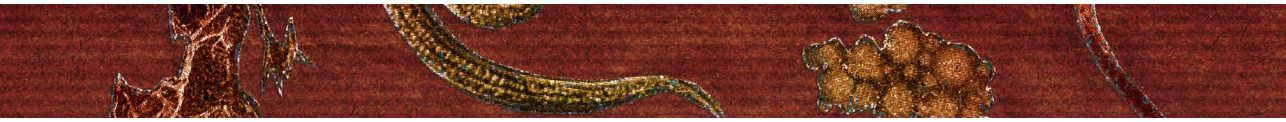
Figure S3. Absolute (µg CO_2-C g^{-1} h^{-1}) and relative (%) substrate utilization for six substrates measured in soil from each of ten long-term field experiments. *CH1* Frick trial, *CH2* Aesch trial, *CH3* DOK trial, *NL2* De Peel trial, *NL1* BASIS trial, *SL1* Tillorg trial, *PT1* Vitichar trial, *ES4* Pago trial, *HU1* Keszthely trial, *HU4* Keszthely trial.

CHAPTER 7

7

General Discussion

Giulia Bongiorno



This chapter has been accepted, subject to minor revision, by Frontiers in Agricultural Science and Engineering.

7.1 Introduction

Intensive agricultural management of soil can have detrimental effects on dynamic chemical, physical and biological soil properties, which in turn can affect soil processes, and ultimately soil-based ecosystem services (Giller et al., 1997; Stoate et al., 2001; Bünemann et al., 2006; Smith et al., 2016). 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. This can ultimately lead to the evaluation and the adoption of alternative agricultural practices that effectively sustain agricultural production and environmental resilience (White et al., 2012; Sandén et al., 2018; Barão et al., 2019).

This thesis aimed to investigate the suitability of a range of soil biological and biochemical parameters as novel soil quality indicators for agricultural management. In my selection of indicators, based on a thorough review of the literature (Chapter 2; Bünemann et al., 2018), I accounted for different but complementary dimensions of the biological and biochemical soil characteristics, namely soil labile organic carbon, soil disease suppressiveness, soil free-living nematode community characteristics and soil microbial functionality. We assessed their sensitivity to soil management consisting in contrasts of tillage (reduced vs conventional) and organic matter addition (low vs high), and their linkage with traditionally measured soil quality indicators (e.g. total organic carbon, pH, water stable aggregates, microbial biomass etc.). By screening the novel indicators in ten European long-term field experiments, this thesis may contribute to detect promising tools that can be added to, or partly replace, soil indicators measured in current soil quality assessment schemes, and also to pinpoint the knowledge gaps in realising their adoption. In this final chapter, the main findings from the previous experimental chapters (Chapters 3, 4, 5 and 6; Bongiorno et al., 2019a, b, c; Bongiorno et al., in preparation) are summarized, contextualized and discussed, in respect to the main objectives outlined in Chapter 1. Subsequently, recommendations for future research are summarized, pointing out where scientific research can help the development of soil quality assessments, which ultimately should be used by farmers and land managers.

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

Reduced tillage and addition of organic matter are widespread agricultural practices applied to reduce the impact of soil management on soil properties and processes (Smith et al., 2016), counteracting multiple soil threats, such as soil organic matter depletion, soil erosion and compaction (Seitz et al., 2018).

As hypothesised in Chapter 1, across the ten European long-term field experiments (LTEs) studied, the novel soil quality indicators were sensitive to changes induced by these agricultural practices, despite the large site effects of the LTEs. In particular, compared to the other indicators, permanganate oxidizable carbon (POXC), hot-water extractable carbon (HWEC) and particulate organic matter carbon (POMC) (Chapter 3; Bongiorno et al., 2019b), were, indeed, sensitive to 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 (Chapter 4, 5 and 6; Bongiorno et al., 2019b,c; Bongiorno et al., submitted). Using random forest analysis[1], I tested which of the novel indicators were the most important in classifying the different combinations of soil management practices (i.e. CT-Low, CT-High, RT-Low, RT-High). As expected from the results of the experimental chapters, the labile carbon fractions were the most sensitive according to the % mean decrease in accuracy (variable importance metric of the random forest model), which measures the average decrease in the model accuracy on the out of bag (OOB) samples (samples that were not selected to be part of bootstrapped samples used to create the trees of the forest) when the values of the respective variable are randomly permuted (Archer and Kimes, 2008) (**Figure 7.1**, out of bag (OOB) estimate of error rate = 52.48%). In particular, POXC was the most important variable in classifying soil management (**Figure 7.1**).

Previous studies already highlighted labile organic carbon, measured with various methodologies, as a very sensitive fraction of the total soil organic carbon (Ghani et al., 2003b; Haynes, 2005b; Mirsky et al., 2008; Culman et al., 2012). This is probably due to the fact that labile organic carbon is dependent on soil aggregation, aggregate turnover, microbial biomass and residue input (Six et al., 1999). Therefore, soil labile carbon concentrations 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 measured indicators (Culman et al., 2012; Culman et al., 2013; Fine et al., 2017; Thoumazeau et al., 2019).

In my work, tillage exerted a stronger effect on the novel soil indicators than organic matter addition and this was particularly evident for soil suppressiveness, nematode communities and microbial catabolic profiles (Chapters 4, 5 and 6; Bongiorno et al., 2019a, b, c; Bongiorno et al., submitted). Conventional tillage destroys soil aggregates, making resources available that boost microbial activity in the short-term (van Capelle et al., 2012). Moreover, conventional tillage entails a destruction of the soil as a habitat for organisms, where these are directly killed and exposed to predators by the mechanical

1 The materials and methods of all the analyses performed in the sections of Chapter 7 are given at the end of the Chapter.

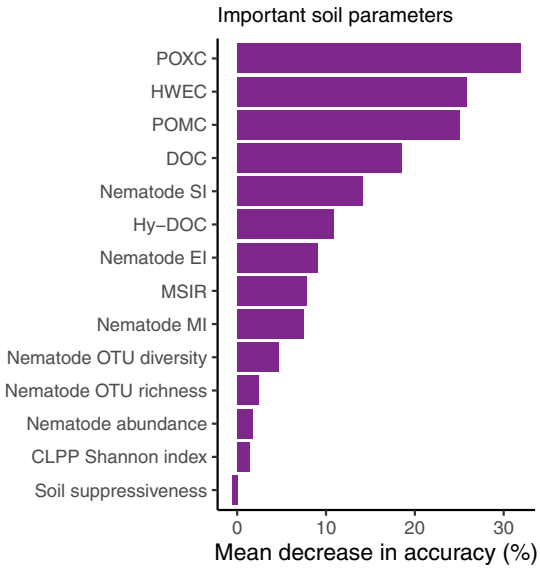


Figure 7.1. Variable importance metric (% mean decrease in accuracy) from random forest classification analysis of the novel soil quality indicators for the discrimination of different combinations of tillage and organic matter addition (i.e. CT-Low OM, CT-High OM, RT-Low OM, RT-High OM). The mean decrease in accuracy measures the average decrease in the model accuracy on the out of bag (OOB) samples (samples that were not selected to be part of bootstrapped samples used to create the trees of the forest) when the values of the respective variable are randomly permuted. *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* hydrophylic dissolved organic carbon, *EI* enrichment index, *MSIR* multiple substrate induced respiration, *MI* maturity index, *OTU* operational taxonomic unit, *CLPP* community level physiological profiling.

action of the plough (Kladivko, 2001; Kibblewhite et al., 2008b). In my research, reduced tillage practices had a positive effect on various soil processes increasing the quantity of available carbon for microbial activity (Chapter 3; Bongiorno et al., 2019b) and creating a stable environment which sustained soil suppressiveness (Chapter 4; Bongiorno et al., 2019c), nematode diversity and richness (Chapter 5; Bongiorno et al., 2019a) and microbial decomposition capacity and functional diversity (Chapter 6; Bongiorno et al., submitted). Previous studies have already shown the beneficial effect of reduced tillage, compared to conventional, on soil quality (Alvear et al., 2005; Melero et al., 2011; Stavi et al., 2011; Aziz et al., 2013; Laudicina et al., 2015). Reduced tillage increased relative and absolute abundance of herbivorous nematodes (Chapter 5; Bongiorno et al., 2019a), in line with results from previous studies (Treonis et al., 2010; Treonis et al., 2018). The effect of tillage was more evident in the upper than in the lower soil layer, confirming the results of previous studies (Six et al., 1999; Angers and Eriksen-Hamel, 2008; Cooper et al.,

2016). In addition, reduced tillage caused a stratification of various soil properties, and soil compaction in the lower soil layer, also confirming previous studies (Needelman et al., 1999; Cooper et al., 2016). These results underline the importance of studying the effect of tillage on soil quality at different depths, as already stressed by Peigné et al. (2018). The plough layer of the reference system may serve as a minimum sampling depth, but further distinction of layers within the plough layer may improve the understanding of reduced tillage effects.

The weaker effect of organic matter additions on the novel soil quality indicators could be due to the heterogeneous nature of the organic matter added in the different long-term field experiments, including biochar, compost, biowaste and farmyard manure (**Figure 4.1**). The quantity and the composition of the added organic matter and the soil organic matter already present are important factors for soil organisms and the soil processes they perform (Bending et al., 2002; Lejon et al., 2007; Wessén et al., 2010; Kallenbach and Grandy, 2011; Wienhold et al., 2013; Bonanomi et al., 2018c) because the latter are associated with changes in abundance and function of the soil microbial community (Bending et al., 2002; Giacometti et al., 2013). Organic matter addition will increase microbial biomass and activity preferentially if it brings to the soil labile and available C and N components (Kallenbach and Grandy, 2011; Cotrufo et al., 2013).

Based on my results and those of previous studies, I conclude that farmers and land managers will generally benefit from the adoption of reduced tillage and increased organic matter addition for multiple soil processes. However, the implementation of these management measures has to be accompanied with awareness about their limitations, and with careful evaluation of the site-specific conditions for site-specific management and *vice versa* (Six et al., 2004; Sandén et al., 2018), in order to maximize the benefit obtained.

7.3 Soil texture

Soil texture can affect the way tillage and organic matter addition impact on soils (Wiesmeier et al., 2015). In Chapters 3, 4, 5 and 6 (Bongiorno et al., 2019a, b, c; Bongiorno et al., submitted) we did not take soil texture into account because my main aim was to assess the general suitability of novel soil quality indicators across the long-term field experiments. For this reason, we indirectly considered soil texture by including the LTEs as a random factor in the analyses. In this section, I visualise the effect of tillage and organic matter addition on the novel soil quality indicators separately for samples from heavy soil (clay + silt content > 50 %) and light soil (clay + silt content < 50 %) with redundancy analysis (RDA) (**Figure 7.2**).

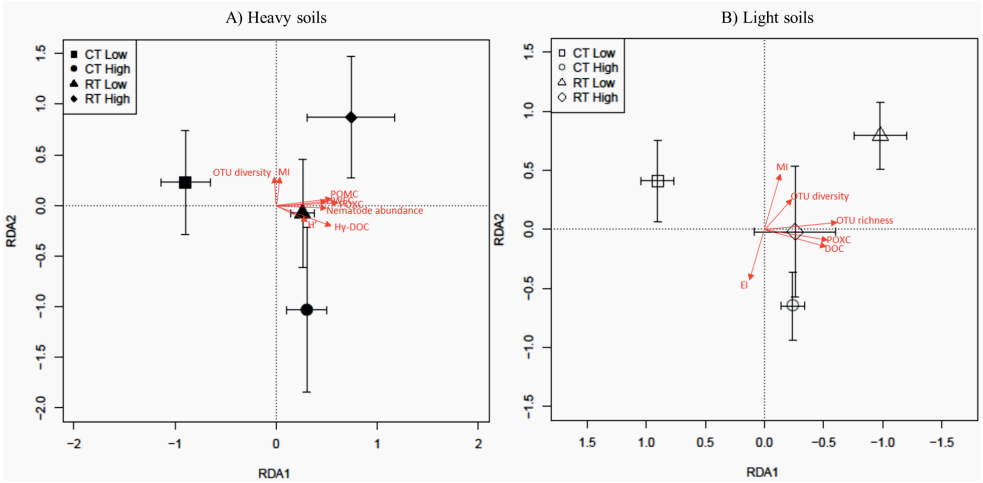


Figure 7.2. Redundancy analysis (RDA) of soil quality indicators assessed in samples with A) heavy soil texture (clay + silt content > 50 %; n=42) and B) light soil texture (clay + silt content < 50 %; n=59. The novel soil quality indicators were used as dependent variables, the management was used as constraining variable, and the long-term field experiment was used as conditional variable. Only the novel soil quality indicators that had a correlation ($p \leq 0.001$) 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, *EI* nematode enrichment index.

For both soil texture classes, the treatments are located in the same position in **Figures 7.2A** and **7.2B**, 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, similarly to the results of Chapter 6 (Bongiorno et al., submitted). This shows the strong negative effect of the most intensive agricultural practice on soil quality.

For the heavy soils (**Figure 7.2A**), tillage and organic matter addition presented different profiles of novel soil quality indicators, and 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 are the ones that discriminate the different treatments most on RDA axis 1 (**Table 7.1**). For the light soils (**Figure 7.2B**) we observed a stronger discrimination of the novel indicators of organic matter addition on RDA axis 2, while tillage had a stronger effect when low organic matter was applied, but similarly to heavy soils, the discrimination was mainly on RDA axis 1. This could be due to the fact that in light soils, which are not very structured and have limited unsaturated soil organic matter protective capacity, the potential for enhancing soil quality is higher with direct

Table 7.1. RDA Scores, Pearson correlation coefficient (r) and related *p*-values of the novel soil quality indicators on the first two RDA axes for heavy texture (clay + silt content > 50 %, n=42) and light texture soils (clay + silt content < 50 %, n=59).

	Heavy soils (clay + silt content > 50 %)						**Light soils** (clay + silt content < 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	*
Maturity index	0.03	-0.05		0.24	0.55	**	0.13	0.26	*	0.45	0.76	***
Structural index	0.04	0.002		0.12	0.36	*	0.12	0.16		0.33	0.39	*
Enrichment index	-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	

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

additions of organic matter than applying reduced tillage, which is more focused on increasing physical properties (Needelman et al., 1999; Chivenge et al., 2007; Abbott and Manning, 2015). However, reduced tillage might be particularly effective when organic matter addition in the soil is low, because of a higher potential for improvement. In addition, for the light soils the nematode indicators (MI, EI, OTU richness and diversity) were more important in discriminating the treatments than in heavy soils, especially on RDA axis 2 (Table 1). Still, the labile carbon fractions were also important, similarly to the heavy soils. I conclude that soil texture did not have a dramatic influence on the results of experimental work presented in the experimental Chapters 3, 4, 5 and 6 (Bongiorno et al., 2019a, b, c; Bongiorno et al., submitted). However, we found some differences, in particular for the nematode indicators, confirming results of Quist et al. (2019), which

confirm that soil texture influences the impact of soil management on soil quality and warrants more investigation in future research.

7.4 The unique role of labile organic carbon

In Chapter 3, labile carbon, in particular POXC, was correlated with various soil parameters from the iSQAPER MDS related to nutrient cycling (total nitrogen, cation exchange capacity, P, Mg, available K, soil respiration), soil structure (water stable aggregates, water holding capacity, bulk density), carbon sequestration (total organic carbon) and habitat provision (microbial biomass carbon, soil respiration) (Bongiorno et al., 2019b). In addition, labile carbon was tightly linked to the other novel indicators assessed, showing its potential as overarching indicator linking different quality aspects of agricultural soils (Chapter 4, 5 and 6; Bongiorno et al., 2019a, c; Bongiorno et al., submitted). Labile organic carbon is also closely linked to total organic carbon (TOC), and it constitutes the primary energy source for soil organisms, being the fuel for their activities and processes (Haynes, 2005b). Various studies found labile carbon to be linked with soil quality parameters, and POXC and HWEC were particularly linked with biological parameters (Ghani et al., 2003b; Melero et al., 2009; Culman et al., 2010a). Labile organic carbon and its aromaticity have been connected with changes in taxonomic microbial community composition (Lejon et al., 2007), which are likely to bring changes in soil functionality. Therefore, not only the total organic carbon is important for soil processes, but also the nature of the organic compounds that compose the soil organic matter can have a strong impact on soil processes, especially in terms of microbial-related processes (Kallenbach and Grandy, 2011; Giacometti et al., 2013; Pezzolla et al., 2013).

The other novel indicators were not strongly correlated with each other, even if, in general, they were enhanced by reduced tillage and addition of organic matter, being positively correlated with various soil quality indicators, such as total organic carbon, total nitrogen, microbial biomass and activity (Chapter 4, 5 and 6; Bongiorno et al., 2019a, b, c; Bongiorno et al., submitted). This shows that the novel indicators could be used together in a complementary way in soil quality assessment, without being redundant in the information they provide.

In my study, causal links between the indicators cannot be proved. However, for novel indicators to be adopted in practice, their links with functions and ecosystem services have to be established (Adhikari and Hartemink, 2016; Rinot et al., 2019). For this reason, I used structural equation modelling (SEM) to confirm hypothesised mechanistic relationships between indicators (Eisenhauer et al., 2015). The SEM models in Chapters 3 and 6 confirmed our hypothesised primary role of labile organic carbon in sustaining soil disease suppressiveness and microbial functional diversity (Chapter 4 and 6; Bongiorno et

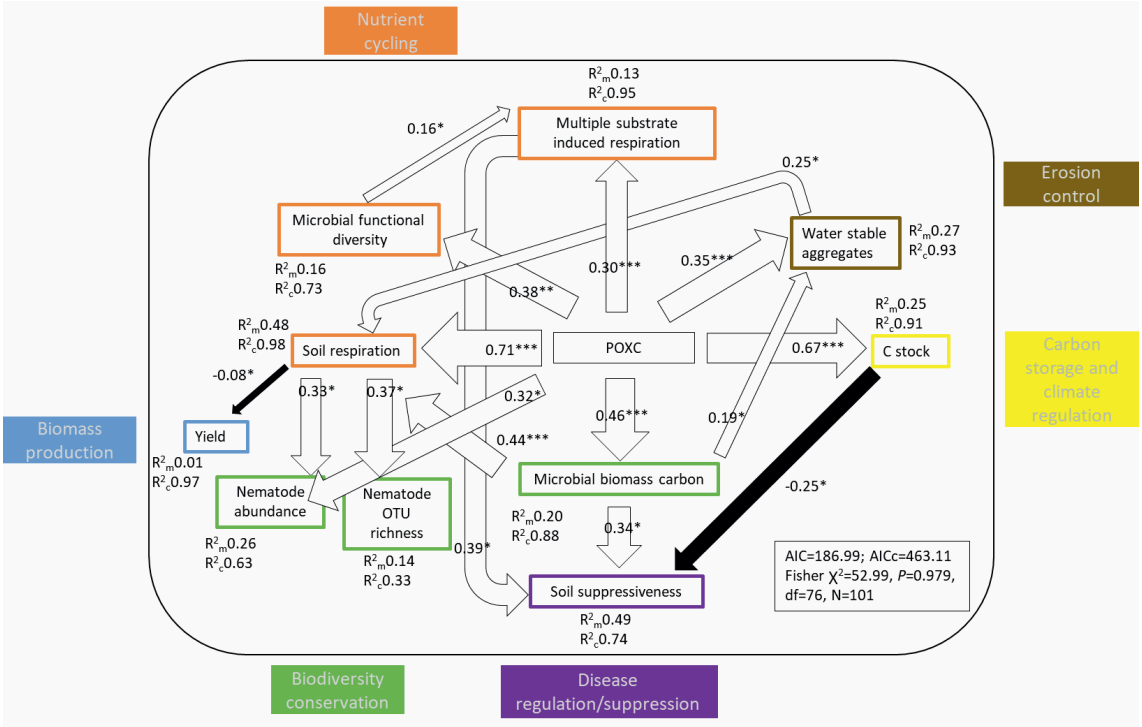


Figure 7.3. Piecewise structural equation model (SEM) showing the central role of POXC as a predictor for various ecosystem processes and services, which are placed in coloured boxes outside of the SEM frame. White boxes within the SEM frame represent measured variables, and arrows represent the unidirectional relationship between the parameters. The colour of the border of the boxes specifies the ecosystem process or service they indicate. White arrows indicate positive relationships; black arrows indicate negative relationships. Numbers on the side of the arrows indicate standardized effect size (reported as path coefficient), and the width of the arrow is proportional to the strength of the path coefficient. 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) 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. Variables lacking the R_m^2 and the R_c^2 acted only as predictor. In the box adjacent to the figure the Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AIC_c), Fisher chi-square (Fisher χ^2), *p* value (P) of the test, degrees of freedom (df), and the number of observation used for the analysis (N) are indicated. SEM models with a χ^2 with a $p \geq 0.05$ are considered to be statistically significant. *POXC* permanganate oxidizable carbon, *C stock* carbon stock, *OTU* operational taxonomic unit.
$^{0}p \leq 0.1$, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

al., 2019c; Bongiorno et al., submitted). In addition to these models, I tested and visualized the central role of labile organic carbon (POXC) in sustaining various soil ecosystem services in an extended SEM model (**Figure 7.3**).

Confirming the results presented in the other chapters of my thesis, labile organic carbon was found to have a multifunctional role in agricultural soils (Chapter 3, 4,5 and 6; Bongiorno et al., 2019a, b, c; Bongiorno et al., submitted). Labile organic carbon had a direct positive link with carbon sequestration, nutrient cycling, biodiversity

conservation and erosion control. POXC has already been proposed as an indicator for carbon sequestration in previous studies (Culman et al., 2012; Hurisso et al., 2016). In addition, labile carbon contributed indirectly to erosion control through its positive effect on microbial biomass carbon. This association has already been established by previous studies (van Leeuwen et al., 2015; Griffiths et al., 2018b). In particular, fungal biomass has been positively associated with micro-aggregate stability (Cosentino et al., 2006; Spurgeon et al., 2013; Manici et al., 2019). 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 competitive ability against the pathogen of the microbial community (i.e. microbial biomass carbon and MSIR). This last finding is in line with results from Dignam et al. (2019) which found that higher carbon availability selected for a richer and potentially more competitive and suppressive community against *Rhizoctonia solani*. Similarly to our results, Bastida et al. (2016) have shown a key role of labile carbon measured as dissolved organic carbon in the multifunctionality of soil microbial communities.

Many positive links between labile organic carbon and ecosystem services were found, underlying the synergies between soil processes. However, even though weak, labile carbon had an indirect negative effect on biomass production through soil respiration. Our results contrast with those from previous studies which found a positive relationship between labile carbon fractions and yield (Weil et al., 2003a; Lucas and Weil, 2012; Culman et al., 2013; Hurisso et al., 2016), but are nevertheless important because they show that trade-offs between different ecosystem services are present, and they have to be accounted for when managing soils. Often, agricultural practices considered to be sustainable, such as organic farming, reduced tillage, and cover cropping show increased values for soil quality indicators with linkages to environmental functions at the expense of productivity (Mäder et al., 2002; Emmerling, 2007; Larsen et al., 2014; Cooper et al., 2016; Wittwer et al., 2017; Knapp and van der Heijden, 2018; Sandén et al., 2018; Kopittke et al., 2019). In particular, this is often the case when soil management aims to sustain multiple functions (Smith et al., 2015).

7.5 Pro and cons of the novel soil quality indicators

I identified the following positive and negative aspects for each of the soil quality indicators:

- The labile carbon fractions were the most sensitive to long-term management practices (**Figure 7.1**), while other studies also showed their sensitivity to short-term management (Culman et al., 2013). In addition, they showed to be suitable

multifunctional indicators (**Figure 7.3**). In particular, permanganate-oxidizable carbon (POXC) appeared to be the most suitable fraction for assessing soil quality in tillage and organic matter addition systems across Europe (Chapter 3, Bongiorno et al., 2019b). Measuring POXC is fast, high-throughput, easy and cheap, and it can also be performed in the field (Weil et al., 2003a; Mirsky et al., 2008). The POXC protocol is used especially in North America, where the method is routinely used in the Cornell Soil Health assessment (CASH) (Idowu et al., 2008). However, caution has to be taken in that the amount of POXC quantified depends on the concentration of total organic carbon in the soil, on the amount of soil used and on the sieving size (Gruver, 2015); recent studies are trying to refine the methodology based on this (Wade et al., in preparation). Sample pre-treatment conditions (e.g. storage and sieving) are known to affect, in general, all the other labile carbon fractions (Haynes, 2005b; Sun et al., 2015). The estimation of the other labile carbon fractions (Chapter 3; Bongiorno et al., 2019b) is more expensive and time-consuming than POXC, but established protocols are available. However, specific laboratory protocols often vary, making the measurements less comparable. The biggest challenge is the interpretation of the labile carbon fractions, especially of POXC, because it is not clear which part of the carbon they quantify. There is evidence that POXC is not only quantifying the labile part of carbon, but also more processed (Culman et al., 2012) and recalcitrant compounds like lignin (Tirol-Padre and Ladha, 2004; Romero et al., 2018).

- The cress-*Pythium* soil suppressiveness bioassay used in Chapter 4 (Bongiorno et al., 2019c) was a highly reproducible, fast and easy assay for assessing the capacity of the soil to suppress pathogens (Thuerig et al., 2009; Tamm et al., 2010). This system can give a primary idea of the general soil suppressiveness of the soil close to *in situ* conditions, but it is an assessment of the *potential* suppressiveness. Our results showed that, in general, systems with higher labile organic carbon and microbial biomass are less conducive to disease and have a higher capacity to suppress disease incidence of a host plant (Chapter 4, Bongiorno et al., 2019c). Microbial biomass, activity and labile carbon have already been found to be positively linked with soil suppressiveness (Postma et al., 2008; Bonanomi et al., 2010; Dignam et al., 2018). Thus, a higher value of these parameters could already be used as a first indication of higher soil suppressive capacity. However, while for reduced tillage we found an overall positive effect on soil suppressiveness, for the case of organic matter addition we did not find an effect, possibly due to the variable nature of the organic matter added to the soil. Therefore, it seems that more factors, which we did not quantify, e.g. microbial community composition, organic matter composition, are affecting soil suppressiveness, leaving

many unanswered questions. In addition, this method is a general characterisation of the antagonistic potential of the microbial community, which does not take into account the specificity of a particular host-pathogen interaction in the field. Various mechanisms in fact are working for different host-pathogen combinations (Kariuki et al., 2015) and these might differ from the mechanisms active in the *Pythium*-cress model system. For this reason bioassays done with different pathogens often yield contrasting results (Bonanomi et al., 2010). Therefore, if an evaluation of the specific pathogenic problems in the field has to be done, I suggest that a bioassay of the potential for general disease suppressiveness should be combined with *in situ* characterisation of disease severity and/or with a bioassay using the crop and the pathogen that are present in the area and cause disease (Bonanomi et al., 2018a).

- The results of Chapter 5 (Bongiorno et al., 2019a) confirm previous evidence that soil free-living nematode communities are suitable soil quality indicators (e.g. sensitive to changes, ubiquitous, characterized in functional groups etc.) (Wilson and Kakouli-Duarte, 2009). In addition, modern molecular techniques gave similar results than older, more established microscopic techniques. This suggests that the extensive knowledge on nematode community composition in assessing soil ecosystems can be used also with molecular methods. Molecular characterization of nematodes in soil will become cheaper, faster and with higher throughput than traditional morphological characterisation (Ahmed et al., 2016; Geisen et al., 2018). A very interesting benefit of the characterization of nematode communities is that next to taxonomy-related information (alpha and beta diversity), also functional and ecological aspects of the food web can be revealed (Ferris et al., 2001; Gardi et al., 2009; van Capelle et al., 2012). This makes the taxonomic assessment of nematode communities a more informative tool for soil quality assessment than the molecular characterisation of other organisms, for example bacteria and fungi. For microbial communities, in fact, the functional meaning of shifts in diversity and community composition is less clear (Kuyper and Giller, 2011).

  The most challenging limitations of using nematode communities studied with molecular methods as indicator of soil quality are the optimization, and possibly the standardization, of the method: primer selection, database completeness and bioinformatic analysis workflow. However, I am convinced that these elements will develop in the next years, increasing the potential for an efficient community characterisation, and for finding indicator species or genera. Also, at the moment, the issue of the number of copies of targeted genes and standardization of the sequencing results is still open (Griffiths et al., 2018a). Despite these drawbacks, in Chapter 5 we

were able to give a sound interpretation to the changes in the nematode soil food-web indices (Bongiorno et al., 2019a).

- The microbial catabolic profile measured through community level physiological profiling (CLPP) has the advantage of combining both functional diversity and degradation rates (Nannipieri et al., 2003). Functional characterization of microbial communities has the potential to give more meaningful information about soil processes than other microbiological parameters assessed through taxonomy or "black box" approaches (e.g. microbial biomass and soil basal respiration) (Brussaard et al., 2004b; Barrios, 2007). CLPP is an easy and practical way to assess the functionality of the microbial community compared to other methods, and is not prohibitively expensive (Emmerling et al., 2002). Being a sensitive parameter, at least in terms of absolute utilization rate, and directly related with ecosystem processes (i.e. nutrient cycling), the microbial catabolic profile measured with MicroResp™ could be added to soil quality assessment schemes (Chapter 6; Bongiorno et al., submitted). Obviously this will be possible only upon proper interpretation of the results obtained, which is more challenging in terms of relative utilization rate (i.e. role of alpha-ketoglutaric acid utilization in more intensive agricultural systems). A major drawback is that this method selects only parts of the microbial community, i.e. species adapted to rapid growth on simple substrates (Campbell et al., 2003). In addition, the choice of the substrates to use should be carefully done, since in some cases there can be complications and some of the substrates have to be abandoned (as in our case we could not use oxalic acid, for its strict correlation with the pH and most likely abiotic release of CO_2). Another aspect is that the same amount of carbon source is added to the soil, not the same amount of carbon, and that final values are highly dependent on a lab-specific calibration line, making the comparison between the substrate utilization measured in different labs problematic.

I also elucidated what may hamper the use of the novel indicators in soil quality assessment.

First of all, standardization of the methodologies, also in terms of sampling time, should be addressed in order to make comparisons possible (Morvan et al., 2008; Philippot et al., 2012; Griffiths et al., 2018b), with the consensus between different laboratories being the most difficult element (Faber et al., 2013). However, it has to be recognized that standardization is not always possible and that sometimes methods tailored for specific conditions are more effective (Wander et al., 2019).

Second, the interpretation of the values obtained remains challenging, also due to seasonal and spatial variation. This is particularly true for biological indicators which can

be very variable (Debosz et al., 1999). This task is related to data collection and availability, necessary for, e.g., developing thresholds, scoring curves, reference values, benchmarks (Jones et al., 2017; Rutgers et al., 2019), and for making information about the state and the changes of soils more precise (Bone et al., 2014a). Data collection should allow for the local variation of biological functioning as affected by multiple factors such as pedoclimatic zone and land use (Abbott and Manning, 2015). Moreover, data availability can affect the predictive power of models simulating soil processes (Wieder et al., 2015; Orgiazzi and Panagos, 2018) and facilitate the link between parameters and soil functions (Römbke et al., 2016).

Finally, but not less important, it has to be ensured that farmers and land managers will understand, relate and get familiar with the indicators measured in soil quality assessment and their outcomes. In this respect, visual soil quality assessments have the benefit to be closer to farmers and land managers. Translation from indicator measurement to meaning for soil quality could also be mediated by researchers, scientists or extension services. In any case, the measurements have to bear the potential to be translated into suggestions for the implementation of sustainable agricultural practices through policies (Robinson et al., 2017b; Vogel et al., 2018). My selection of indicators seems to be particularly suitable in this respect, as they can be all be well related with soil conditions and ecosystem processes.

7.6 General remarks and suggestions for future research

My approach to the identification and measurement of novel soil quality indicators required simplification of the management practices in the 10 long-term field experiments (LTEs) in broad categories (tillage and organic matter addition). This type of generalization can be important for the development of soil conservation policies (El Mujtar et al., 2019). However, site-specificity is important as well for the measurement of supply and demand of soil functions, as these depend on soil type, land use and specific management (Six et al., 2004; van Capelle et al., 2012; Schulte et al., 2014b; O'Sullivan et al., 2015). There is, in fact, a need for more regional and management-specific soil quality assessments (Wade et al., 2016), which can eventually develop context-specific solutions in agriculture (Plassart et al., 2019; Veen et al., 2019).

In addition, I want to stress 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 pros and cons of the potential indicators is needed. In my opinion, this is especially true for molecular methods (Prosser, 2012; McLaren and Callahan, 2018).

Having said that, the novel soil quality indicators assessed in this thesis offer the potential to be added to existing soil quality assessment schemes because of their sensitivity to management, and linkages with soil processes. A more in depth time and cost analysis should be done to evaluate if also these aspects make them appropriate elements of soil quality assessment schemes. At the moment, the addition of POXC measurement seems the most feasible possibility. However, the cons mentioned in section 7.5 have to be taken into account for effectively adding the novel indicators to soil quality assessments, in particular remarks about the standardization and interpretation of the indicators.

In this respect, I suggest several research opportunities:

- Future studies should focus on i) elucidating which part of the total organic carbon labile fractions are measured, ii) developing a fast and easy way to assess organic carbon quality, iii) elucidating the relationship between labile carbon, different organic carbon compounds and functions. Spectroscopic methods seem very promising in this respect, e.g. mid-infrared photoacoustic spectroscopy (FTIR-PAS) (Chenu et al., 2015), and diffuse reflectance Fourier transformed mid-infrared spectroscopy (DRIFTS); they could also be related to microbiological characteristics (Giacometti et al., 2013).
- Elucidating which methodologies could help, and how, to assess effective indicators of soil disease suppressiveness. In this regard, sequencing, transcriptomics and quantitative PCR techniques are promising (Toyota and Shirai, 2018). However, the link between potential antagonistic activity of the microbial community measured with molecular methods (e.g. presence of antagonistic genes) and the actual soil suppressiveness measured with bioassays needs to be established.
- Validation of the results of food-web indices calculated with sequencing results from free-living nematode community analysis is needed, together with the optimization of databases, pipelines for the method (primer selection, bioinformatics analysis), and eventual standardization of the sequencing results for obtaining corrected relative abundances (Griffiths et al., 2018a).
- Better interpretation and validation of the MicroResp™ results is needed in order to make sure that, especially in terms of relative utilization rate, 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 should also consider other management practices, such as crop rotation, intercropping, cover crops, and also more specific organic matter input practices (e.g. farmyard manure, slurry, compost, biochar etc.). In addition, the effect of soil

texture should be further considered, in order to give more site-specific management recommendations.

- Involvement of different stakeholders in the validation of the applicability and the use of the novel soil quality indicators by farmers and land managers in soil quality assessment could help to render more effective research activities done in the field of soil quality indicators.

7.7 Materials and methods

Statistical analysis

All statistical analyses were performed using R version 3.6.0 (R Development Core Team, 2013). The samples used for all the analysis were from the 0-10 cm layer for samples where tillage was one of the treatment investigated, and the 0-20 cm layer for samples where the only treatment investigated was organic matter addition. The total number of samples used in the analysis was 101. I used only these layers, and not the 10-20 cm layer, because only in the upper layers all the novel indicators were measured, in addition, all the samples from the 0-20 cm layer were exposed to conventional tillage and therefore I did not expect large differences between the 0-10 and 10-20 cm layer in these sites because of the mixing exerted by ploughing (Bongiorno et al., 2019a, b).

Classification random forest 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, RT-High) (Breiman, 2001). Classification random forest 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-third of the observation) from the entire dataset (bagging or bootstrapped aggregation), and at each node of the tree a random subset of variables is selected. The class of a sample will be determined by the majority of the votes of all the trees in the forest. For the random forest model the function *randomForest* from the package *randomForest* was used (Liaw and Wiener, 2002), using 2000 trees and the default value of *m*= (in our case 4). A high number of trees is needed to get a stable estimate of variable importance (Archer and Kimes, 2008). The variable importance measure reported is the mean decrease in model accuracy (%) on the out of bag (OOB) samples (samples that were not selected to be 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 was 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 soils with heavy (clay + silt content < 50 %) and light (clay + silt content > 50 %)

texture. Long-term field experiments (LTEs) CH1, CH2, CH3, SL1 and ES4 were characterised as heavy soils (n=42), while LTEs NL1, NL2, PT1, HU1 and HU4 were characterised as light soils (n= 59). For the RDA, the function *rda* in the *vegan* package was used (Oksanen et al., 2018), with the novel indicators as dependent variables, the soil management as constraining variables, 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. Thereafter, we correlated the soil quality parameters with the first two RDA axes to check their association with the agricultural management. In the RDA graph we reported dependent variables with a correlation with $p \leq 0.001$ with either one of the two RDA axis.

Piecewise structural equation modelling (SEM) was used to evaluate the direct and the indirect effects of permanganate oxidizable carbon (POXC) on various ecosystem services, taking into account the dependent structure of the data coming from the same LTE (Lefcheck, 2016). We established an *a priori* model of the relationship between labile carbon and ecosystem services, where the hypothesised relationships acted as a framework for the optimization of the piecewise SEM. The data matrix was fitted using the log-transformed variables, soil suppressiveness was logit transformed, nematode abundance was squared 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 (Shipley, 2013). 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 (Lefcheck, 2016). We calculated and reported the total standardized effects of the predictors on the soil quality indicators. For the structural equation model the *lavaan* and *piecewiseSEM* package was used (Rosseel, 2012; Lefcheck, 2018), the results were considered statistically significant at $p \leq 0.05$. In this SEM model, I used microbial functional diversity (Shannon functional diversity index, H'), soil respiration and multiple substrate induced respiration (MSIR) as measures of *nutrient cycling*, water stable aggregates as a measure of *erosion control*, C stock (Mg C ha^{-1}) as a measure of *carbon storage and climate regulation*, soil suppressiveness as a measure of *disease regulation/suppressiveness*, microbial biomass carbon, nematode abundance and nematode richness as measures of *biodiversity conservation*, and yield (dry yield from 2016 in ton ha^{-1} measured as part of the iSQAPER MDS, and only for the PT1 trial fresh yield in ton ha^{-1} was used because dry yield was not available, and we used models that took into account the LTE as random factor) as a measure of *biomass production*. We used POXC as labile organic carbon fraction in the model, as it was the most sensitive of the labile fractions, and better correlated with various soil quality parameters. In addition, while

comparing SEM models performed with the other labile carbon fractions, the model with POXC was the best fitting one, although the results of the modelling were similar for all the other labile carbon fractions.

7.8 Conclusions

Assessing biological soil quality indicators is essential to monitor the status and the changes of soil processes as affected by anthropogenic pressure. In this thesis, I demonstrated the potential of different soil parameters, 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. Reduced tillage in particular, and organic matter addition to a lesser extent, affected these different dimensions of soil quality, which helped in the identification of more sustainable agricultural management. Permanganate oxidizable carbon (POXC), and to a lesser extent also the other labile carbon fractions, was found to be a particularly suitable indicator, as apparent from the quantitative analysis of its sensitivity to soil management and of its direct and indirect contributions in sustaining multiple soil ecosystem services. The novel indicators assessed have the benefit of being linked to functionality, and thus are valuable in translating soil quality assessment into agricultural management options. Moreover, in addition to being sensitive indicators for long-term agricultural management effects, as found in my thesis, I speculate that they can serve as sensitive indicators for short-term agricultural management effects as well. My thesis contributes to the development of soil quality assessments, adding information about the suitability of novel techniques to measure soil quality. Future work should focus on the validation and optimization of the indicators studied in this thesis, in order to facilitate their implementation in soil quality assessment, in combination with, or substitution of, traditionally measured soil quality indicators.

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Summary

The measurement and monitoring of the status and the changes in soil properties can indicate the effect of agricultural management on soil quality, defined as the capacity of a soil to perform multiple functions. With advances in knowledge and technological developments in the field of soil biology and soil organic matter, the floor is opening up for the use of novel biological soil quality indicators. My thesis is motivated by the need of assessing the suitability of novel indicators to better understand the impact of agricultural soil management on soil quality in the search for sustainable management practices.

Chapter 1 provides an introduction on soil multifunctionality, the soil quality concept, and soil quality indicators. In addition, I introduce the novel indicators selected for investigation in my thesis: labile carbon fractions, soil disease suppressiveness, soil free-living nematode communities, and microbial catabolic profiles. I highlight their relevance for soil quality assessments by referring to the broad body of literature on the topic, and by showing their conceptual link with multiple soil processes. This selection was based on the outcome of Chapter 2 (Bünemann et al., 2018), a critical review of the soil quality concept, to which I contributed especially with a review novel soil quality indicators.

In the next experimental chapters (3,4,5, and 6; Bongiorno et al., 2019b, c, a; Bongiorno et al., submitted) these soil parameters were screened for their suitability as novel soil quality indicators. This was done by assessing their sensitivity to tillage (conventional *vs* reduced) and organic matter addition (low *vs* high) in ten European long-term field experiments, and by linking them to a range of traditionally measured soil quality indicators, selected for their association with soil processes as the minimum data set (MDS) in the Horizon 2020 project iSQAPER (interactive Soil Quality Assessment in Europe and China for Productivity and Environmental Resilience).

In Chapter 3, five different labile carbon fractions were measured: hydrophilic dissolved organic carbon (Hy-DOC), dissolved organic carbon (DOC), permanganate-oxidizable carbon (POXC), hot water extractable carbon (HWEC), and particulate organic matter carbon (POMC), ordered here from the smallest to the largest proportion of the total organic carbon (Bongiorno et al., 2019b). The labile fractions were increased by reduced tillage and high organic matter addition, even more than total organic carbon. In particular, POXC was found to be one of the most sensitive fractions, together with POMC and HWEC, and to be linked with various existing chemical, physical and biological soil quality indicators. These results showed that POXC, more than other fractions, has the

potential to be a fast, cheap, and meaningful indicator of soil quality, applicable in soil management.

Chapter 4 aims at assessing the soil disease-suppressive capacity of the management systems with a bioassay using Cress-*Pythium* as a model pathosystem, and at elucidating the mechanistic relationship between labile carbon and soil suppressiveness (Bongiorno et al., 2019c). Overall, reduced tillage increased soil disease suppressiveness, but organic matter addition did not have an effect. Microbial biomass carbon was the soil parameter that most explained the variation in soil disease suppressiveness, while the labile carbon had an indirect effect on soil suppressiveness through a positive effect on microbial biomass.

In Chapter 5, I assessed soil free-living nematode communities with sequencing methods, obtaining information about alpha and beta diversity and the total nematode abundance with qPCR (Bongiorno et al., 2019a). Feeding groups relative and total abundances and food-web soil quality indices were also calculated. Reduced tillage increased richness and diversity of nematodes, caused a shift in community structure, and increased maturity, stability, and the fungal-decomposition channel of the food web. Organic matter addition had a weaker effect on nematode communities than tillage and created a more favourable environment for bacterivorous nematodes. Nematode communities were tightly linked with labile organic carbon fractions, available K, and microbial parameters (microbial biomass carbon and soil respiration).

In Chapter 6, the MicroResp™ system was used to measure microbial catabolic profiles in response to adding carbon substrates of different complexity to the soil (Bongiorno et al., submitted). Catabolic profiles were expressed in absolute and relative utilization rate and the Shannon microbial functional diversity index (H') was calculated. Organic matter addition and reduced tillage increased the utilization rate of all the carbon substrates, but only reduced tillage increased the microbial functional diversity. In conventional tillage a higher proportional utilization of alpha-ketoglutaric acid was found, which suggest a more important role of organic acids in more intensive systems. A key direct positive role of labile carbon in sustaining microbial functional diversity was found, which points at the importance of carbon availability for sustaining microbial functionality.

Finally, in Chapter 7, I brought together the results of the four experimental chapters 3, 4, 5 and 6 (Bongiorno et al., in preparation). POXC, and to a less extent the other labile carbon fractions, was found to be the most sensitive indicator of the effects of tillage and organic matter addition. Because of its strong link found with other soil quality parameters, including soil disease suppressiveness, nematode communities, and microbial functional diversity, we modelled the role of POXC (a *proxy* for labile carbon) in sustaining multiple ecosystem services by Structural Equation Modelling (SEM). POXC

was found to have a central role in *nutrient cycling*, *carbon sequestration*, *biodiversity conservation*, *erosion control* and *disease regulation/suppression*. Beside this synergistic effect between ecosystem services, labile organic carbon was found to have an indirect negative link with *biomass production* through soil respiration. This result shows a trade-off between functions sustaining agricultural productivity and environmental resilience.

The novel soil quality indicators were not redundant, and gave different and relevant information about the impact of tillage and organic matter input on soil processes. In particular, POXC has a high potential as a fast, cheap and multifunctional soil quality indicator.

Reduced tillage clearly 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 had a weaker role in sustaining soil quality, possibly due to the different compositions of the organic matter inputs in the different long-term field experiments.

Future studies should focus on understanding which parts of the total carbon are assessed with the different labile fractions, in particular POXC, in order to better establish linkages with soil processes. In addition, the methodologies of measurement of the novel indicators proposed in my thesis will have to be improved and validated to enhance their usefulness in soil quality assessments for agricultural management.

Samenvatting

Het meten en monitoren van de status en de veranderingen in de bodemeigenschappen kan het effect van landbouwbeheer op de bodemkwaliteit aangeven, gedefinieerd als het vermogen van een bodem om meerdere functies te vervullen. Met de vooruitgang in kennis en technologische ontwikkelingen op het gebied van bodembiologie en organische stof in de bodem, opent de vloer zich voor het gebruik van nieuwe biologische bodemkwaliteitsindicatoren. Mijn proefschrift is ingegeven door de noodzaak om de geschiktheid van nieuwe indicatoren te beoordelen om de impact van landbouwkundig bodembeheer op de bodemkwaliteit beter te begrijpen in de zoektocht naar duurzame beheerspraktijken.

Hoofdstuk 1 geeft een inleiding over de multifunctionaliteit van de bodem, het bodemkwaliteitsconcept en de bodemkwaliteitsindicatoren. Daarnaast introduceer ik de nieuwe indicatoren die ik in mijn proefschrift heb geselecteerd voor onderzoek: labiele koolstoffracties, onderdrukking van bodemziekten, bodemlevende nematodegemeenschappen en microbiële katabole profielen. Ik benadruk hun relevantie voor bodemkwaliteitsbeoordelingen door te verwijzen naar de brede literatuur over het onderwerp, en door hun conceptuele link met meerdere bodemprocessen te tonen. Deze selectie was gebaseerd op de resultaten van hoofdstuk 2 (Bünemann et al., 2018), een kritisch overzicht van het bodemkwaliteitsconcept, waaraan ik vooral met een overzicht van nieuwe bodemkwaliteitsindicatoren heb bijgedragen.

In de volgende experimentele hoofdstukken (3,4,5 en 6; Bongiorno et al., 2019b, c, a; Bongiorno et al., ingediend) zijn deze bodemparameters gescreend op hun geschiktheid als nieuwe bodemkwaliteitsindicatoren. Dit is gedaan door hun gevoeligheid voor grondbewerking (conventioneel versus gereduceerd) en toevoeging van organische stof (laag versus hoog) te beoordelen in tien Europese langlopende veldproeven en door deze te koppelen aan een reeks traditioneel gemeten bodemkwaliteitsindicatoren, geselecteerd voor hun associatie met bodemprocessen als minimale dataset (MDS) in het Horizon 2020 project iSQAPER (interactieve Bodemkwaliteitsevaluatie in Europa en China voor Productiviteit en Veerkracht in het Milieu).

In hoofdstuk 3 werden vijf verschillende labiele koolstoffracties gemeten: hydrofiele opgeloste organische koolstof (Hy-DOC), opgeloste organische koolstof (DOC), permanganaat-oxiderende koolstof (POXC), warmwater-extraheerbare koolstof (HWEC), en fijnstofkoolstofkoolstof (POMC), hier geordend van het kleinste tot het grootste deel

van de totale organische koolstof (Bongiorno et al., 2019b). De labiele fracties werden verhoogd door minder grondbewerking en een hoge toevoeging van organische stof, zelfs meer dan de totale organische koolstof. Met name POXC bleek een van de meest gevoelige fracties te zijn, samen met POMC en HWEC, en gekoppeld aan verschillende bestaande chemische, fysische en biologische bodemkwaliteitsindicatoren. Deze resultaten toonden aan dat POXC, meer dan andere fracties, de potentie heeft om een snelle, goedkope en zinvolle indicator van de bodemkwaliteit te zijn, die toepasbaar is in het bodembeheer.

Hoofdstuk 4 richt zich op het beoordelen van het bodemziekte-onderdrukkend vermogen van de beheersystemen met een bioassay met behulp van Cress-Pythium als model-pathosysteem, en op het verhelderen van de mechanistische relatie tussen labiele koolstof en bodemonderdrukkendheid (Bongiorno et al., 2019c). Over het geheel genomen heeft een verminderde grondbewerking geleid tot een toename van de onderdrukking van bodemziekten, maar de toevoeging van organische stof heeft geen effect gehad. Microbiële biomassakoolstof was de bodemparameter die de variatie in de onderdrukking van bodemziekten het meest verklaarde, terwijl de labiele koolstof een indirect effect had op de onderdrukking van bodemziekten door een positief effect op microbiële biomassa.

In hoofdstuk 5 heb ik met behulp van sequencemethoden de bodemlevende nematodegemeenschappen beoordeeld, waarbij ik met Qpcr informatie heb verkregen over de alfa- en bèta-diversiteit en de totale aaltjesdichtheid (Bongiorno et al., 2019a). Ook werden voedergroepen relatieve en totale abundanties en voedselweb bodemkwaliteitsindices berekend. Verminderde grondbewerking verhoogde de rijkdom en diversiteit van de nematoden, veroorzaakte een verschuiving in de structuur van de gemeenschap, en verhoogde de rijpheid, stabiliteit en het schimmelafbraakkanaal van het voedselweb. Toevoeging van organische stof had een zwakker effect op nematodengemeenschappen dan grondbewerking en creëerde een gunstiger klimaat voor bacterie¨le nematoden. Nematodengemeenschappen waren nauw verbonden met labiele organische koolstoffracties, beschikbare K, en microbiële parameters (microbiële biomassakoolstof en bodemademhaling).

In hoofdstuk 6 werd het MicroRespTM -systeem gebruikt om microbiële katabole profielen te meten als reactie op het toevoegen van koolstofsubstraten van verschillende complexiteit aan de bodem (Bongiorno et al., ingediend). De katabole profielen werden uitgedrukt in absolute en relatieve bezettingsgraad en de Shannon microbiële functionele diversiteitsindex (H') werd berekend. Toevoeging van organische stof en minder grondbewerking verhoogden de bezettingsgraad van alle koolstofsubstraten, maar alleen een verminderde grondbewerking verhoogde de microbiële functionele

diversiteit. Bij conventionele grondbewerking werd een hoger proportioneel gebruik van alfa-ketoglutaarzuur gevonden, wat wijst op een belangrijkere rol van organische zuren in meer intensieve systemen. Een belangrijke directe positieve rol van labiele koolstof in het ondersteunen van de microbiële functionele diversiteit werd gevonden, wat wijst op het belang van de beschikbaarheid van koolstof voor het ondersteunen van de microbiële functionaliteit.

Tenslotte heb ik in hoofdstuk 7 de resultaten van de vier experimentele hoofdstukken 3, 4, 5 en 6 samengebracht (Bongiorno et al., in voorbereiding). POXC, en in mindere mate de andere labiele koolstoffracties, bleek de meest gevoelige indicator te zijn voor de effecten van grondbewerking en toevoeging van organische stof. Vanwege het sterke verband met andere bodemkwaliteitsparameters, waaronder de onderdrukking van bodemziekten, nematodengemeenschappen en functionele diversiteit van micro-organismen, hebben we de rol van POXC (een proxy voor labiele koolstof) in het ondersteunen van meerdere ecosysteemdiensten gemodelleerd door middel van Structural Equation Modelling (SEM). POXC bleek een centrale rol te spelen in nutriëntencyclus, koolstofvastlegging, behoud van biodiversiteit, erosiecontrole en ziekte-regulatie/onderdrukking. Naast dit synergetische effect tussen ecosysteemdiensten, bleek labiele organische koolstof een indirect negatief verband te hebben met de productiviteit door middel van bodemademhaling. Dit resultaat toont een afweging tussen functies die de landbouwproductiviteit in stand houden en de veerkracht van het milieu.

De nieuwe bodemkwaliteitsindicatoren waren niet overbodig en gaven andere en relevante informatie over de impact van grondbewerking en organische stofinput op bodemprocessen. Met name POXC heeft een groot potentieel als snelle, goedkope en multifunctionele bodemkwaliteitsindicator.

Minder grondbewerking heeft de beschikbaarheid van koolstof, de onderdrukking van ziekten, de rijkdom en diversiteit van aaltjes, de stabiliteit en de rijpheid van het voedselweb, en de microbiële activiteit en functionele diversiteit duidelijk doen toenemen. De toevoeging van organische stof heeft een zwakkere rol gespeeld bij het handhaven van de bodemkwaliteit, mogelijk als gevolg van de verschillende samenstellingen van de organische stofinputs in de verschillende langlopende veldproeven.

Toekomstige studies zouden zich moeten richten op het begrijpen welke delen van de totale koolstof worden beoordeeld met de verschillende labiele fracties, in het bijzonder POXC, om zo beter een verband te leggen met bodemprocessen. Bovendien zullen de meetmethodes van de nieuwe indicatoren die in mijn proefschrift worden voorgesteld, moeten worden verbeterd en gevalideerd om hun bruikbaarheid bij de beoordeling van de bodemkwaliteit voor landbouwbeheer te vergroten.

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
- Soil quality: a critical review (2018)

Writing of project proposal (4.5 ECTS)
- Indicators of soil quality for agricultural management

Post-graduate courses (8.4 ECTS)
- Soil ecology; PE&RC (2016)
- Soil fauna and SOM dynamics; Cost action (2016)
- Basic statistics; PE&RC (2016)
- Introduction to R for statistics; PE&RC (2016)
- Linear models; PE&RC (2017)
- Generalized linear models; PE&RC (2017)
- Mixed linear models; PE&RC (2017)
- Microbiota data analysis workshop; Genetic Diversity Centre, Zürich (2019)

Laboratory training and working visits (0.4 ECTS)
- Ergosterol extraction; NIOO, Wageningen (2017)
- DNA quantification; Genetic Diversity Centre, Zürich (2018)

Invited review of (unpublished) journal manuscript (8 ECTS)
- Nematology: labile carbon and nematode communities (2016)
- AMBIO: sol quality assessments (2016)

- Land degradation and development: effect of continues cropping on chemical properties and bacteria community structure (2017)
- Science of the total environment: long-term effect of nitrogen and sulphur on bacterial communities (2017)
- Applied soil ecology: soil biological indicators (2018)
- Geoderma: cover crops effect on soil quality (2019)
- Organic agriculture: soil organic amendment effect on soil properties (2019)
- Geoderma: soil quality assessments (2019)

Competence strengthening / skills courses (3.9 ECTS)
- The essential of scientific writing and presenting; WGS (2015)
- Reviewing a scientific paper; WGS (2016)
- Information literacy including Endnote; Wageningen Library (2016)
- Scientific writing; WGS (2017)
- Writing scientific proposals; WGS (2019)

PE&RC Annual meetings, seminars and the PE&RC weekend (3.85 ECTS)
- PE&RC First year weekend (2016)
- Workshop plant-soil-microbe relations (2016)
- Tipping points in pest management (2016)
- PE&RC Days (2016-2017)
- PhD Carousel (2017)
- PhD Symposium (2017)
- Frontiers in soil ecology: soil-plant interphase (2017)

Discussion groups / local seminars / other scientific meetings (10 ECTS)
- Soil pathogens and soil microorganisms (2015-2017)
- Mycorrhiza seminar (2016)
- Seminar Cornell University test (2016)
- Seminar Biolog (2016)
- Soil plant relation (2016-2017)
- Mycology seminar Zürich (2017)
- MolDiversity seminar Zürich (2018)
- MolBio seminar FIBL (2018-2019)

International symposia, workshops and conferences (9.7 ECTS)
- SomMic- Soil microbial community and SOM dynamics; Leipzig, Germany (2016)
- European Union (EGU); Vienna, Austria (2017)
- Soil conference; Wageningen, the Netherlands (2017)
- Bonares conference; Berlin, Germany (2018)
- Soil conference; Wageningen, the Netherlands (2019)
- SoilMan conference; Braunschweig, Germany (2019)

Societally relevant exposure (1.6 ECTS)
- Infographics and videos (2016-2019)
- Article in Dutch farmers' website: Beter Bodembeheer (2019)

Lecturing / supervision of practicals / tutorials (5.85 ECTS)
- Nematology (2016, 2017)
- Global guest program WUR (2017)
- Ecological aspect of bio-interaction (2017)
- Training course on soil quality indicators (2018-2019)
- Presentation Basel Blokkurs (2018-2019)
- Field visit in the DOK experiment (2019)
- Workshop BIOSIS (2019)

Supervision of MSc students (12 ECTS)
- Labile carbon and microbial functional diversity
- Enzymes and microbial community structure assessed with PLFA
- Microbial parameters studied with molecular methods (qPCR and sequencing of mycorrhizal communities)

Curriculum Vitae

Giulia Bongiorno was born on the 29th of April 1990, and grew up, in Milan, Italy. After obtaining a diploma in humanities she decided to start a bachelor's degree in Plant Production and Protection at the Agricultural faculty of the University of Milan. She completed her bachelor's degree in 2012, and in 2013, ready to explore the world, she moved to The Netherlands where she enrolled at Wageningen University in the Plant Science master's program (Plant pathology end Enthomology). During the two years of her MSc Giulia started to get very interested in sustainable agriculture and the role of soil biota in its development. In 2015 she completed her MSc with a thesis in the Nematology department, regarding the effect of organic and conventional agriculture on soil microorganisms and free-living nematodes. This work made her more interested in monitoring and counteracting the negative effects of intensive agricultural practices on the soil biota. For this reason, Giulia continued working on this subject during her PhD, which was a collaboration between the Soil Biology group at Wageningen University and the Soil Sciences department at FIBL (Research Institute of Organic Agriculture) in Switzerland. Her PhD research was conducted as part of the project iSQAPER (Interactive Soil Quality Assessment in Europe and China for Productivity and Environmental Resilience), funded by the Horizon 2020 program of the European Commission. For the first two years of her PhD Giulia worked in Wageningen, and in 2017 she moved to Switzerland where she completed her doctoral research. Her work during these years ignited her interest in the role of agriculture in the whole economic and societal context. Giulia is constantly looking for her role in society and wants to contribute to a world where resources are shared and taken care of.

Publications

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Wade, J., Maltais-Landry, G., Lucas, D.E., **Bongiorno, G.**, Bowles, T.M., Calderón, F.J., Culman, S., Daughtridge, R., Ernakovich, J.G., Fonte, F., Giang, D., Herman, B.L., Guan, L., Jastrow, J., Loh, B.H.H., Kelly, C., Mann, M.E., Matamala, R., Miernicki, E.A., Peterson, B., Pulleman, M., Scow, K.M., Snapp, S, Thomas, V., Tu, X., Wang, D., Jelinski, N.A., Liles, G.C., Barrios-Masias, F., Rippner, D.A., Silveira, M., Margenot, A.J. 2020. Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: an interlab comparison across soils. *Geoderma*. Accepted.

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