



The fate of biological soil quality
functional diversity and realised niche width

Carmen Vázquez Martín

Propositions

1. Community level specialisation of soil organisms does not respond to disturbance in the same manner as that of above-ground organisms.

(this thesis)

2. Without governmental intervention, soil biodiversity will continue to decline in favour of primary productivity.

(this thesis)

3. The role of science in achieving environmental sustainability is smaller than that of society.

4. Research should be overseen by statisticians.

5. Teaching is the most effective way of affecting change.

6. The term expat is used to classify migrants according to socioeconomic status.

Propositions belonging to the thesis, entitled

The fate of biological soil quality: functional diversity and realised niche width

Carmen Vazquez Martin

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**The fate of biological soil quality:
functional diversity and realised niche width**

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The fate of biological soil quality: functional diversity and realised niche width

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Chapter 1

General introduction

C. Vazquez

1.1. Soil multifunctionality

It is thanks to soils, and the processes that take place within the soil matrix that life can take root on the earth's surface. Soils hold the water and nutrients that plants then use to grow, and we in turn harvest. But the benefits that we derive from the soil, and the attributes and processes through which the soil system can sustain a range of environmental functions (**soil-based ecosystem services or soil functions**) go much further than just the delivery of food and fibre (Dominati *et al.* 2010). Agricultural soils in particular deliver five main soil functions: primary productivity, water regulation and purification, climate regulation and carbon sequestration, provision and cycling of nutrients and biodiversity and habitat provision (Schulte *et al.* 2015) (Box 1.1).

Box 1.1. Definitions of the five main agricultural soil functions

Function	Definition
Primary productivity	The capacity of a soil to produce plant biomass for human use, providing food, feed, fibre and fuel within natural or managed ecosystem boundaries (Sandén <i>et al.</i> 2019)
Water regulation and purification	The capacity of a soil to remove harmful compounds from the water that it holds and to receive, store and conduct water for subsequent use and the prevention of both prolonged droughts and flooding and erosion.
Climate regulation and carbon sequestration	The capacity of a soil to reduce the negative impact of increased greenhouse gas (i.e., CO ₂ , CH ₄ , and N ₂ O) emissions on climate
Provision and cycling of nutrients	The capacity of a soil to receive nutrients in the form of by-products, to provide nutrients from intrinsic resources or to support the acquisition of nutrients from air or water, and to effectively carry over these nutrients into harvested crops (Schulte <i>et al.</i> 2015; Schröder <i>et al.</i> 2016).
Biodiversity and habitat provision	The multitude of soil organisms and processes, interacting in an ecosystem, making up a significant part of the soil's natural capital, providing society with a wide range of cultural services and unknown services (van Leeuwen <i>et al.</i> 2019).

Traditionally soil quality was seen as a reflection of the soil's capacity to produce food, fuel and fibre. In more recent schemes, however, soil quality has been defined as the capacity of a soil to function (Bünemann *et al.* 2018). In this way, low soil quality occurs when a soil functions sub-optimally, not just in the delivery of one, but several functions

and high soil quality occurs when a soil functions in such a way that it can match demands of several soil functions (Kibblewhite *et al.* 2008). The idea that soils can perform several functions simultaneously is referred to as **multifunctionality**. The number and identity of the soil functions that a soil is expected to deliver should depend on the soil properties, the environmental conditions and the associated soil management (Schulte *et al.* 2015).

1.2. Synergies and trade-offs

Soil functions do not occur in isolation to one another and processes that determine one can in turn affect another. To increase primary productivity in agricultural soils farmers and governments have relied on external inputs (e.g. fertilizers and pesticides), intensive crop rotations and management practices that are disruptive to the soil system (e.g. tillage, heavy machinery) (Emmerson *et al.* 2016). To put this in terms of soil functionality: the use of fertilizers benefits primary productivity, but has direct negative impacts on climate regulation, due to associated emissions of greenhouse gasses, and biodiversity and habitat provision due to acidification of the soil that renders the soil uninhabitable for some organisms (Tsiafouli *et al.*, 2015). Yet, the relationship between all soil functions is not fully understood, in part due to the earlier focus on primary productivity, and in part due to the difficulty of measuring several functions simultaneously (Schulte *et al.* 2014).

If we want to simultaneously meet agronomic and environmental objectives it is important that we understand the **trade-offs** and **synergies** that occur between soil functions, as well as what management decisions and environmental conditions promote multifunctionality.

1.3. The role of soil biota on soil functioning

Soils develop from different materials, under different climatic and physical conditions, creating numerous opportunities for niche partitioning and habitat specialization. This is one of the reasons why soil ecosystems are amongst the most biologically diverse in the planet (Orgiazzi *et al.* 2016a; Bardgett 2002). **Soil biota** (the organisms living all or part of their lives in the soil) are fundamental to the functioning of the soil ecosystem and the delivery of ecosystem services (Bardgett and van der Putten 2014; Ferris and Tuomisto 2015; Haygarth and Ritz 2009; Kibblewhite *et al.* 2008). Soil biota can be classified according to their role in soil functioning as: decomposers, nutrient transformers, ecosystem engineers and bio-controllers (Kibblewhite *et al.* 2008). Microbes are the most important **decomposers** and **nutrient transformers** in the soil,

making nutrients available to plants (Pulleman *et al.* 2012). Earthworms, enchytraeids and microbes, for example, can all act as **ecosystem engineers** by restructuring soil material, which directly affects erosion control and water quality and supply and improves the accessibility of other organisms to food resources. Soil structure can also affect the climate regulation capacity of soils (Porre *et al.* 2016; Pulleman *et al.* 2012). **Bio-controllers** can affect the delivery of ecosystem services by for example grazing on bacteria, or plant parasitic organisms (Anderson *et al.* 1981; Chen and Ferris 1999). Changes to the soil food-web (Figure 1.1) induced by grazing or by changes to the environment, can add up and have an impact on all soil functions (Bardgett and van der Putten 2014, Haygarth and Ritz 2009; Pulleman *et al.* 2012). There is, however still a lot to discover regarding the ecological roles of many soil animals (Briones 2014).

1.4. Human impacts on soil biota

The intensification of agricultural land is one of the largest threats to soil biodiversity in Europe (Turbé *et al.* 2010; Orgiazzi *et al.* 2016a). The impacts related to agricultural intensification take many shapes, and can therefore lead to many diverse effects on the soil biota. Some impacts, like the use of pesticides, lead to mortality of specific soil groups (Korthals *et al.* 1996) while others, like the addition of manure, lead to a change in the energy flows within the soil community by disproportionately and positively affecting some groups while having very little impact on others (Ettema and Bongers 1993).

Species develop a degree of tolerance to different environmental and biological constraints that translate into species specific **niche widths** (Futuyma and Moreno 1988; Devictor *et al.* 2010). In this thesis we follow the ecological niche definition of Hutchinson (1957), who defined niche as the space in the multi-dimensional set of ecological variables within which a species (or taxon) can maintain a viable population. Above ground, species with a narrow niche width (**habitat specialists**) are more likely to disappear from a habitat that has gone through changes than those with a wider niche width (**habitat generalists**) (Clavel *et al.* 2011). In fact, the loss of specialist species as a consequence of land use intensity has been documented for many above ground organisms, where human impact and agricultural intensification were correlated with a loss of specialist species (Fried *et al.* 2010; Clavel *et al.* 2011). The loss of specialists is a problematic issue, since it leads to the loss of functional diversity (Clavel *et al.* 2011; Ibarra and Martin 2015), which could translate to a loss of ecosystem services being delivered. Human activity affects the habitats of soil organisms: the use of heavy machinery, for example, can cause compaction, which translates into a decrease in the pore space that actively excludes larger organisms from these soils. Some species have

very specific requirements in terms of the soil pH, and changes following the repeated application of inorganic fertilizers (for example) can over time render the soil uninhabitable for such a species (Chen *et al.* 2015; Graefe and Beylich 2003). And yet, we do not know whether the impacts associated with agricultural practices have a larger effect on soil specialists than generalists, amongst others because the quantification of niche width in soil organisms is very complex (Bardgett 2002).

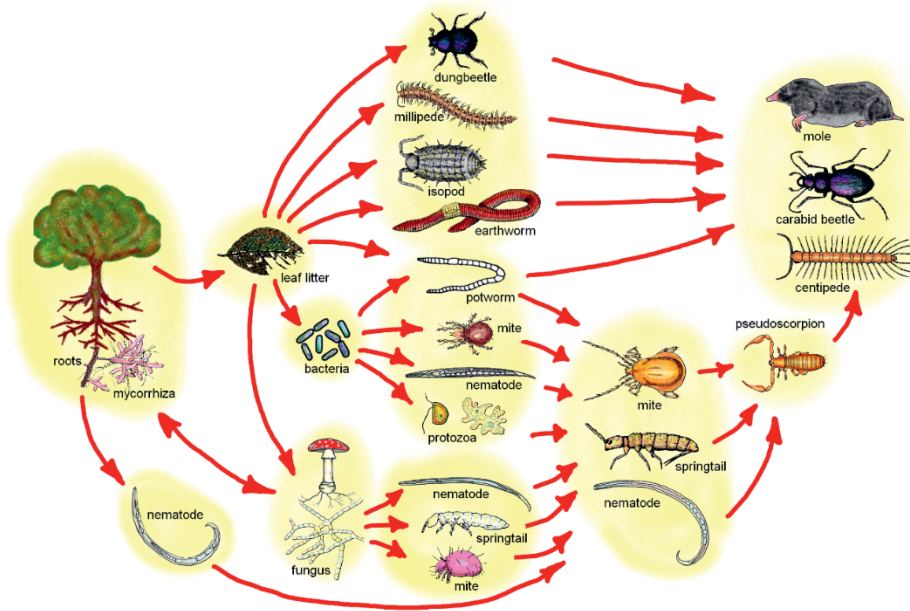


Figure 1.1. The Soil Food Web. Simplified scheme of the energy flows within the soil community from the first trophic level (with plants as photosynthesizers) to higher level predators such as centipedes and moles. Image courtesy of R.G.M de Goede.

1.5 From community structure to indicator

By tracking the soil's community, scientists can derive a lot of information about the type of disturbances and limitations that are being imposed on the soil community (Ferris *et al.* 2001; Pelosi and Römcke 2016; Pulleman *et al.* 2012; Rutgers *et al.* 2009). Nematode community composition, for example, has often been used as an indicator of biological soil quality (Bongers and Ferris 1999). There are several reasons behind this: nematodes can be classified according to their position across a colonizer-persister (CP) scale (Bongers 1990, 1999), a classification that can be used to track the maturity of a nematodes community, and the degree of disturbance suffered by the community. Additionally, nematodes are present at diverse trophic levels of the soil

food web (Figure 1.1), and tracking changes in the abundance of the different trophic groups, together with changes in the maturity of the community can help reveal aspects of soil functioning (Ferris *et al.* 2001).

At one end of the scale are the colonizers, nematodes that grow fast and reproduce in high numbers. At the other end of the scale are the persisters, which are slow growing nematodes, more likely to have fewer and larger offspring (Pianka 1970). Using this scale one can track the successional stage of a soil: a young soil is more likely to be occupied by colonizers, and as the soil matures, the food-web becomes more complex and can support nematodes that are more sensitive to disturbances or depend on less readily available food sources. Disturbance can lead the soil community to one that resembles an earlier successional stage (Bongers 1990, 1999). But the potential of soil organisms as indicators of soil function is not limited to nematodes. Many other soil groups are sensitive to human disturbance (e.g., earthworms, enchytraeids, microarthropods, microbes) and have important roles in soil functioning as well as key positions in the soil food-web indicating a high potential as indicator organisms. There is, however, still much that we do not know about their ecology. Due to the vast diversity of soil organisms and the variety and complexity of the soil system, understanding the biology and ecology of soil organisms is a complicated endeavour (Bardgett 2002). Finding cost-effective ways to derive information about the ecology of soil organisms could be of great benefit to the study of soil ecology. A way forward in this regard is be the study of already existing datasets coming from soil biological surveys and experimental sites, using methods of analysis that have yet to be applied to the analyses of soil communities, such as for example the calculation of niche width or the study of the community's nestedness (Fridley *et al.* 2007; Staniczenko *et al.* 2013; Strona *et al.* 2014).

1.6. Research objectives of this thesis

The main aim of this thesis is to study the effects of land use on different aspects of the soil system, particularly the effects on soil biodiversity. More specifically, I:

- a) calculate the habitat niche width of soil organisms and classify them from specialists to generalists,
- b) study the effect of land use intensity on the community weighted specialisation of soil organisms,
- c) study the effects of long-term agricultural practices on biological soil quality, and
- d) study the multifunctionality of agricultural soils, and the synergies and trade-offs that occur between biodiversity, primary productivity and nutrient cycling.

To complete objectives (a) and (b) I used a dataset gathered by the Netherlands Institute for Public Health and the Environment (RIVM) as part of an effort to monitor soil quality and health across different systems in the Netherlands (Rutgers *et al.* 2009) to calculate the realised niche widths of soil nematodes, followed by the calculation of the community level specialisation of the nematode community. Additionally, using data gathered across Europe by the EcoFinders project (EU Seventh Framework Programme funded) (Stone *et al.* 2016), I calculated the realised niche widths of soil enchytraeids, and subsequently calculated the community level specialisation of the Enchytraeid community. My initial hypothesis was that like above ground, we would observe a decrease in the abundance of specialist species with an increase in land use intensity.

In order to assess the effects of long-term farming on the soil community (objective c)) I carried out a sampling campaign in 2018 that included farms that had previously been visited by Rutgers *et al.* (2009), as well as two experimental farms, where different management practices had been tested for close to a decade (Hoek *et al.* 2019; Korthals *et al.* 2014). I hypothesized that over time I would observe a decrease of biological soil quality of conventional agricultural systems, but this trend would either not exist or be flipped in organic systems. Lastly, in order to calculate soil functions (objective d)), I complemented the data obtained from the RIVM with economic data from the same farms gathered by Wageningen Economic Research in order to calculate the performance of several soil functions. I hypothesized that while agricultural soils would deliver more than one function simultaneously, I would observe a trade-off between biodiversity and primary productivity.

1.7. Outline of this thesis

This thesis contains 6 chapters: In Chapter 1, I have provided a short introduction to the topics tackled in this thesis; chapters 2-5 present the results of the analyses performed; and Chapter 6 presents a general discussion of the results obtained. Chapters 2 and 3 present the relative niche width of nematode genera and enchytraeid species respectively, as well as an analysis of the community level specialisation of each nematodes and enchytraeids in relation to land use intensity. I then (Chapter 4) explore the long-term evolution of biological soil quality in arable soils over time under different agricultural management, particularly I studied the differences between organic and conventional systems, as well as the long-term effects of different tillage practices. In Chapter 5 I calculate soil multifunctionality in agricultural land in the Netherlands, explore the synergies and trade-offs between soil functions and explore the

management decisions that promote multifunctionality. Finally, in chapter 6 I summarize and discuss the results of the thesis.

Chapter 2

The effects of increasing land use intensity on soil nematodes: A turn towards specialism

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Abstract

The ecosystem services that humans obtain from the soil are strongly linked to the soil's biota. There is ample evidence that intensive agriculture has a negative effect on the soil's biological diversity. While in other ecosystems habitat specialists are at a higher risk of extinction due to human impacts than generalists, we have no evidence of whether this holds true for soil biota. We calculated the realised niche width for soil nematodes using co-occurrence data. We compared these with ecological traits. We then calculated an index of community specialisation and tested whether land use intensity leads to decreases in the index of community specialisation, taxon richness, diversity, and to changes in nematode abundance.

The resulting realised niche widths did not correlate with ecological traits such as feeding group, body mass or c-p class. While it is possible that there are no relationships between these traits and the realised niche width, it is likelier that food availability, pH tolerance, or host breadth are more important factors in explaining niche width.

Contrary to our expectations, the lowest community specialisation levels were found in soils with the lowest human intervention (shrubland-woodland ecosystems), while grasslands, dairy farms, and arable farms had an overall higher level of specialisation. Weather variables and land use intensity explained 66% of the variation in the index of community specialisation in sandy soils. We found highest richness and diversity at intermediate levels of disturbance (grasslands and dairy farms). The lowest abundances were found on shrubland-woodland systems. Dairy farms on sand and clay had similar indices of community specialisation, whereas peaty soils fostered a higher proportion of habitat specialists.

We argue that farmland supposes a stable environment for organisms with shorter lifespans. Agricultural management strives to lower disturbances, allowing shorter lived organisms to escape pressures otherwise present in nature, such as drought or nutrient deficiencies during the growing season. In very disturbed systems, however specialists may also suffer from negative effects of land use intensity.

This co-occurrence method to assess niche width opens the door to estimating the soil community's niche breadth, for which resource-based methods are difficult to implement.

Keywords: effects of land use intensity, Fridley's co-occurrence method, generalist species, index of community specialisation, nematode community, realised niche width, specialist species.

2.1 Introduction

Humans derive multiple benefits from the soil system (Sarukhan et al. 2005). The delivery of these 'ecosystem services' depends on a number of soil processes driven by different groups of soil dwelling fauna (Kibblewhite et al. 2008; Ferris and Tuomisto 2015; Haygarth and Ritz 2009). Soil biota act as decomposers, nutrient transformers, ecosystem engineers and bio-controllers (Kibblewhite et al. 2008). For example, earthworms, enchytraeids and fungi can act as ecosystem engineers (functional group) by restructuring soil material (ecosystem process) which in turn affects erosion, water quality and water supply (ecosystem services) (Pulleman et al. 2012). Microbes are also important nutrient cyclers and decomposers, and changes in the microbial community can lead to reduced decomposition rates, affecting the provision of food, fibre and water, as well as the capacity of the soil to reduce pollutant concentrations (Bardgett and van der Putten 2014). The soil food web drives these nutrient transformations, but can also be impacted by (human induced) changes in soil quality and resilience, which in turn can reduce the capacity of soil processes, functions and ecosystem service delivery (Haygarth and Ritz 2009; Wagg et al. 2014).

There is little question that a human disturbance affects soil biota. Management practices, chemical stresses (pollution), and soil compaction are some of the most pertinent threats to soil biota (Orgiazzi et al. 2016a; Turbé et al. 2010). Land use change has also been documented to impact the diversity of predatory mites, nematodes, earthworms and enchytraeids (Postma-Blaauw et al. 2010). Crop rotation and cover crops affect microbial composition (Pankhurst et al. 1995). The application of pesticides and pollutants lead to decreases in the abundance of earthworms, isopods, enchytraeids and collembolans (Bardgett et al. 1994; Blakely et al. 2002; Yeates et al. 1994; Paoletti and Hassall 1999; Korthals et al. 1996). Physical impacts such as tillage and compaction affect root pathogenic fungi, protozoa, collembola and earthworm communities (Chan 2001; van Capelle et al. 2012), and this sequence is not at all exhaustive. Across Europe, agricultural intensification has led to a decrease in functional diversity, a shorter food-web and a lower community weighted biomass (Tsiafouli et al. 2015).

Species with a narrow niche width (habitat specialists), are often more vulnerable to disturbance, and more likely to be endangered than those that can inhabit a broader range of habitats (habitat generalists) (Clavel et al. 2011; Ducatez et al. 2014). Population decreases in habitat specialists have been observed in birds (Devictor et al. 2008; Ibarra and Martin 2015); carabid beetles (Kotze and O'Hara 2003); bees, Heteroptera, spider communities (Dormann et al. 2007); and even agricultural weeds (Fried et al. 2010), all related to an increase in human disturbances. While there have been many attempts to quantify the effect of land use change and land use intensity (LUI) on the diversity of

soil biota, none, have distinguished these effects on the proportion of generalists and specialists, most probably due to the difficulty in establishing a soil organisms' niche width (Bardgett 2002).

Traditional methods of calculating a species fundamental niche width (the set of environmental conditions a species can potentially live and reproduce in) require measuring the “variance in performance measured in common garden or controlled experiments” (Futuyma and Moreno 1988; Venail *et al.* 2008). There are two reasons why this approach is not appropriate for soil biota: firstly, due to the high physical and chemical heterogeneity and microclimatic characteristics at small scales in the soil, which result in a myriad of niches (Bardgett 2002; Ettema and Wardle 2002); and secondly more than 5000 species (belonging to more than 770 genera) of soil and fresh water dwelling nematodes have been described worldwide (Andrassy 1992). Selecting and manipulating the soil variables that limit species occurrence and setting up individual controlled experiments would become a daunting (time and resource consuming) task. There are examples, however, of classifications of soil organisms according to traits that are often associated with niche widths. Nematode families, for example, have been classified into the c-p scale, from colonizers to persisters (Bongers 1990). Bongers (1990) considered nematodes that rapidly increase in number under favourable conditions, with a short-life cycle, high colonization ability and a high tolerance to disturbance as colonizers. At the other end of the spectrum are the 'persisters', nematodes with a low reproduction rate, a long life-cycle, a low colonization ability and sensitive to disturbance. This classification has served as a starting point to calculate diverse ecological indices to assess, for example, the successional stage, disturbance level, or nutrient status of the soil (Bongers 1990; Ferris *et al.* 2001). Colonizers fit the typical description of generalists, while persisters are better associated with specialism. There is, however, a lack of consensus regarding the directionality of the relationship between biological traits and specialisation (Büchi and Vuilleumier 2016). This classification into the c-p scale might not be representative of the niche width of the different nematode families, since other factors (such as pH tolerance, host breadth, resting phase) might also limit niche width.

A different method of estimating niche width is calculating the realised niche width (RNW; the set of conditions a species occupies) rather than the fundamental niche width, using diversity metrics or multivariate techniques (Futuyma and Moreno 1988; Devictor *et al.* 2010). This approach is not biased by the choice of measured variables or the availability and ease of collection of environmental data (Fridley *et al.* 2007), problems that are often found when using resource-based methods (see Gaston *et al.* (1997) for a review). One such approach uses large scale co-occurrence matrices under

the assumption that extreme specialist species will occur always in the company of the same species, while extreme generalists will occur in very different communities (Fridley *et al.* 2007; Zelený 2009; Manthey and Fridley 2009). Co-occurrence, resource and trait-based methods, can lead to similar niche width estimates, but deviations can occur when one species is limited by a resource that is not limiting to others (Pannek *et al.* 2016; Carboni *et al.* 2016).

While co-occurrence methods provide no direct insight into the underlying mechanisms that determine the target species' niche width, they offer the opportunity to study the effects of LUI on communities' overall specialism by calculating an index of community specialisation (I_{CS}) which can be used as an indicator of agricultural intensification (Fried *et al.* 2010). In this paper we calculated the RNW of different nematode taxa using data gathered in multiple habitats in the Netherlands. We explored its relationship with the aforementioned c-p scale, as well as other functional traits, in order to understand what determines nematode niche width. We then studied nematode richness, abundance, diversity, and the I_{CS} in different habitats in the Netherlands. Finally, we assess the effects of LUI on these four indices and hypothesize that with increasing LUI, there will be a decrease in the I_{CS} , diversity and richness.

2.2 Methods

2.2.1 Dataset

Most of the data used for this study was gathered as part of the Netherlands Soil Monitoring Network (NSMN (Rutgers *et al.* 2009); Table 2.1). This monitoring network incorporated abiotic and biotic measurements taken in sites representative of the more common land use/soil texture categories in the Netherlands (Rutgers *et al.* 2008; Rutgers *et al.* 2009). Due to the overwhelming number of samples belonging to dairy farms on sand (115 out of the NSMN 458 sites), and to prevent bias in the niche width calculation deriving from an uneven site selection (Fridley *et al.* 2007), the dataset was complemented with data (195 sites) from survey studies carried out in the Netherlands in different habitats (Table 2.1). For sites with several replicates, only one was chosen at random to be part of the dataset. Soil samples were taken from the top 10-20 cm, and nematode extraction was done using an Oosterbrink elutriator. Further information on the sampling procedures can be found in the literature cited on Table 2.1.

Each sample was categorized according to its land use and soil texture category, resulting in a combination of arable farms, organic and conventional dairy farms, horticulture, city parks, extensively managed grasslands, heathland, dune systems, and coniferous and deciduous forests in a combination of loess, sandy, clayey, loamy, and

Table 2.1. Land use type, soil texture and number of independent sites used to calculate nematodes' realised niche width (preliminary set), to study the variables that affect the nematode's index of community specialisation (test set), and related references.

Soil type	Land use	Sites in preliminary set	Sites in test set	Reference
Clay	Arable farm (conventional)	10	37	Rutgers et al. 2009
	City park	1		Rutgers et al. 2009
	Dairy farm conventional	10	49	Bongers et al. 1989;
	organic	10		Rutgers et al. 2009;
	Forest	2		Keidel, 1998
	Horticulture	10		Bongers et al. 1989;
	Semi-natural grassland	2		Rutgers et al. 2009
Loam	Forest coniferous	1	23	Bongers et al. 1989;
	deciduous	10		de Goede and Bongers 1994;
				Rutgers et al. 2009
Loess	Dairy farm conventional	7		Rutgers et al. 2009
	organic	4		
Peat	Arable farm (conventional)	6		Rutgers et al. 2009
	Dairy farm conventional	10	28	Rutgers et al. 2009
	organic	10		
	Horticulture	1		Rutgers et al. 2009
	Semi-natural grassland	10		Rutgers et al. 2009
Sand	Arable farm conventional	10	14	Rutgers et al. 2009
	organic	10		
	City park	10		Rutgers et al. 2009
	Dairy farm conventional	10	89	Bongers et al. 1989;
	organic	10	12	Rutgers et al. 2009
	Dune systems coastal	10	6	de Goede et al. 1993;
	inland	5		Bongers et al. 1989;
	Forest coniferous	10	38	Verschoor et al. 1998
	deciduous	10	81	Bongers et al. 1989;
	Heathland	10	16	de Goede and Bongers 1994;
	Horticulture	10		Rutgers et al. 2009
	Semi-natural grassland	10	33	Rutgers et al. 2009
				de Goede and Ogg, 1998;
				Keidel, 1998;
				Rutgers et al. 2009
Total :		229	426	

peaty soils (Table 2.1). We split the dataset (655 sites) into two sets, one was used to calculate the RNW of the target nematode taxa (preliminary set; 229 sites), and the other to test the hypotheses (test set; 426 sites, of which different subsets were selected for further analysis depending on the question at hand). Site selection for the preliminary set is further discussed in section 2.2.4 of the methods.

2.2.2 Weather data

Soil biota goes through seasonal changes in abundance and composition. In arable and grass fields, microbial and nematode biomasses are highest in the early summer (Buchanan and King 1992), and lowest in the winter (Bardgett *et al.* 1997; Bardgett *et al.* 1999). Water content in the soil can affect nutrient availability, and strong rainfall can lead to nutrient losses through leaching (Bhadoria *et al.* 1991; Kuchenbuch *et al.* 1986). The effects of temperature and rainfall on the soil's condition may further depend on soil texture (Bhadoria *et al.* 1991) or the presence of vegetation (Green *et al.* 1984).

To account for differences in sampling season and year, each data point was complemented with information on the long-term (three months) and short-term (a week) weather prior to sampling. Average daily temperature (°C), number of freezing days and cumulative precipitation (mm) for the two periods of interest were extracted from the Royal Netherlands Meteorological Institute (KNMI) from the closest available weather station. Due to strong collinearity between short- and long-term weather, only long-term average daily temperature, long-term cumulative precipitation, and log-transformed short-term cumulative precipitation were used as explanatory variables in the models (Eq. 2 and Eq. 3). For five of the sites in the test set, exact coordinates were not available, so rather than local weather data, we used national averages.

2.2.3 Nematode data

Nematode taxa were classified according to Bongers (1988). Identification was not always possible beyond the family level. If members of a specific family were rarely identified to genus level, further calculations were done at family level (this was the case for Criconematidae, Dolichodoridae, Neodiplogasteridae, Qudsianematidae, Rhabditidae, Thornenematidae and Trichodoridae). If, however, members of a family had been identified to genus level more often than to family level, the nematodes identified into family level were allocated to the genera (within said family) present in the same site. Calculations were done at genus level. This was done to prevent an overestimation of taxon diversity. *Prodorylaimus* and *Mesodorylaimus* were grouped prior to analysis. Dauer larvae were analysed as a separate taxon, since they represent a common response to a stressor or environmental cue.

Nematode taxa were assigned a c-p value (Bongers and Bongers 1998), feeding group (Yeates *et al.* 1993), functional guild (Ferris *et al.* 2001), metabolic footprint (Ferris 2010b), which were extracted from Nemaplex (Ferris (1999); last accessed November 2018). For average body mass values, we used values reported by Mulder and Vonk (2011), which include the weight of males, females and juveniles extracted from soils belonging to the NSMN. The averages reported by Ferris (2010b) are unlikely to be representative of our sample, since they are based on average female weights (which in the case of endoparasitic nematodes cannot be extracted following the procedures in the present work) and have recently been reported to grossly overestimate the average size of nematodes extracted from the soil (Zhao *et al.* 2019)). *Filenchus*, *Aphelenchoides* and *Ditylenchus* were classified as fungus feeders. Body mass was log-transformed prior to analysis.

2.2.4 Realised niche width

To quantify the nematodes' RNW, we used the protocol developed by Fridley *et al.* (2007) with some adjustments. Our data selection for the preliminary set is a fair representation of Dutch habitats. The Netherlands uses up to 60% of its land for agriculture, and only slightly above 12% of the country is considered to be woodland or nature (CBS 2016). To prevent bias towards one or another habitat (Fridley *et al.* 2007) the preliminary set was made out of no more than 10 sites per land use/soil texture category (Table S1; resulting in a total of 229 sites), under the assumption that different soil textures and land use types and management styles (organic vs. conventional) provide distinct habitats for soil life (Freckman and Ettema 1993; Jiao *et al.* 2015; de Goede and Bongers 1994; Quist 2017; Quist *et al.* 2016).

This protocol is known to be biased when the community is or appears saturated, that is, when an increase in landscape (gamma) diversity does not lead to an increase in local (alpha) diversity (Manthey and Fridley 2009; Zelený 2009). To check this, we calculated average alpha and gamma diversities in 100 random subsets of 20 sites each from the preliminary set, which showed a significant positive relationship (F -statistic=22.55, adjusted $r^2=0.18$, p -value<0.001). Following recommendations from Manthey and Fridley (2009), we quantified RNW using Jaccard's pairwise dissimilarity (Jaccard 1912). Jaccard's dissimilarity can range from 0 (all species are the same in the compared sites) to 1 (no overlap in the species composition).

Nematode taxa present in at least 10% of the sites were considered target taxa. For each target taxon, we selected all sites in which it was present. Then, a random subset of 20 sites was used to calculate pairwise Jaccard's dissimilarity after presence/absence standardization (calculated using function 'vegdist' from the 'vegan' package for R

(Oksanen *et al.* 2018)). This random selection procedure was repeated 100 times, and we took the average Jaccard's dissimilarity in these 100 repetitions as the target taxon's RNW (theta (θ) in the initial protocol (Fridley *et al.* 2007)). As a consequence, taxa present in more sites will have a more accurate estimate of RNW.

We analysed differences between RNW and the aforementioned traits using either Spearman's rank order correlation (using the '*cor.test*' function in R; (R Core Team 2019)) for continuous variables, or Kruskal-Wallis' rank sum test (Hollander and Wolfe 1999) in the case of categorical variables (using the '*kruskal.test*' function in the same R package; (R Core Team 2019)).

Nematode taxa were then classified as either generalists (higher RNW) or specialists (lower RNW) by splitting the RNW values into two groups using Jenks' natural breaks optimization (Jenks 1967). This division into groups is intended to facilitate the calculation of the I_{CS} . Nematodes classified into specialists simply have a narrower niche width than those classified as generalists. Goodness of variance fit (GVF), a measure based on sum of squares deviation between values and mean, which ranges from 0 (worst fit) to 1 (perfect fit) was used to evaluate the split. Both tests were carried out using the '*classInt*' package for R (Bivand 2017).

2.2.5 Nematode diversity indices

To monitor the nematode community, we calculated nematode abundance (in number of nematodes per 100 g fresh weight), nematode richness (defined as the number of taxa present in a site), nematode diversity and the I_{CS} .

We calculated taxon diversity using the Shannon-Weaver index (H'), such that $H' = \sum_{i=1}^S p_i \times \ln p_i$; where p_i is the proportional abundance of taxon i (in number of nematodes per 100g fresh weight) and S is the total number of taxa identified per site (the site's richness; Hill (1973)). We used the function 'diversity' from the vegan package (Oksanen *et al.* 2018).

The I_{CS} was calculated such that:

$$I_{CSi} = \frac{s_i}{s_i + g_i} \quad \text{Eq. 1}$$

where s_i is the abundance of specialist nematodes in site i , and g_i is the abundance of generalist nematodes in site i .

We selected (from the test set) land use/soil texture combinations with 10 or more replicates and tested whether different land use/soil texture combinations have a different I_{CS} using the Kruskal-Wallis' rank sum test for categorical variables (Hollander and Wolfe, 1973) (using the afore mentioned function in R). We assessed differences

between the groups using Dunn's test for multiple comparisons with Bonferroni adjustment for p -values using the '*posthoc.kruskal.dunn.test*' function within the PMCMR package (Dunn 1964; Holm 1979; Pohlert 2014).

2.2.6 Effects of land use intensity on the nematode community

While the test set did not permit complete combinations of all land use categories and soil textures, it did allow to test differences in nematode diversity indices due to LUI in (a) sandy soils, where data was available for land use classes with ascending land use intensity (shrubland-woodland (131 sites), semi-natural grasslands (33 sites), dairy farms (101 sites) and arable farms (14 sites)); and (b) within dairy farms on soils with different textures (32 sites on clayey soils, 16 on peaty soils and 61 on sandy soils), where nutrient availability acted as proxy for LUI, since a higher cattle density results in an increase in available phosphorus and organic matter in the soil (Mulder *et al.* 2003).

Data were analysed following the protocols proposed by Zuur *et al.* (2010) and Zuur and Ieno (2016). Collinearity between explanatory variables was assessed using correlation plots. Variance inflation factors (VIF) were calculated for the remaining independent variables using the '*corvif*' function for R, which was below 3 for all variables, and none were removed (Zuur *et al.* 2009). The initial models were such that:

$$\begin{aligned} \text{Response} \sim LUI_i + LTAvgT_i + STCPP_i + LTAvgT_i * LTCPP_i \\ + LTAvgT_i * LUI + LTCPP_i * LUI \end{aligned} \quad \text{Eq. 2}$$

$$\begin{aligned} \text{Response} \sim Ctot_i + P - Al_i + LTAvgT_i + LTCPP_i + STCPP_i \\ + LTAvgT_i * LTCPP_i + LTCPP_i * Ctot_i \\ + LTCPP_i * PAL_i \end{aligned} \quad \text{Eq. 3}$$

where Response is either I_{CS} , abundance, richness or diversity; LUI_i , is the LUI category in sandy soil, $LTAvgT_i$ and $LTCPP_i$ are the long-term average temperature and cumulative precipitation, and $STCPP_i$ is the short-term cumulative precipitation (log-transformed), $Ctot_i$ is total carbon (%), determined by thermogravimetric analysis), PAL_i is the extractable phosphorus (determined using an ammonium lactate extraction and expressed in mg $P_2O_5/100g$ dry soil), in site i .

Model selection processes were done following (Zuur *et al.* 2009), starting with all variables under study and ecologically motivated interactions, terms were dropped using the AIC criterion. Model assumptions were verified by plotting residuals versus fitted values, versus each covariate in the model and versus each covariate not in the model, as well as exploring diagnostic plots.

Since I_{CS} is restricted from 0-1, we used beta regressions (Ferrari and Cribari-Neto 2004) to test the relationship between I_{CS} and explanatory variables in Equations (1) and (2), using the ‘*betareg*’ package in R (Cribari-Neto and Zeileis 2010). Beta distributions are restricted from 0-1, but include neither of these values, thus we transformed I_{CS} so that zeroes and ones became numbers close to 0 and 1 respectively, such that:

$$I_{CS_i} = \frac{I'_{CS_i} \times (n-1) + 0.5}{n} \quad \text{Eq. 4}$$

where I_{CS} is the I'_{CS} without zeroes or ones, I'_{CS} is the index of community specialisation calculated using Equation (1), and n is the total number of sites in the analysis (Cribari-Neto and Zeileis 2010).

To study the relationships between the explanatory variables and (a) taxon richness, (b) nematode abundance and (c) taxon diversity, we used for (a) a Poisson generalized linear model, using the ‘*glm*’ function of the ‘*stats*’ package (R Core Team 2019); for (b) a negative binomial generalized linear model, and models were fit using the ‘*glm.nb*’ function of the ‘*MASS*’ package (Venables and Ripley 2002); in the case of (c), we fit different regressions to test the models presented in Equations (2) and (3). After checking the residual plots, and performing a log likelihood ratio test to compare models (L. Ratio, p -value), a model allowing for variable variances per land use type (fit using the ‘*varIdent*’ function of the ‘*nlme*’ package (Pinheiro *et al.* 2017) such that 1 | LUI_i was preferable for Equation (2), while a linear regression was used to test Equation (3).

When LUI was a significant explanatory variable in the resulting models, we carried out a Wald test to compare two linearly restricted models to assess whether LUI classes were different from one another (Fox 1997) using the ‘*linear.hypothesis*’ function from the ‘*car*’ package (Fox *et al.* 2012).

2.3 Results

2.3.1 Nematode realised niche width

There were 45 target taxa (occurring in at least 23 sites) belonging to 26 families. These target nematode taxa occurred with an average of 19 other taxa per site (alpha diversity) and can co-occur with an average of 69 taxa in 20 sites (gamma diversity). RNW (quantified using Jaccard’s dissimilarity) was 0.63, ranging from 0.524 (*Psilenchus*) to 0.689 (*Heterocephalobus*) (Table S1). Realised niche width showed no significant relationships with putative feeding, c-p value, herbivore guild or the average taxon mass (Figure 2.1).

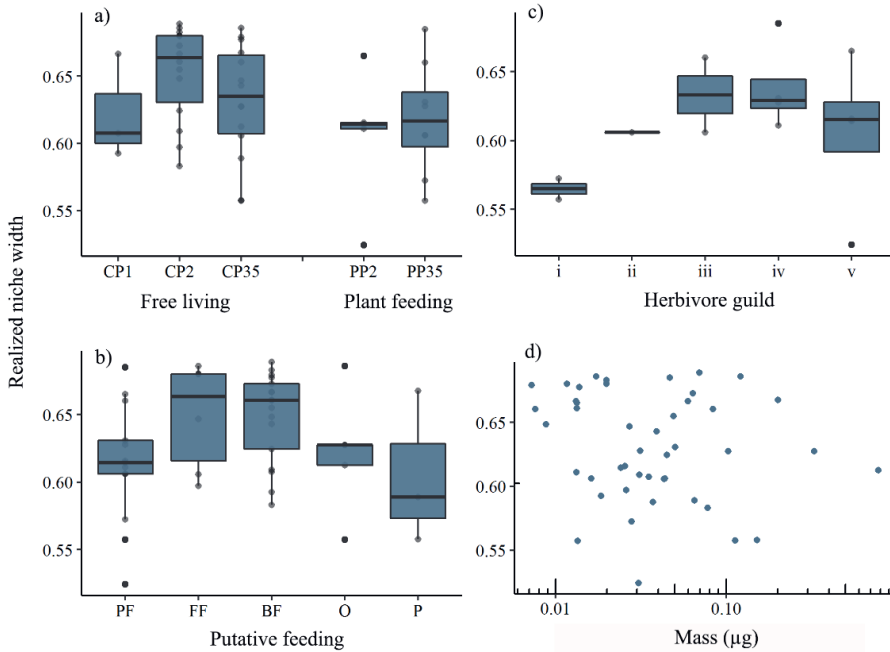


Figure 2.1. Relationship between realised niche width (RNW) and nematode ecological traits. On the left column are boxplots for group comparisons of RNW and (a) c-p and PP values; (b) Putative feeding where BF are bacterial feeders, FF are fungal feeders, O are omnivores, PF are plant feeders, and P are predators; and (c) Herbivore guild, where *i* are sedentary endoparasites, *ii* are migratory endoparasites, *iii* are facultative endoparasites, *iv* are ectoparasites and *v* are epidermal/root hair feeders. On the right-hand column, the relationship between RNW and (d) Body mass (as measured by Mulder and Vonk (2011)). All ecological traits, except for body mass (Mulder and Vonk 2011), were extracted from Nemaplex ((Ferris 1999); accessed November 2018).

2.3.2 Community specialisation

Classification into two groups, namely habitat generalists and habitat specialists, yielded 24 (relative) specialist and 21 generalist taxa (Table S1; Jenks natural breaks optimization GVF=0.73).

Mean community specialisation (I_{CS}) was lowest in forests, followed by heathland, semi-natural grasslands, arable fields and highest in dairy farms (Figure 2.2; Kruskal Wallis, $\chi^2 = 284$, $df = 10$, p -value < 0.01). We observed different mean I_{CS} in different land use/texture classes (Dunn's test for multiple comparisons with Bonferroni adjustment for p -values; Figure 2.2). Variations in I_{CS} were driven by an increase in nematodes with a narrower niche width (Figure S1).

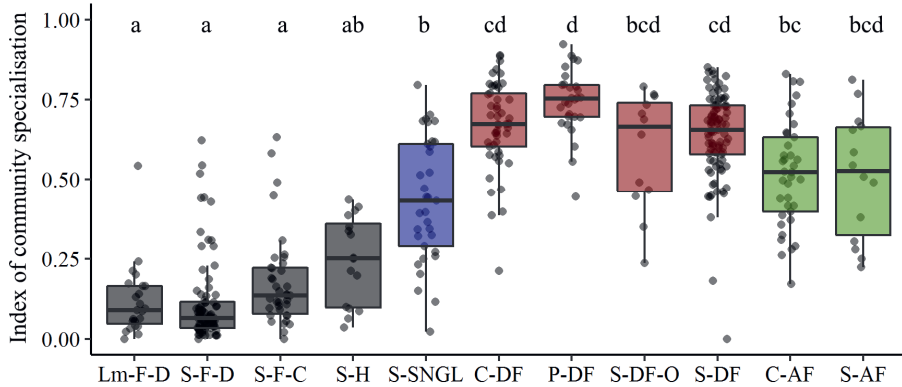


Figure 2.2. Index of community specialisation (I_{CS}) in different land use types. Sites are organized in order of increasing land use intensity first, and then alphabetically according to soil texture categories. The first letters denote soil texture (loam (Lm), sand (S), clay (C) and peat (P)), the second the land use type (forest (F) (deciduous (D) and coniferous (C)), heathland (H) (in black), semi-natural grassland (SNGL) (in blue), dairy farm (DF) (some organic (-O); in red), and arable farm (AF; in green). Highest I_{CS} is observed in dairy farms, while the lowest appears on forests. Semi natural grasslands and arable farms show high variability. There are significant differences in the I_{CS} between groups (Kruskal-Wallis $\chi^2 = 287.45$, p -value < 0.01). Letter's denote the result of pairwise comparisons (Dunn's test of multiple comparisons of independent samples).

2.3.3 Land use intensity in sandy soils

After initial model validation, a point with a very large Cook's distance and high generalized leverage (an extensive dairy farm on sandy soil with 0 generalists) was taken out and the model was re-fit. This had no strong effects on the coefficients, but increased the precision parameter. Further model validation showed no underlying problems. In sandy soils, long term daily average temperature as well as long term cumulative precipitation had a significant effect on the proportion of specialist nematodes in the community (beta regression, Pseudo $R^2 = 0.66$, log likelihood of 228.2 on 16 degrees of freedom; Table S2), such that drier, warmer conditions relate to the highest I_{CS} (Figure 2.3a). I_{CS} was significantly lower in shrubland-woodland systems than in the rest of the land use types (Table 2.2). This land use type had a higher proportional abundance of generalist nematodes (Figure S2a). Non-target taxa (taxa for which we did not calculate a RNW, since they were not present in enough sites) made up a higher proportion of the total population in shrubland-woodland systems (46 taxa), than in semi-natural grasslands (33 taxa), dairy farms (34 taxa) and arable farms (7 taxa) (Figure S2).

Table 2.2. Differences in the nematode index of community specialisation (I_{CS}), taxon richness, abundance (nematodes per 100gr of fresh weight) and Shannon diversity between different land use categories in sandy soils.

		Shrubland-woodland		SN- Grassland		Dairy farming	
		<i>z</i> - value	<i>p</i> -value	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value
I_{CS}	Shrubland-woodland			-	-	-	-
	SN-Grassland	4.66	<0.001			-	-
	Dairy farming	6.70	<0.001	1.35	0.25		
	Arable farming	6.516	<0.001	0.008	0.93	3.36	0.07
Richness	Shrubland-woodland			-	-	-	-
	SN-Grassland	4.51	<0.001			-	-
	Dairy farming	8.00	<0.001	0.10	0.32		
	Arable farming	0.31	0.78	5.58	0.02	10.57	0.001
Abundance	Shrubland-woodland			-	-	-	-
	SN-Grassland	4.96	<0.001			-	-
	Dairy farming	8.14	<0.001	0.10	0.75		
	Arable farming	5.63	<0.001	0.17	0.68	0.90	0.34
		<i>t</i> - value	<i>p</i> -value	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value
Shannon diversity	Shrubland-woodland			-	-	-	-
	SN-Grassland	2.06	0.04			-	-
	Dairy farming	2.11	0.03	1.14	0.28		
	Arable farming	-2.49	0.01	14.17	<0.001	24.03	<0.001

Note: For comparisons between shrubland-woodland and other groups, we present the parameters and *p*-values of the respective regression models (a beta regression, a generalized linear model, a negative binomial and a Poisson generalized linear model). For comparisons between the rest of the sites, we present the χ^2 and *p*-value for Wald-test-based comparisons between the groups. SN-grassland: semi natural grassland; *p*-values <0.05 are given in bold

2.3.4 Land use intensity in dairy farms

Within dairy farms, I_{CS} increased slightly with increasing PAL and precipitation, but there was a significant negative interaction between long-term cumulative precipitation and PAL (beta regression, Pseudo $R^2 = 0.39$; log likelihood of 105.7 on 10 degrees of freedom; Figure 2.4a). I_{CS} was significantly higher on peaty soils, which tend to have a lower proportion of generalists than clayey and sandy soils (Table S3; Figure S3).

None of the studied independent variables could explain the variations in nematode richness (Poisson, Null dev. = 55.45 (271 *df*); Res. Dev. = 52.46 (270 *df*); Figure 2.4b). Nematode abundance could be partially explained by soil texture (where clay soils had lowest abundance, sandy soils the highest, and peaty soils had high variability) and total

carbon (Ctot) (NB, Null dev. = 144 (108 *df*); Res. Dev. = 112 (105 *df*); Figure 2.4c). Taxon diversity significantly decreased with increasing PAL (Linear regression, adjusted $R^2 = 0.093$; $F = 12.1$; p -value < 0.001 ; Figure 2.4d).

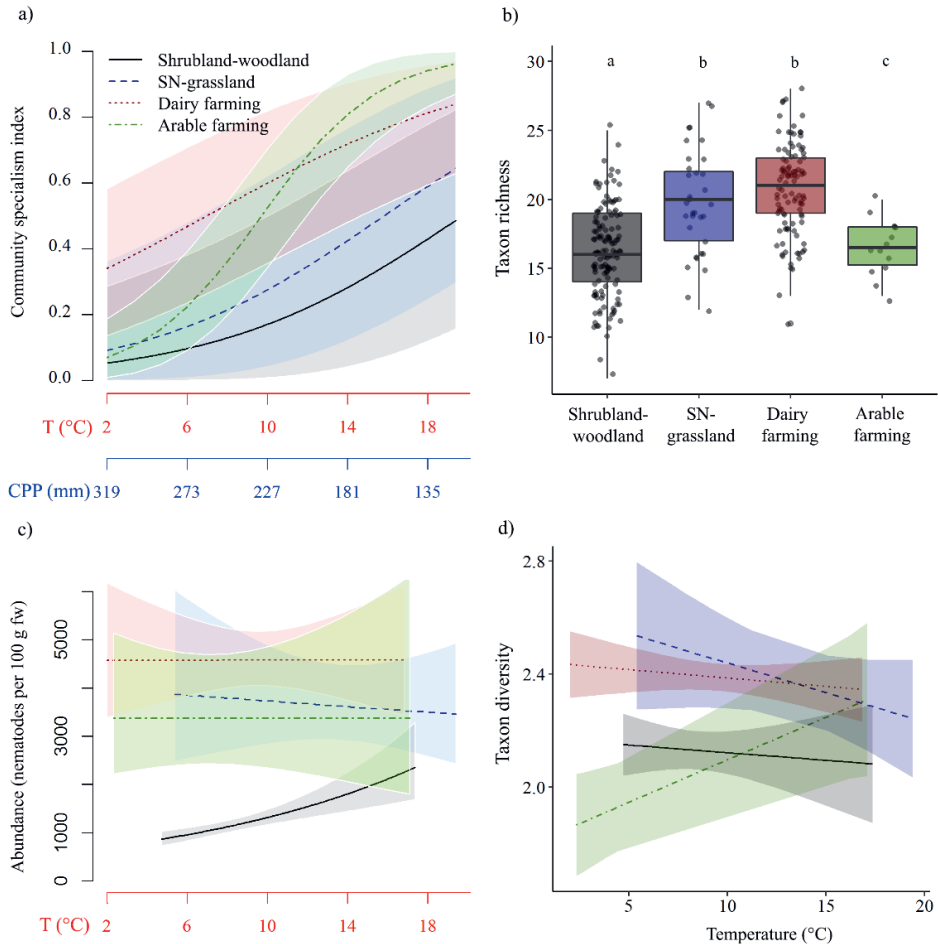


Figure 2.3. Expected index of community specialisation (I_{CS} ; a), abundance (c) and diversity (d); and observed richness (b), under different land use intensities. Lines correspond to the predicted values in different models, with significant explanatory variables in the x axis: average temperature (a, c, d) and cumulative precipitation (CPP) (a) in the three months prior to sampling. Shaded areas represent the 95% confidence interval for each model. Letters denote significant differences between groups through pairwise comparisons of linearly restricted models.

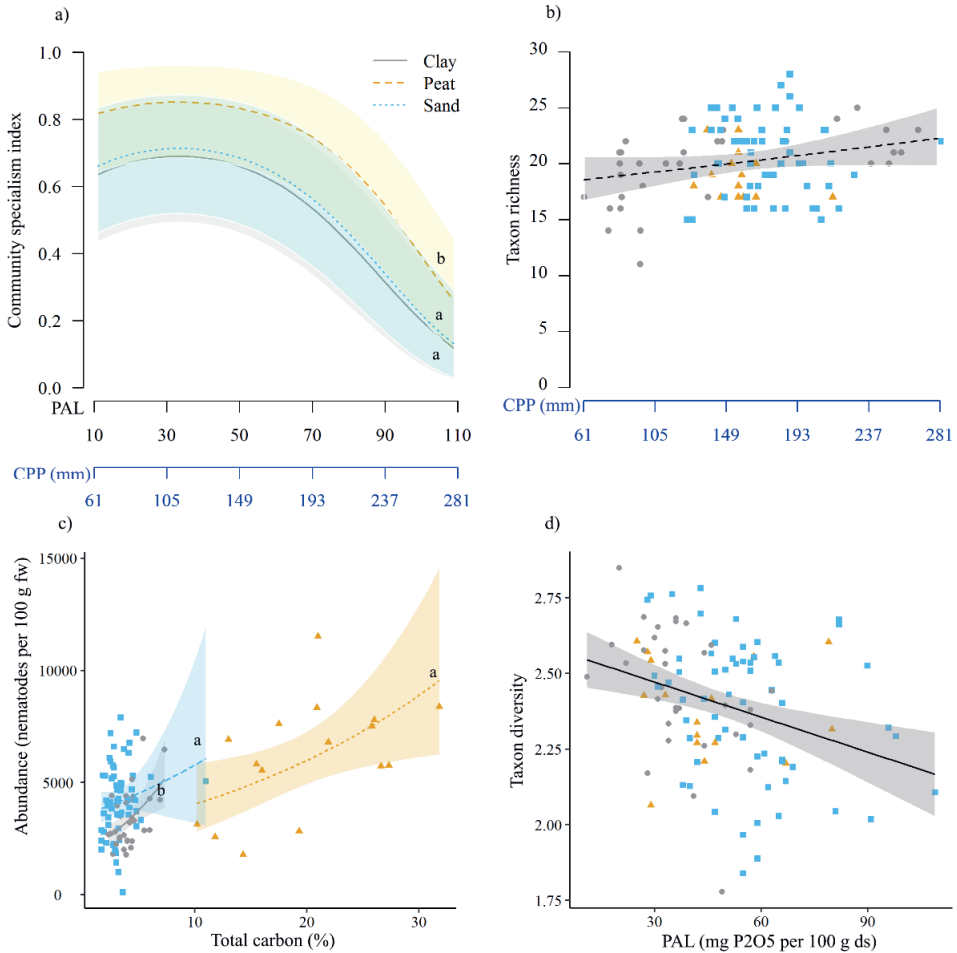


Figure 2.4. Expected index of community specialisation (I_{CS} ; (a), abundance (c) and diversity (d); and observed richness (b), in dairy farms against weather related and nutrient availability gradient. X-axes denote the most relevant explanatory variable(s), colours and shapes denote different soil textures. Letters in panels (a) and (c) denote significant differences between groups through pairwise comparisons of linearly restricted models. In panel (d) the black solid line indicates a significant relationship between PAL and taxon diversity, while the dashed line in panel b indicates lack of significance in the model.

Within dairy farms, I_{CS} increased slightly with increasing PAL and precipitation, but there was a significant negative interaction between long-term cumulative precipitation and PAL (beta regression, Pseudo $R^2 = 0.39$; log likelihood of 105.7 on 10 degrees of freedom; Figure 2.4a). I_{CS} was significantly higher on peaty soils, which tend to have a lower proportion of generalists than clayey and sandy soils (Table S3; Figure S3). None

of the studied independent variables could explain the variations in nematode richness (Poisson, Null dev. = 55.45 (271 *df*); Res. Dev. = 52.46 (270 *df*); Figure 2.4b). Nematode abundance could be partially explained by soil texture (where clay soils had lowest abundance, sandy soils the highest, and peaty soils had high variability) and total carbon (C_{tot}) (NB, Null dev. = 144 (108 *df*); Res. Dev. = 112 (105 *df*); Figure 2.4c). Taxon diversity significantly decreased with increasing PAL (Linear regression, adjusted $R^2 = 0.093$; $F = 12.1$; p -value < 0.001 ; Figure 2.4d).

2.4 Discussion

In this paper, we estimated the niche width of soil nematodes using co-occurrence data, studied the effects of land use on the nematode community, and the vulnerability of specialist nematodes to LUI. Habitat generalists were similarly abundant in all studied systems, while nematodes with a narrower niche width (opposite to our expectations) dominated agricultural landscapes. The I_{CS} was lowest in forests, and higher in the other land use types. The communities' specialisation in dairy farms increased with increasing PAL, but the overall role of nutrient availability in determining I_{CS} appears to be dependent on external factors such as weather conditions.

2.4.1 Realised niche width

Although the protocol to calculate RNW using co-occurrence matrices was initially developed and applied to tree communities, it has since been used to calculate the RNW of, for example, vertebrates (Ducatez *et al.* 2014). The suitability of this method to calculate the RNWs of such different organisms resides in the simplicity of the idea behind it: a habitat specialist, will occur in the company of species that can inhabit the same habitat. A generalist might appear in this and other habitats, in the company of diverse sets of species. It is widely accepted that nematode communities differ from one another under different environmental conditions, even in environments that are already extreme (Kerfahi *et al.* 2017), implying at least community level habitat specialisation. As such, the protocol could also be used to assess the RNW of other soil fauna for which similar trends have been observed (such as earthworms (Decaëns *et al.* 2008), enchytraeids or collembolans (van Dijk *et al.* 2009)). It is, however, not suitable for communities that are or appear saturated (which might be the case for soil bacteria (Raynaud and Nunan 2014)) although Zelený (2009) proposed a Beals transformation of the zeroes in the co-occurrence matrix prior to the calculation of the niche width of saturated communities.

There were no significant relationships between RNW and the studied traits, (i.e. nematode life-history and feeding groups). While there is a possibility that there is no

relationship between these factors, the sample size of the groups in our study may be too small to pick up any sort of significant pattern. The target taxa contained, for example, only three predatory nematodes and five omnivores. A decrease in dispersal ability has been associated with specialism in the past, particularly in the case of seed dispersal (Fridley *et al.* 2007). Similarly to seeds, smaller nematodes are more likely to be wind-blown than larger nematodes (Ptatscheck *et al.* 2018), but what this might mean to dispersal rates is not clear. There is no knowledge on how wind dispersal compares to crawling, or how either of these compare to other forms of passive dispersal. Furthermore, while dispersal might play a role in RNW, it might be small compared to that of food availability, pH tolerance, or host breadth in the case of plant parasites.

Niche breadth is a result of differences in environmental stability: a stable environment leads to specialisation, a heterogeneous environment will lead to different generalist strategies depending on the time scale at which organisms experience disturbance (Levins 1968; Clavel *et al.* 2011). This means that the same disturbance is experienced differently between below and above ground organisms, but also by soil free-living nematode species with different lifespans. For example, a species with a 7 day-lifespan (*Rhabditis terracoli*) or one with a 138 day-lifespan (in *Cephalobus dubius*) (Gems 2000), will experience a week of flooding as either the nature of the environment (in the first case) or a temporary disturbance (in the latter), and this event will have a very different impact in their evolutionary history.

Contrary to previous studies (Ducatez *et al.* 2014; Carboni *et al.* 2016; Fridley *et al.* 2007), community level specialisation increased with increasing LUI. Lowest levels of community specialisation were observed on shrubland-woodland systems. These are characterized by having a very low human impact, due in part to the poor nutrient availability and acidity of the soils. Nematode habitat generalism might be a reflection of any of these characteristics, a tolerance to a wide pH range or the possibility to survive under different nutrient regimes.

2.4.2 Land use intensity on sandy soils

Once we accounted for weather variations, we discovered that the I_{CS} was still lowest in shrubland-woodland systems, but did not differ significantly between semi-natural grasslands, dairy farms and arable farms in sandy soils. The management of productive soils in the Netherlands strives to provide stable conditions for the plant's growing season, conditions that will also favour nematode growth by maintaining high nutrient inputs, and minimizing impacts such as drought or flooding. In this context, a growing season in a managed system might represent an unstable environment for organisms

with lifespans longer than a growing season, but a stable one for nematodes, which might explain a more specialised community.

Take for example the case of Rhabditidae; a taxon classified as a generalist. A flush of nutrients often leads to a rapid increase in the number of Rhabditidae (Ettema and Bongers 1993). When the nutrients are scarce, the new generation might go into a resting stage (dauer larvae) to wait for better conditions. The ability to go into a temporary developmental stage is one of the expected outcomes of evolution in a system with coarse environmental variability (a disturbance regime that affects only some members of the population at a time) (Levins 1968). Dauer larvae, which we treated as its own target taxon, had a relatively narrow niche width. This might be a result of farmland management practices, which stimulate the growth of Rhabditidae, and the subsequent appearance of Dauer larvae, while conditions in natural systems rarely allow for a flush of Rhabditidae. To confirm this, we calculated the I_{CS} without Dauer larvae and did not observe any changes in the observed trends (Figure S4).

2.4.3 Land use intensity in dairy farming

Dairy farms on peat soils (with a higher carbon content) had a more specialised community than those on sand or clay. Increased soil carbon content was also related to an increase in nematode abundance, in line with previous results (Ferris *et al.* 1996; Briar *et al.* 2007). We used PAL as a proxy for LUI. Although there are other important measurements of LUI other than PAL (i.e. pesticide use or frequency of tillage), we did not have access to such information. Phosphorus application comes either as a result of a higher cattle density grazing and/or a higher frequency of mechanical manure applications, both of which could lead to compaction of the soil, but also an increase in nitrogen in the soil (Bilotta *et al.* 2007; Mulholland and Fullen 1991; Scholefield and Hall 1986; Matches 1992). We found a slight positive relationship between PAL and I_{CS} but also an interaction between PAL and long-term cumulative precipitation that led to a decrease in I_{CS} . Manure applications have been shown to lead to an increase in the total number of nematodes (Forge *et al.* 2005), which, can lead to unevenness in the nematode community (which will result in a decrease in diversity) if some nematodes are benefited more than others (in line with our results). While previous studies have observed an interaction between the effect of nitrogen additions and rainfall to the nematode communities, such that an increase of these two factors led to a decrease in nematode abundance and a change in composition (Sun *et al.* 2013), we could find no studies linking PAL and precipitation with any effects on the nematode community, nor can we provide a suitable explanation without further study.

2.5 Conclusions

Co-occurrence based methods of niche width estimation offer a great opportunity for soil ecology, as well as a potential tool for biological soil quality assessment. Soil biota is often difficult to culture and manipulate, and much of its ecology is still to be discovered. Contrary to our expectations, the highest levels of community specialisation were found on farmland systems. The average taxon composition in farmland highlights the environmental homogeneity of such environments (particularly during the growing season), a fact also supported by the decrease in species rarity in these systems. We provide the first look into the realised niche width of soil nematodes, a soil biota group with a relatively well known ecology, but we suggest that future work should look into the niche width of other soil biota groups. From our work, there are strong indications that below-ground community level specialisation is a result of human activity, but that different activities might have different effects on the overall specialisation of the community.

2.6 Acknowledgements

This work was supported by the Netherlands Organisation for Scientific Research (NWO) as part of the ALW Green Top Sector Project 870.15.090, entitled ‘SQUASH – a Soil Quality Universally Applicable Soil Health assessment system’. Activities within RIVM were commissioned by the Netherlands Ministry of Housing, Spatial Planning and the Environment (VROM), currently Ministry of Infrastructure and Water Management (I&W) and took place within project M/607604, entitled ‘Soil ecosystems – monitoring, data management and integration’. We gratefully acknowledge Eurofins Agro and Koppert Biological Systems B.V. for their financial support, and we thank the two anonymous reviewers and the journal’s editors that provided valuable commentary, particularly on the discussion of this paper.

2.7 Data accessibility

The data that support this work were mainly collected by the Netherlands National Institute for Public Health and the Environment (RIVM) and are protected by confidentiality agreements. Data not belonging to the NSMN (Nationwide soil monitoring network) are published in the provided literature.

2.8 Authors’ contributions

C. V. and R. G. M. G. conceived the presented idea, and received important feedback from all co-authors. M. R. and A. J. S provided the data necessary to perform the analyses. C.V. led the writing of the manuscript and the analysis of the data. R. G. M.

G. verified the computations. All authors discussed the methods and results and contributed to the final manuscript.

Supplementary information

Table S1. Feeding group, cp-class, herbivore guild, mass, metabolic footprint, relative niche width, and niche width (RNW) class for target nematode taxa. The taxa are ordered in ascending relative niche width (as quantified using average Jaccard's Dissimilarity), with specialist nematodes on top, and generalists at the bottom of the table.

Taxa	Family*	Putative Feeding*	Herbivore guild***	CP or PP class*	Mass (μ g, Ferris 2010)*	Mass (μ g, Mulder & Vonk 2011)	Metabolic footprint (Ferris, 2010)*	RNW Classification
Psilenchus	Tylenchidae	Plant feeder	v	2	0.489	0.031	0.181	Specialist
Meloidogyne	Heteroderidae	Plant feeder	i	3	52.076	0.013	6.874	Specialist
Thornemematidae	Thornemematidae	Omnivore		5	3.430	0.113	0.727	Specialist
Triplyla	Triplylidae	Predator		3	3.560	0.151	0.787	Specialist
Heterodera	Heteroderidae	Plant feeder	i	3	41.921	0.028	5.716	Specialist
Anaplectus	Plectidae	Bacteria feeder		2	1.497	0.078	0.432	Specialist
Dauer larvae						0.037	0.588	Specialist
Mylonchulus	Mylonchulidae	Predator		4	1.776	0.065	0.452	Specialist
Neodiplogasteridae	Neodiplogasteridae	Bacteria feeder		1	1.601	0.019	0.520	Specialist
Aphelenchus	Aphelenchidae	Fungus feeder		2	0.231	0.026	0.102	Specialist
Tyloaimophorus	Diphtherophoridae	Fungus feeder		3	0.872	0.043	0.275	Specialist
Helicotylenchus	Hoplolaimidae	Plant feeder	iii	3	0.286	0.044	0.112	Specialist
Pratylenchus	Pratylenchidae	Plant feeder	ii	3	0.141	0.016	0.067	Specialist
Panagrolaimus	Panagrolaimidae	Bacteria feeder		1	0.660	0.035	0.261	Specialist
Eucephalobus	Cephalobidae	Bacteria feeder		2	0.243	0.031	0.105	Specialist
Paratylenchus	Tylenchulidae	Plant feeder	iv	2	0.060	0.013	0.036	Specialist
Aporcelaimellus	Aporcelaimidae	Omnivore		5	8.927	0.777	1.527	Specialist
Aglenchus	Tylenchidae	Plant feeder	v	2	0.104	0.024	0.055	Specialist
Costlenchus	Tylenchidae	Plant feeder	v	2	0.110	0.026	0.057	Specialist
Acrobeles	Cephalobidae	Bacteria feeder		2	0.711	0.045	0.230	Specialist
<i>Mesodorylaimus</i>	Dorylaimidae	Omnivore		4	1.369	0.328	0.372	Specialist
<i>Prodorylaimus</i>	Thornemematidae	Omnivore		4	5.162	0.103	0.995	Specialist
Dolichodoridae	Dolichodoridae	Plant feeder	iv	3	0.602	0.031	0.186	Specialist

Taxa	Family*	Putative Feeding*	Herbivore guild***	CP or PP class*	Mass (µg, Ferris 2010)*	Mass (µg, Mulder & Vonk 2011)	Metabolic footprint (Ferris, 2010)*	RNW	Classification
Trichodoridae	Trichodoridae	Plant feeder	iv	4	0.895	0.050	0.251	0.631	Specialist
<i>Alaimus</i>	Alaimidae	Bacteria feeder		4	0.534	0.039	0.163	0.643	Generalist
<i>Diphtherophorida</i>	Diphtherophoridae	Fungus feeder		3	0.504	0.027	0.178	0.647	Generalist
<i>Wilsonema</i>	Plectidae	Bacteria feeder		2	0.054	0.009	0.033	0.648	Generalist
<i>Cephalobus</i>	Cephalobidae	Bacteria feeder		2	0.266	0.049	0.113	0.655	Generalist
<i>Ropylenchus</i>	Hoplolaemidae	Plant feeder	iii	3	1.076	0.084	0.311	0.660	Generalist
<i>Teratocephalus</i>	Teratocephalidae	Bacteria feeder		3	0.084	0.008	0.045	0.660	Generalist
<i>Eumonystera</i>	Mononysteridae	Bacteria feeder		2	0.241	0.013	0.097	0.661	Generalist
<i>Malenchus</i>	Tylenchidae	Plant feeder	v	2	0.066	0.013	0.038	0.665	Generalist
Rhabditidae	Rhabditidae	Bacteria feeder		1	5.287	0.060	1.370	0.667	Generalist
<i>Cervidellus</i>	Cephalobidae	Bacteria feeder		2	0.157	0.013	0.075	0.667	Generalist
<i>Clarkus</i>	Mononchidae	Predator		4	4.389	0.202	0.892	0.668	Generalist
<i>Plectus</i>	Plectidae	Bacteria feeder		2	0.909	0.064	0.277	0.673	Generalist
<i>Prismatolaimus</i>	Prismatolaimidae	Bacteria feeder		3	0.487	0.014	0.154	0.678	Generalist
<i>Metateratocephalus</i>	Teratocephalidae	Bacteria feeder		3	0.073	0.007	0.041	0.679	Generalist
<i>Aphelenchoides</i> **	Aphelenchoiidae	Fungus feeder		2	0.162	0.012	0.076	0.680	Generalist
<i>Filenchus</i> **	Tylenchidae	Fungus feeder		2	0.093	0.020	0.047	0.680	Generalist
<i>Acroboloides</i>	Cephalobidae	Bacteria feeder		2	0.148	0.020	0.071	0.683	Generalist
Criconematidae	Criconematidae	Plant feeder	iv	3	0.602	0.047	0.200	0.685	Generalist
<i>Diitylenchus</i> **	Anguinidae	Fungus feeder		2	0.555	0.017	0.194	0.686	Generalist
Qudsianematidae	Qudsianematidae	Omnivore		4	2.876	0.121	0.629	0.686	Generalist
<i>Heterocephalobus</i>	Cephalobidae	Bacteria feeder		2	0.356	0.070	0.141	0.689	Generalist

* Trait values were extracted from Nemaplex (Ferris 1999), accessed on 10-07-2018. Plant feeders are allocated to a PP-class, other taxa to a CP-class.

** Classified as fungus feeders

*** Herbivore guilds:

i= sedentary endoparasitic females;

ii= migratory endoparasites;

iii= facultative endoparasites;

iv= ectoparasites;

v= root associates, epidermal/root hair feeders

Table S2. Estimated regression parameters, standard errors, *z/t-values*, *p-values* and model statistics for four models with response variables: Index of community specialisation (I_{CS}), taxon richness, nematode abundance (nematodes per 100gr of fresh weight), and taxon diversity, and explanatory variables that include 4 land use intensity classes on sandy soils, as well as weather variables. Intercept values are calculated for the lowest management intensity: Shrubland woodland.

Response parameter	Estimate	Std. error	<i>z-value</i>	<i>p-value</i>	
<i>Coefficients (mean model with logit link)</i>					
I_{CS}	Intercept	-2.016	0.371	-5.43	<0.001
	Average Temp (3 months)	0.117	0.024	4.80	<0.001
	Cumulative PP (3 months)	-0.003	0.002	-2.28	0.02
	Land use: SN-Grassland	3.896	0.836	4.66	<0.001
	Land use: Dairy farming	2.981	0.445	6.70	<0.001
	Land use: Arable farming	3.975	0.610	6.52	<0.001
	Average Temp : Land use: SN-Grassland	-0.123	0.037	-3.35	<0.001
	Average Temp : Land use: Dairy farming	-0.056	0.030	-1.89	0.06
	Average Temp : Land use: Arable farming	0.020	0.033	0.60	0.55
	Cumulative PP : Land use: SN-Grassland	-0.010	0.004	-2.39	0.02
	Cumulative PP : Land use: Dairy farming	-0.002	0.002	-0.95	0.34
	Cumulative PP : Land use: Arable farming	-0.012	0.003	-3.59	<0.001
	<i>Phi coefficients (precession model with log link)</i>				
	Intercept	1.948	0.127	15.38	<0.001
Land use: SN Grassland	0.053	0.265	0.20	0.84	
Land use: Dairy farming	0.804	0.187	4.30	<0.001	
Land use: Arable farming	1.382	0.393	3.52	<0.001	
Pseudo $R^2 = 0.66$; LL = 228.2; d.f. = 16					
Richness	<i>Coefficients (mean model with logit link)</i>				
	Intercept	2.786	0.02	128.44	<0.001
	Land use: SN-Grassland	0.202	0.04	4.52	<0.001
	Land use: Dairy farming	0.246	0.03	8.00	<0.001
Land use: Arable farming	0.021	0.07	0.31	0.76	
Null dev. = 241.49 (278 d.f.); Res. Dev. = 170.94 (275 d.f.)					
Abundance	<i>Coefficients (mean model with log link)</i>				
	Intercept	6.392	0.165	38.63	<0.001
	Average Temp (3 months)	0.079	0.018	4.33	<0.001
	Land use: SN-Grassland	1.912	0.385	4.97	<0.001
	Land use: Dairy farming	2.036	0.250	8.14	<0.001
	Land use: Arable farming	1.733	0.307	5.64	<0.001
	Average Temp : Land use: SN-Grassland	-0.087	0.031	-2.85	0.004
	Average Temp : Land use: Dairy farming	-0.079	0.026	-3.04	0.002
	Average Temp : Land use: Arable farming	-0.079	0.033	-2.39	0.017
<i>Theta</i>	2.536	0.203			
Null dev. = 579 (278 d.f.); Res. Dev. = 297 (271 d.f.)					

Effects of land use intensity on soil nematodes: a turn towards specialism

	Estimate	Std. error	<i>t</i> -value	<i>p</i> -value
<i>Coefficients</i>				
Intercept	2.176	0.104	20.92	<0.001
Average Temp (3 months)	-0.005	0.011	-0.47	0.640
Land use: SN-Grassland	0.472	0.229	2.06	0.040
Land use: Dairy farming	0.269	0.127	2.12	0.035
Land use: Arable farming	-0.380	0.152	-2.49	0.013
Average Temp : Land use: SN-Grassland	-0.016	0.018	-0.85	0.399
Average Temp : Land use: Dairy farming	-0.001	0.014	-0.04	0.967
Average Temp : Land use: Arable farming	0.035	0.016	2.15	0.032
	Shrubland			
	d-	SN-	Dairy	Arable
	Woodland	Grassland	farming	farming
	d			
<i>Variance parameters</i>	1	0.93	0.62	0.68
	LL = -100.4; d.f. = 279; R.s.e = 0.39			

Temp: temperature (C°), PP= precipitation (mm) , SN-grassland: semi natural grassland,
 LL: Log-Likelihood, d.f.: degrees of freedom. R.s.e: residual standard error. p-values <0.05 are given in bold

Table S3. Estimated regression parameters, standard errors, *z/t-values*, *p-values* and model statistics for four models with response variables: Index of community specialisation (I_{CS}), taxon richness, nematode abundance (nematodes per 100gr of fresh weight), and taxon diversity in dairy farms, and explanatory variables that include nutrient availability, soil texture, as well as weather variables. Intercept values are calculated for clayey soils.

Response parameters	Estimate	Std. error	<i>z-value</i>	<i>p-value</i>	
<i>Coefficients (mean model with logit link)</i>					
I_{CS}	Intercept	-0.750	0.666	-1.10	0.270
	PAL	0.032	0.001	2.76	0.005
	Total carbon	0.126	0.008	1.66	0.097
	Cumulative PP (3 months)	0.009	0.004	2.47	0.014
	Log-Cumulative PP (7days)	0.052	0.036	1.43	0.153
	Soil texture: Peat	0.948	0.317	2.99	0.003
	Soil texture: Sand	0.112	0.150	0.98	0.328
	PAL : Cumulative PP (3 months)	-0.0002	0.0001	-3.53	<0.001
	Total carbon : Cumulative PP (3 months)	-0.0009	0.0005	-1.82	0.068
	<i>Phi coefficients (preciddion model with log link)</i>				
Φ (Phi)	23.478	3.125	7.51	<0.001	
Pseudo $R^2 = 0.39$; LL = 105.7; d.f. = 10					
<i>Coefficients (mean model with logit link)</i>					
Richness	Intercept	2.871	0.0815	35.24	<0.001
	Cumulative PP (3 mo.)	0.0008	0.0005	1.73	0.08
Null dev. = 55.45 (271 d.f.); Res. Dev. = 52.46 (270 d.f.)					
<i>Coefficients (mean model with log link)</i>					
Abundance	Intercept	7.918	0.097	81.27	<0.001
	Total carbon	0.048	0.015	3.17	0.002
	Soil texture: Peat	-0.187	0.274	-0.68	0.495
	Soil texture: Sand	0.255	0.093	2.75	0.006
	Theta	5.660			
Null dev. = 144 (108 d.f.); Res. Dev. = 112 (105 d.f.)					
Estimate Std. error <i>t-value</i> <i>p-value</i>					
<i>Coefficients</i>					
Shannon diversity	Intercept	2.587	0.0576	44.91	<0.001
	PAL	-0.004	0.0011	-3.48	<0.001
Adjusted $R^2 = 0.093$; $F = 12.1$; d.f. = 107; p -value <0.001					

Temp: PP= precipitation (mm) , LL: Log-Likelihood, d.f.: degrees of freedom.

P-values <0.05 are given in bold

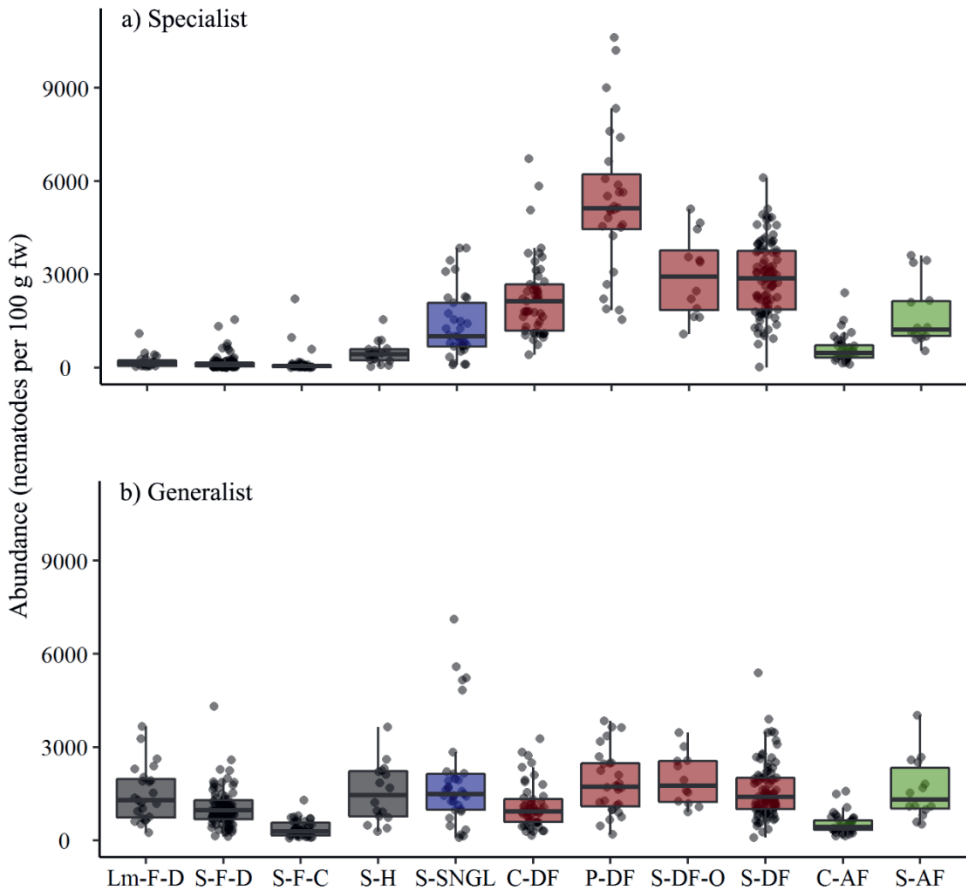


Figure S1. Total number of specialist (a) and generalist (b) nematodes per 100 g of fresh weight per land use and soil texture. Sites are organized in order of increasing land use intensity first, and then alphabetically according to soil type. The first letters denote soil texture (loam (Lm), sand (S), clay (C) and peat (P)), the second the land use type (forest (F) (deciduous (D) and coniferous (C)), heathland (H) in black; semi-natural grassland (SNGL) in blue; dairy farm (DF) (some organic (-O) in red; and arable farm (AF) in green. Generalist nematodes have similar numbers in all land use types, except for coniferous forests in sandy soils and arable farms on Clay. Dairy farms on peat have highest numbers of specialist nematodes, while forests have the lowest.

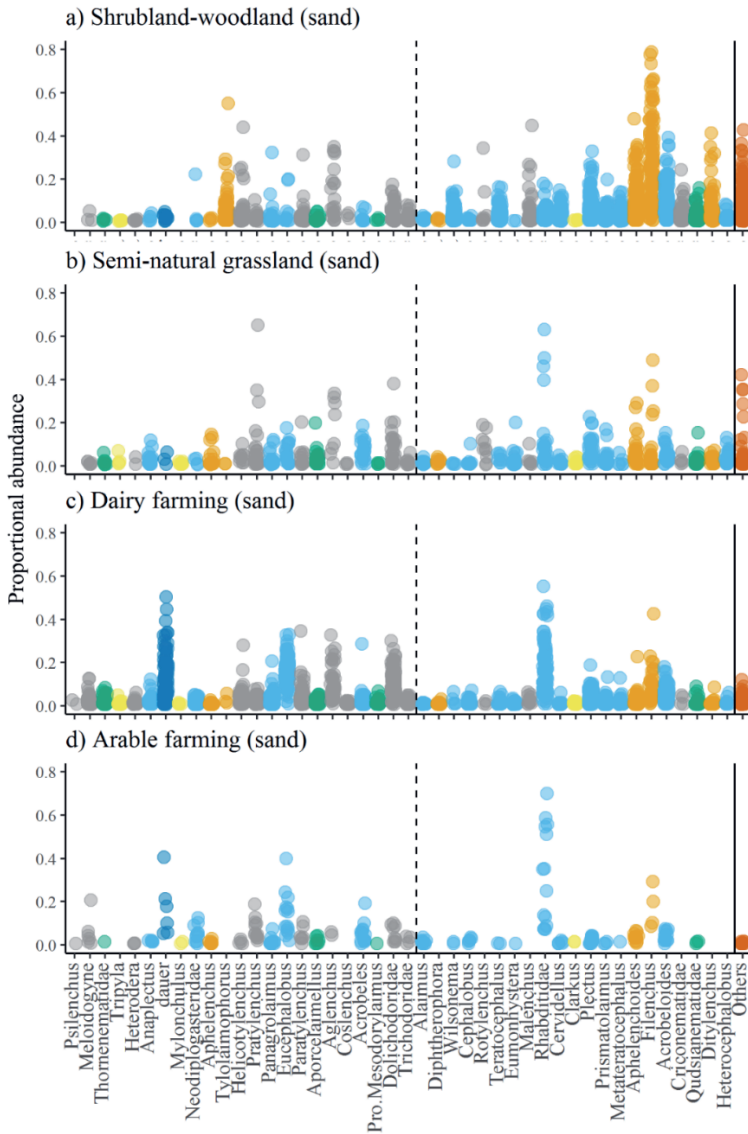


Figure S2. Proportional abundance of each target taxon in different sites in sandy soils, in order of increasing niche width. Colours on the left of the solid dividing line reflect putative feeding (Yeates et al. 1993) with plant feeders (●), fungal feeders (●), bacterial feeders (●), omnivores (●), predators (●), and dauer larvae (●). On the right of the solid dividing line are non-target taxa (●). Generalists (on the right of the dashed dividing line), are proportionally more abundant in Shrubland-woodland systems, particularly generalist fungal feeders (a). Also, Shrubland-woodland systems had a higher number of ‘Other’ taxa that are not common enough to be target taxa. Dairy farms (c) have a high proportion of dauer larva compared to all other land use types.

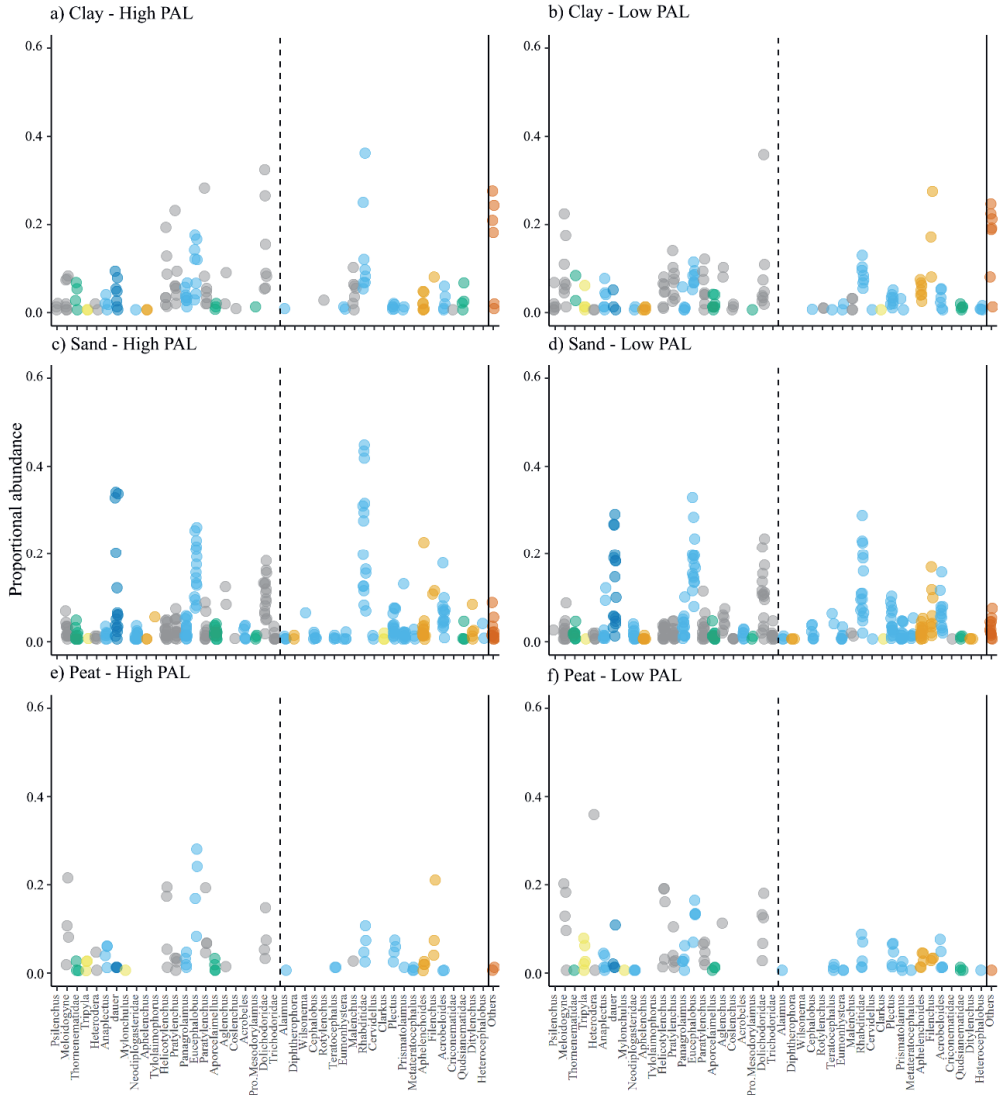


Figure S3. Proportional abundance of each target taxon in order of increasing niche width in dairy farms in different soil textures and phosphorus concentration (PAL). Colours on the left of the solid dividing line reflect putative feeding (Yeates et al. 1993) with plant feeders (●), fungal feeders (●), bacterial feeders (●), omnivores (●), predators (●), and dauer larvae (●). On the right of the solid dividing line, non-target taxa (●). Sites with high (upper quartile) PAL (mg P₂O₅ per 100 grams of soil extracted in an ammonium-lactate solution) are on the left hand column (a, c, e), and those with low PAL (in the lower quartile) are on the right hand column (b, d, f). There are no apparent differences in the nematode composition of the nematode communities between left and right hand columns.

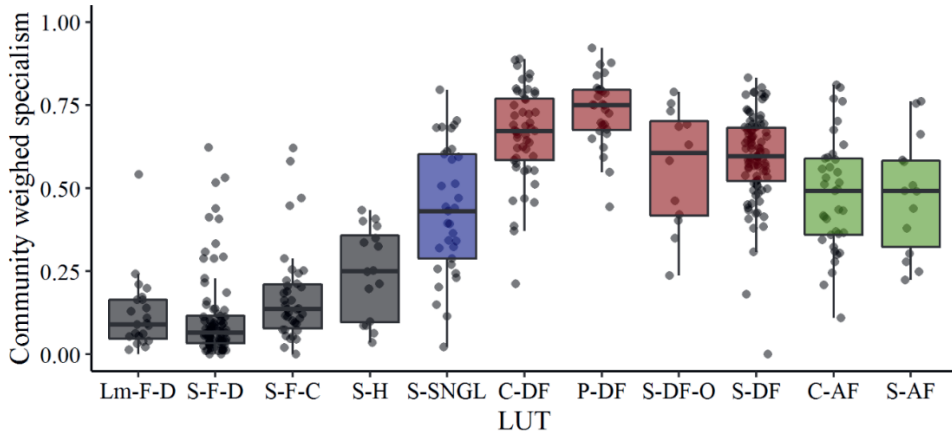


Figure S4. Index of community specialisation (I_{cs}) in different land use types calculated without dauer larvae. Sites are organized in order of increasing land use intensity first, and then alphabetically according to soil texture categories. The first letters denote soil texture (loam (Lm), sand (S), clay (C) and peat (P)), the second the land use type (forest (F) (deciduous (D) and coniferous (C)), heathland (H) (in black), semi-natural grassland (SNGL) (in blue), dairy farm (DF) (some organic (-O); in red), and arable farm (AF; in green).

Chapter 3

Realised niche widths of soil enchytraeid species (Enchytraeidae, Clitellata)

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Abstract

Enchytraeids support several ecosystem services via processes such as the formation of soil structure and their effects on organic matter dynamics. Consequently, changes in the enchytraeid community can in turn affect the delivery of said ecosystem services. While above ground, human disturbances often lead to the loss of habitat specialists we do not yet know whether this holds true for underground organisms. In fact, the opposite trend was recently found in soil nematodes. In this study we classified several enchytraeid species according to their degree of habitat specialisation using co-occurrence matrices. We used data gathered from 81 sites in five bio-climatic regions (Alpine, Atlantic, Boreal, Continental and Mediterranean) and three land use types of increasing land use intensity (forestry, grassland and arable land). Using simulations, we tested possible biases and the sensitivity of our calculations. We then calculated an Index of Community Specialisation (I_{CS}) for each site and tested whether increasing land use intensity led to a decrease of the I_{CS} . From the 23 species for which habitat specialisation was quantified, *Cognettia sphagnetorum* was the most specialised and *Enchytronia parva* the least. Tolerance to pH might explain at least the position of these two extreme species in the generalist to specialist scale: *C. sphagnetorum* is known to prefer acid soils and *E. parva* is one of the few species that is present in communities typical of acid soils as well as those in neutral pH. The resulting niche width ranks were robust to scenarios simulating under-sampling, but the tests underlined the importance of using the same sampling effort in all sites when calculating niche width from co-occurrence methods. We found no evidence to support the hypothesis that land use intensity had an effect on the level of community specialisation of enchytraeids, and none of the explored variables were able to explain the variations in the I_{CS} . We hypothesize that enchytraeid habitat specialism is a result of a myriad of environmental conditions, and that in our dataset, community specialism may therefore respond to multiple environmental scales, leading to a seemingly random degree of community specialisation.

3.1 Introduction

The delivery of ecosystem services such as the production of food, fibre and fuel, nutrient cycling or water purification and regulation depends on processes that are driven by soil biota (Haygarth and Ritz 2009; Kibblewhite *et al.* 2008; Bünemann *et al.* 2018). Enchytraeids in particular play a strong role in several of these processes. They affect the cycling of nutrients and soil organic matter directly through the ingestion of decomposing organic matter and indirectly through modifications to the soil structure as well as the microbial community (Briones and Ineson 2002; Pelosi and Römbke 2016; Porre *et al.* 2016). While they are smaller than earthworms in size, their burrowing activities can have similar effects by improving aggregate stability and hydraulic conductivity, an effect that is particularly important under soil conditions that are unsuitable to earthworms (Van Vliet *et al.* 1993; Didden 1993; Porre *et al.* 2016).

There is evidence that enchytraeid abundance as well as community composition are affected by changes in land use, and that communities under arable farming are in fact different to those found in grasslands or forests (Pelosi and Römbke 2016; Postma-Blaauw *et al.* 2012). However, the effects of specific land management practices on the enchytraeid community are not always clear. For example, enchytraeid density was found to be similar in long term arable fields when compared to nearby shelterbelts, but the arable fields fostered smaller and younger individuals than the shelterbelts (Nowak 2007). Studies into the effect of conventional tillage practices (with a rotary plough), when compared with reduced tillage or even no tillage have also led to mixed results in measurements of enchytraeid abundance and biomass (Pelosi and Römbke 2016). The effect of pesticides depends on the pesticide load and can be species specific. In fact, low pesticide loads sometimes lead to an increase in enchytraeid abundance due to a loss in competitive pressure. This effect is lost at higher loads where a decrease in total abundance is observed, although it is sometimes accompanied by an increase in the abundance of specific species (Römbke *et al.* 2017).

Human disturbances can often lead, specifically, to the loss of habitat specialists (Devictor *et al.* 2008; Ibarra and Martin 2015; Kotze and O'Hara 2003; Fried *et al.* 2010). The explanation is that species which have evolved to thrive in very specific environmental conditions (habitat specialists) are more likely to be filtered out of a community when these conditions change than those which have evolved to survive in a broad set of environmental conditions (habitat generalists), because human disturbance acts as an environmental filter (Clavel *et al.* 2011). Whether increased land use intensity impacts specialist soil organisms more than generalists is still unclear, in fact, the opposite trend was observed on soil nematodes (Vazquez *et al.* 2019; Chapter 2). Increased land use intensity led to a higher abundance of nematodes classified as

habitat specialists, which could be a result of agricultural land providing the stable soil conditions necessary for the success of habitat specialists (Vazquez *et al.* 2019; Chapter 2).

Defining the habitat niche width of a species is complicated because the important environmental gradients that determine the occurrence of species are often unknown (Fridley *et al.* 2007). Niche width in enchytraeids, and their degree of specialization in particular, has not yet been addressed explicitly, even though enchytraeids have been classified according to their association with environmental conditions. Graefe and Schmelz (1999) scored enchytraeid species based on their association with soil moisture, salinity, acidity as well as their life strategy (either K or r selected species), and occurrence in humus. Later Jänsch and Römcke (2003) and more recently Römcke *et al.* (2013) studied the level of association between enchytraeid species and specific land uses, humus forms and soil texture. These approaches could be used to capture the species' realized niche width (RNW in the following), as they address the real-world conditions which a species actually occupies, but they include a bias if the measured environmental variables do not cover the entire set of abiotic and biotic conditions that define the niche width of a given species (Devictor *et al.* 2010). To circumvent this bias, various authors have proposed to disregard these variables altogether for the calculation of RNW by analysing different aspects of species co-occurrence matrices. The principle is such that species that occur always in the company of the same set of species are considered habitat specialists, while those occurring in relatively different communities are considered habitat generalists (Fridley *et al.* 2007; Manthey and Fridley 2009; Zelený 2009). This method, does not provide direct insight into the underlying mechanisms that determine the target species' niche width, but it allows the calculation of niche width for several target species simultaneously. Several studies have shown for vegetation that this method matches approaches based on environmental variables reasonably well (Fried *et al.* 2010; Manthey and Fridley 2009; Carboni *et al.* 2016), and it can be followed up by a calculation of community weighted specialism (Fried *et al.* 2010; Vazquez *et al.* 2019; Chapter 2).

However, we foresee three problems when estimating RNW of enchytraeids (that may also apply to other soil fauna) via co-occurrence matrices. Firstly, to attain a more representative picture of the species preferred habitats, species for which RNW is calculated should be present in a minimum number of sites (Fridley *et al.* 2007). These species are referred to as target species. If specialists have in fact become rare or extinct due to human intervention (Clavel *et al.* 2011), it is likely that the target species are generalists, unless sampling is done in pristine systems. Secondly, soil faunal groups are extremely species rich. Only in Europe, one can identify up to 206 soil enchytraeid

species visually (Schmelz and Collado 2010), some of which are in fact an assemblage of several species that are not distinguishable under the microscope. Populations in the soil are often patchy or clustered (Ettema and Wardle 2002; Decaëns 2010), and while steps are often taken during the sampling procedure to prevent biases derived from this patchiness, it is likely that rare and less abundant species go under-sampled. Lastly, the presence of a species on a site might not be a sufficient measure of habitat suitability. Enchytraeids can survive in unsuitable habitats for years after a land use change event (Postma-Blaauw *et al.* 2012; Beylich *et al.* 2015). In fact, reductions in population due to causes other than mortality have been reported in laboratory tests for specific enchytraeid species, for example, dips in the abundance of test species (e.g., *Enchytraeus albidus* Henle, 1837) due to the presence of organic pesticides have been attributed to reductions in reproductive success (Römbke *et al.* 2017).

In this study we calculate RNW for several enchytraeid species using data gathered throughout Europe in forests, grasslands and arable land. We then study the sensitivity of the calculation of RNW under specific scenarios designed to explore the aforementioned problems. Lastly, we calculate an Index of community specialisation (I_{CS}), and investigate the effects of land use on the I_{CS} , as well as the possible drivers of the I_{CS} . We hypothesize that (1) specialist species are more likely to be slow growing species with a longer life span and a smaller number of offspring (*K - selected*) and strongly associated with specific environmental conditions compared to generalists, (2) the calculation of the RNW will be robust to scenarios involving under-sampling, as observed by earlier literature (Fridley *et al.* 2007; Manthey and Fridley 2009; Zelený 2009), but we expect non-target species to be more specialised than target species, which would be indicative of increased rarity of specialist species; and (3) like nematodes (Vazquez *et al.*, 2019; Chapter 2), enchytraeid communities will become more specialised with increasing land use intensity.

3.2 Materials and Methods

3.2.1 Enchytraeid community

As a response to the need to establish baseline values of soil biodiversity in different land use classes, as well as the need to harmonise the methods for collection and measurement of soil biodiversity in Europe, the EcoFinders project (EU Seventh Framework Programme funded), established a transect across Europe to include a varied set of soils and land use classes across Europe which resulted in the sampling of 81 sites spread across 5 bio-climatic zones spreading throughout 11 countries (Denmark, France, Germany, Ireland, Italy, Portugal, The Netherlands, Slovenia, Sweden, Switzerland and the United Kingdom) and covering three land uses: grassland,

arable land and forestry (Table 3.1). The sampling was designed to include a broad range of soil properties (Figure 3.1) and took place between September and November of 2012 (Stone *et al.* 2016).

Table 3.1. Number of locations sampled per land use type and bio-climatic zone

		Bio-climatic zone				
		Alpine	Atlantic	Boreal	Continental	Mediterranean
Land use	Arable land	1	13	0	11	2
	Grassland	9	13	0	11	2
	Forestry	2	7	4	5	1

At each site a representative plot of 2x2 m was selected, loose material was removed from the top and above ground vegetation was cut down to 2 cm height. A minimum of 20 soil cores (27 in highly organic soils) of 5 cm diameter and 5 cm depth were extracted from this plot, three were used for enchytraeid extraction, five to microarthropod extraction and the rest were pooled. Part of this pooled sample was used for nematode extraction, part for DNA extraction, and part was sieved at 2mm and used for chemical analyses of several soil parameters (Table 3.2). All soils used for enchytraeid extraction and chemical analyses were stored at 4 °C until processing, which took no more than 4 weeks in 94% of the cases (Stone *et al.* 2016).

Enchytraeids were extracted from the soil using a combination of cold and hot wet funnel extraction methods to maximize the extraction efficiency, following ISO (2007) guidelines. A soil sample was first submerged in cold water for 6 h, then heated for 3–4 h from room temperature to about 40°C using light bulbs on top, to create a heat gradient which induces the animals to move down into the water. Animals were investigated and identified *in vivo* with a light microscope following Schmelz and Collado (2010). Undescribed species were listed as "sp. 1", "sp. 2", but not named (Table S1).

3.2.2 Relative niche width

To quantify the enchytraeids' RNW we applied a protocol first proposed by Fridley *et al.* (2007) with some adjustments. The initial protocol was based on additive beta diversity metrics, such that the species that co-occurred with the highest number of other species was considered the most extreme generalist and that which co-occurred with the lowest number of species was, therefore, the furthestmost specialist. This calculation of RNW, however, is biased by the size of the species pool at the species

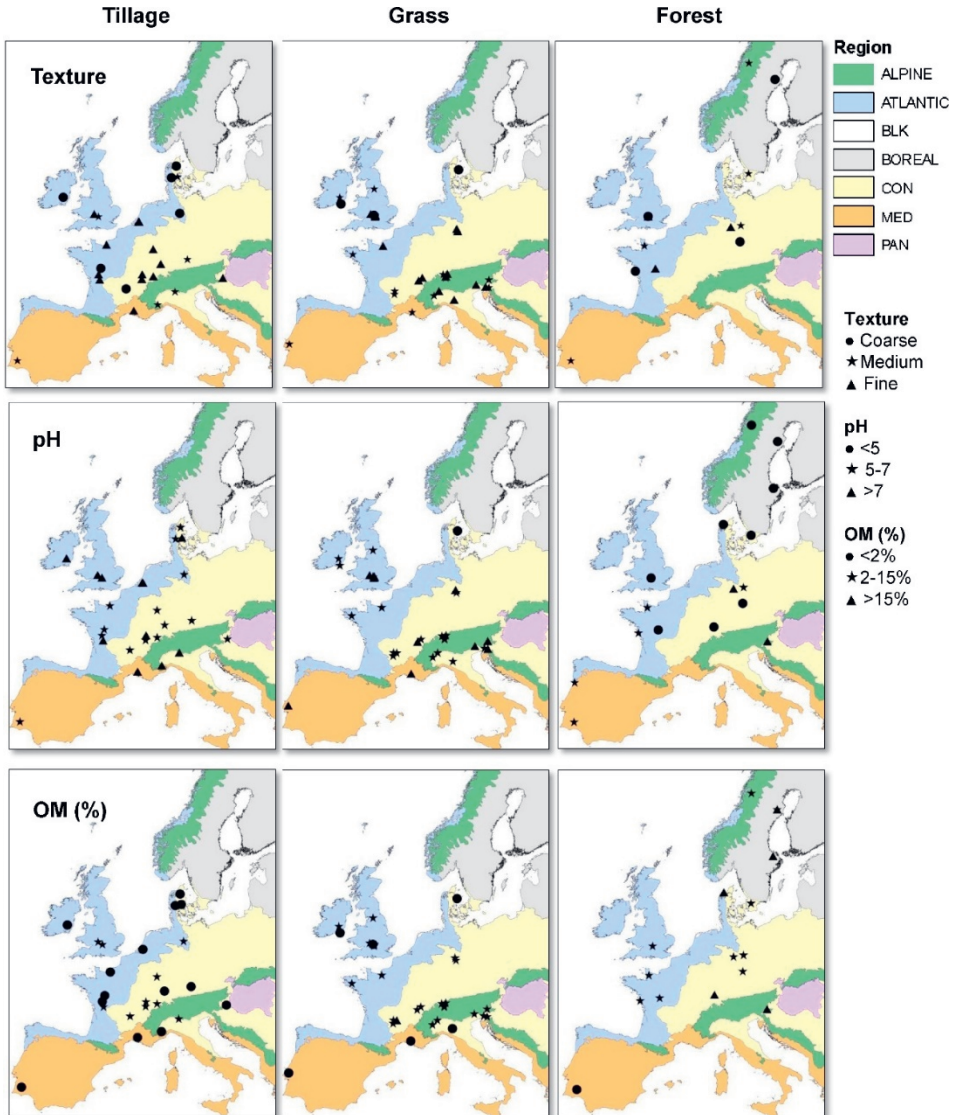


Figure 3.1. 81 Sites sampled within the EcoFinders project during the transect campaign divided into land use categories: arable, grass, and forestry. The sites were located on a range of texture, pH and organic matter content (OM %) represented by symbols. Bio-climatic (Alpine, Atlantic, blank (BLK), Boreal, continental (CON), Mediterranean (MED) and Pannonian (PAN)) are shown on each individual map with different colours. Figure from Stone *et al.* (2016) used with permission.

optima, and gives an inaccurate representation of the RNW when the relationship between local and regional diversity is not linear (Manthey and Fridley 2009; Zelený 2009). To correct for the “species-pool” bias, Manthey and Fridley (2009) proposed the use of dissimilarity metrics calculated using presence absence data. This method, however, does not solve the latter problem, and so before applying the protocol we calculated average local (alpha) and regional (gamma) diversities in 100 random subsets of 20 sites each from the community matrix, which showed a significant positive linear relationship (F-statistic = 58.6, adjusted $R^2 = 0.37$, p -value < 0.001). Consequently, we continued with the calculation of the RNW using Jaccard's pairwise dissimilarity (Jaccard, 1912), which ranges from 0-1 (either all or none of the species overlap in the compared sites, respectively).

Table 3.2. Average values for different soil parameters in five bio-climatic zones (BCZ) in three land use classes (LUC). Bio-climatic zones include Alpine (Alp), Atlantic (Atl), Continental (Con), Boreal (Bor) and Mediterranean (Med). Total nitrogen (N), Total carbon (C), Organic carbon (Org. C), clay, silt and sand content are given in percentages.

BCZ	LUC	WHC	Tot. N	Tot. C	Org. C	Plant Av. P (mg/l)	pH	Clay	Silt	Sand	CEC cmolc/kg
Alp	Arable	46	0.37	4.5	3.2	27	7.78	57	38	5	26.6
	Grass	53	0.62	7.1	6.2	94	6.14	31	41	28	27.3
	Forestry	62	0.82	13.3	12.7	55	5.56	36	38	26	29.0
Atl	Arable	33	0.22	2.9	2.1	137	7.13	23	32	45	18.1
	Grass	48	0.47	5.5	4.5	69	6.24	27	26	47	22.1
	Forestry	46	0.40	7.7	6.3	36	5.15	20	33	46	16.5
Bor	Forestry	80	0.79	31.5	32.1	122	4.02	14	21	65	22.0
Con	Arable	34	0.20	2.4	1.7	156	6.89	25	36	39	15.8
	Grass	49	0.57	7.0	6.2	64	6.36	31	41	28	25.4
	Forestry	54	0.53	9.4	9.1	46	5.20	23	30	47	20.8
Med	Arable	27	0.13	3.6	1.3	18	7.17	22	52	26	10.2
	Grass	32	0.20	2.0	1.7	70	7.41	20	33	47	15.4
	Forestry	31	0.17	1.7	1.4	69	5.16	14	29	57	4.4

Only species present in 10% of the sites were considered for calculation of the RNW, we refer to these species as target species from here onwards. For each target species, we selected all sites in which it was present. Then, a random subset of 8 sites (10%) was used to calculate pairwise Jaccard's dissimilarity (calculated using function ‘vegdist’ function from the ‘vegan’ package for r (Oksanen *et al.* 2018). This random selection procedure was repeated 100 times, as suggested in the literature, and we took the average

Jaccard's dissimilarity in these 100 repetitions as the target taxon's RNW, which corresponds to θ in the initial protocol (Fridley *et al.* 2007). This means that the RNW calculated for species present in a larger number of sites is more accurate than that calculated for species present in only 8 sites, where the calculation of RNW resulted in the same value for each iteration.

3.2.3 Robustness of the RNW calculation

To ensure that the selection of target species (species present in 10% of the sites) was not biased towards the specialists or the generalists, we selected species present in more than 2 sites (i.e., target and non-target species) and calculated the average Jaccard dissimilarity between the sites in which each species was present. We then examined the results and compared them to those obtained while calculating the RNW of the target species.

Second, if the sampling effort is not sufficient to represent the diversity of the entire soil community it is likely that non-dominant species (those that tend to occur with a low abundance) are underrepresented in the community and that their absence from an assemblage is not a reflection of environmental characteristics. Moreover, the inclusion of said species in the calculation of distance metrics such as Jaccard dissimilarity could increase the apparent dissimilarity between two assemblages due simply to the laws of probability. To explore the effects of differing sampling efforts on the community we (a) calculated and compared the RNW of the target species under different scenarios using Spearman's rank correlations (Hollander and Wolfe 1999). The scenarios included the calculation of RNW using the full species matrix, and using a random subset of the matrix including 75, 50 and 25% of the observed individuals. We also (b) compared the RNW calculated when excluding species that are more likely to have been lost from only some of the sites, which were identified during the random sub-setting of the species matrix.

Lastly, enchytraeid species might survive under unfavourable conditions for years (Postma-Blaauw *et al.* 2012; Beylich *et al.* 2015). We therefore compared (also using Spearman's rank correlations) the RNW obtained using the full species matrix to one that portrayed habitat preference. This second species matrix was obtained by using, for each target species, the sites in which it was more abundant than in the lowest quartile of its overall abundance distribution. To be able to compare the effects of this procedure on all the target species, we lowered the number of sites used for the calculation of each iteration of Jaccard's dissimilarity from 8 to 5.

3.2.4 Index of community specialisation

The target species were then classified into groups from specialists (smaller RNW) to generalists (larger RNW) by splitting the RNW values into natural groups using Jenks' natural breaks optimization (Jenks, 1967), and the fit for the grouping was measured using the Goodness of variance fit using the 'classInt' package for R (Bivand 2017), a measure based on sum of squares deviation between the values and the mean for each group, which ranges from 0 to 1 (from worse to perfect fit). This division into groups is intended to facilitate the calculation and interpretation of the I_{CS} . Enchytraeid species appointed to group one (extreme generalist) simply have a wider niche width than those enchytraeids appointed to the higher groups (specialists).

We then calculated an index of community specialisation such that: $I_{CS} = \sum_i^n PA_i \times RNW_i$, where the I_{CS} of each site was the result of multiplying the proportional abundance of target species i relative to the abundance of all target species (PA_i) by its RNW group value (RNW_i).

Lastly, we studied whether land use intensity had an effect on the I_{CS} . We consider land use intensity to increase from forest to grassland to arable land. We included covariates in the initial model that have been linked with the enchytraeid community distribution in the past, namely the percentage of organic matter (OM), soil pH, bio-climatic zone and soil texture (Jänsch *et al.* 2005). We found that average pH increased with decreasing land use intensity, but no other signs of collinearity, and the Variation inflation factor for the variables included in the model calculated with the 'corvif' function for R was below 3 for all variables, indicating no problems of collinearity (Zuur *et al.* 2009). Since the I_{CS} is contained between two numbers (from one to the number of RNW groups) we used a beta regression (Ferrari and Cribari-Neto 2004), which assumes the response variable is above 0 and below 1, using the 'betareg' function for R (Cribari-Neto and Zeileis 2010). Prior to analysis we rescaled the I_{CS} to be limited between 0 and 1, such that $I_{CSRi} = (I_{CSi} - 1) / (n - 1)$ where I_{CS} is the I_{CS} value calculated for site i , n is the number of RNW groups and I_{CSR} is the rescaled value and we transformed the zeroes and ones to values close to zero and one as per Cribari-Neto and Zeileis (2010). Starting with a full model, we dropped one covariate at a time until there were no further decreases in the AIC. Lastly, we verified model assumptions by exploring the diagnostic plots and by plotting the residuals of the best model versus each covariate in the model, as well as those excluded from the model.

3.3 Results and Discussion

3.3.1 *Enchytraeid* community

We identified 113 species in the transect (Tables S1-S2). Two forms of *C. sphagnetorum* (Vejdovský, 1878) *sensu lato* were distinguished, based on morphological differences as indicated in Chalupský (1992) and Schmelz and Collado (2010). The species status of these two forms of *C. sphagnetorum* was demonstrated later (Martinsson and Erséus 2014) and their valid names are now—after a transient change of the genus name into *Chamaedrillus* (Martinsson *et al.* 2015; Schmelz *et al.* 2015; ICZN 2018)—*Cognettia chlorophila* (Friend, 1911) and *Cognettia sphagnetorum*, respectively (Martinsson 2019). *C. sphagnetorum sensu stricto* itself is morphologically indistinguishable from another species, *C. pseudosphagnetorum* Martinsson, Rota & Erséus, 2015; for convenience, these two species are treated here as one.

3.3.2 Realised niche width

Out of the 113 species found in the transect, 23 species occurred in at least 8 sites (Table 3.3), 43 occurred in 2-7 sites and 47 were observed only in one site. The 23 target species occurred with an average of 36 species per 8 sites, with a maximum of 49 co-occurring species for *Fridericia bisetosa* (Levinsen, 1884) and a minimum of 11 for *C. sphagnetorum* (Vejdovský, 1878) *sensu* (Martinsson *et al.* 2015). Per site, each target species co-occurred with an average of 10 other species. *C. sphagnetorum* had the lowest realised niche width (0.56) and *Enchytronia parva* (Nielsen & Christensen, 1959) had the highest (0.83; Table 3.3). We hypothesized that the specialists would be more likely to be *K* strategists than *r* strategists, however, both strategies were common amongst relative specialists and generalists (Table 3.3). Similarly, no significant relationships were found between the RNW of nematodes and traits describing the nematode life strategies (Vazquez *et al.* 2019; Chapter 2). This might be a result of the numerous variables that characterize the soil habitat. The realised niche width of a species can be a response to a number of pressures acting together to define a species niche (Giller 1996).

Interestingly, the RNW of *C. sphagnetorum* was much smaller than that of *C. chlorophila* (0.79) even though both species were previously considered as one (Martinsson and Erséus 2014; Martinsson *et al.* 2015). When taken as one species, their combined RNW was 0.82, higher even than *C. chlorophila*. Moreover, *E. parva*, *F. connata*, and *F. bisetosa* (the three species with the largest RNW) and *Enchytraeus buchholzi* (the fifth largest

Table 3.3. Ecological information on 23 target species regarding their reaction to moisture (M), pH (R), salinity (S), life strategy (Str) (Graefe and Schmelz, 1999) and ecological group (Eco. G.). Species are organised with increasing realised niche width (RNW), from the furthestmost specialist to furthestmost generalist. The categories "epigeic, endogeic", and "indifferent" are equivalent to "litter dweller, soil dweller," and "intermediate" in Römboke et al. (2017), respectively.

Genus	Species	Author	RNW	RNW	M ¹	R ²	S ³	Str ⁴	Eco. G.
				Group					
<i>Cognettia</i>	<i>sphagnetorum</i>	Vejdovsky, 1878	0.563	6	7	2	0	A/F: R	epigeic
<i>Fridericia</i>	<i>schmelzi</i>	Cech & Dózsa-Farkas, 2005	0.719	5	x	7	0	K	endogeic
<i>Marionina</i>	<i>communis</i>	Nielsen & Christensen, 1959	0.725	5	5	7	0	K	epigeic
<i>Henlea</i>	<i>perpusilla</i>	Friend, 1911	0.730	5	x	7	1	K	indifferent
<i>Fridericia</i>	<i>ratzeli</i>	Eisen, 1872	0.733	5	x	7	0	K	endogeic
<i>Henlea</i>	<i>ventriculosa</i>	d'Udekem, 1854	0.735	5	x	7	1	K	indifferent
<i>Fridericia</i>	<i>isseli</i>	Rota, 1994	0.736	5	x	7	0	K	endogeic
<i>Fridericia</i>	<i>ulrikae</i>	Rota & Healy, 1999	0.737	5	x	7	0	K	endogeic
<i>Fridericia</i>	<i>galba</i>	Hoffmeister, 1843	0.737	5	x	7	0	K	endogeic
<i>Fridericia</i>	<i>christeri</i>	Rota & Healy, 1999	0.746	4	x	7	0	R/K	endogeic
<i>Marionina</i>	<i>mendax</i>	Rota, 2013	0.756	4	8	7	1	K	endogeic
<i>Fridericia</i>	<i>tuberosa</i>	Rota, 1995	0.758	4	x	7	0	K	endogeic
<i>Enchytronia</i>	<i>minor</i>	Möller, 1971	0.774	3	5	5	0	K	endogeic
<i>Buchholzia</i>	<i>appendiculata</i>	Buchholz, 1862	0.776	3	x	7	0	R/F	epigeic
<i>Enchytraeus</i>	<i>bulbosus</i>	Nielsen and Christensen, 1963	0.787	2	x	7	0	R/K	indifferent
<i>Fridericia</i>	<i>paroniana</i>	Issel, 1904	0.788	2	x	7	0	K	endogeic
<i>Cognettia</i>	<i>chlorophila</i>	Friend, 1911	0.789	2	5	2	0	A/F: R	epigeic
<i>Achaeta</i>	<i>sp. 3</i>		0.790	2	5	7	0	K	endogeic
<i>Enchytraeus</i>	<i>buchholzi</i>	Vejdovsky, 1879	0.791	2	x	7	x	R	indifferent
<i>Fridericia</i>	<i>bulboides</i>	Nielsen and Christensen, 1959	0.792	2	x	7	0	R/K	endogeic
<i>Fridericia</i>	<i>bisetosa</i>	Levinsen, 1884	0.796	2	x	7	0	K	endogeic
<i>Fridericia</i>	<i>connata</i>	Bretscher, 1902	0.797	2	x	7	0	K	endogeic
<i>Enchytronia</i>	<i>parva</i>	Nielsen & Christensen, 1959	0.827	1	5	6	0	A	endogeic

1. x – indifferent or unknown; 5 – indicator of fresh soils; 7 – indicator of damp soils; 8 – indicator of wet soils

2. 2 – extremely acidic; 5 – occasionally in strongly acid soils; 6 – Between 5 and 7; 7 – Slightly acid to slightly alkaline

3. x – indifferent or unknown; 0 – Not salt supporting; 1 – salt supporting, but mostly in soils poor in salt.

4. R – r-selected opportunist; K – k-selected persistent species; A – stress tolerant; F – asexual reproduction.

RNW) are all considered species complexes, that is each of these is made up of a group of species that are difficult to separate using traditional morphological characters (Schmelz and Collado 2010). The relative behaviour of these species as generalists might be a result of the combination of several relative specialists, or a combination of specialists and relative generalists, as in the case of *Cognettia* spp. described above. In fact, some authors have recommended using species when using enchytraeids as indicators (Jänsch *et al.* 2005; Römbke *et al.* 2013), due to the observed differences in ecological preferences within genera. On the other hand, several species within a genus have similar ecological behaviour; for example, most species of *Fridericia* prefer slightly acid to slightly alkaline soils, and many species of *Enchytraeus* are *r*-strategists (Graefe and Schmelz, 1999). Accurately assessing the impact of not resolving species complexes is therefore not straightforward. The literature researching the ecology of enchytraeid species has so far not separated these species complexes, since this would require the additional use of recently developed molecular taxonomical discrimination tools (Schmelz *et al.* 2017).

While species complexes could explain part of the tendency towards generalism, enchytraeid species might in fact be quite tolerant to different soil characteristics. Römbke *et al.* (2013) gathered the degree of association of several enchytraeid species to different classes of soil organic matter, pH, texture and land use, and while many species showed a higher association with a specific class of one of these variables, they could often be found in sites with very different characteristics. *Henlea perpusilla* (which is the fourth species with the narrowest niche width) in fact showed a significant association with grasslands and croplands, but could on occasion be found in deciduous and coniferous forests. Another example of tolerance to different systems was observed by Postma-Blaauw *et al.* (2012), who observed that half of the species present in a long term grassland were still present in the field three years after a switch to arable farming. There is a jump from the *C. sphagnetorum* to the nearest species in terms of specialism of 0.15, which is a lot, considering the next largest difference between the RNW of two enchytraeid species is that between *E. parva* and *F. connata* of 0.03. This might indicate a larger difference in RNW between *C. sphagnetorum* and the rest of the studied species. In our study, *C. sphagnetorum* occurred only in sites with a pH below 5, while *C. chlorophila* was found in those same sites, but also in some forests with a pH between 5 and 7. In fact, when we calculated the average Jaccard dissimilarity between sites inhabited by non-target species (see section 3.3.2) we found that *Mesenchytraeus flavus*, with an average Jaccard dissimilarity of 0.44, scores as even more specialised than *C. sphagnetorum*; *M. flavus* was found in acid forests (3 sites), which agrees with data available for this species in the Edaphobase database (accessed on the 25th of September, 2020). This could indicate that habitat specialism in enchytraeids (as calculated in this study) is a reflection of tolerance to extreme soil acidity. It has been

shown that soil pH and soil moisture are more important for explaining the occurrence of enchytraeid species than land use (Graefe and Schmelz 1999; Graefe and Beylich 2003; Beylich and Graefe 2007). Strongly acid soils have an almost completely different enchytraeid community than slightly acid or neutral soils; the turning point lies at about a pH of 4.2 (CaCl₂), which coincides with the absence or presence of endogeic and anecic earthworms (Graefe and Beylich 2003). Among the few species that bridge this gap is *E. parva*, which may further explain its score as the most generalist of all enchytraeid target species.

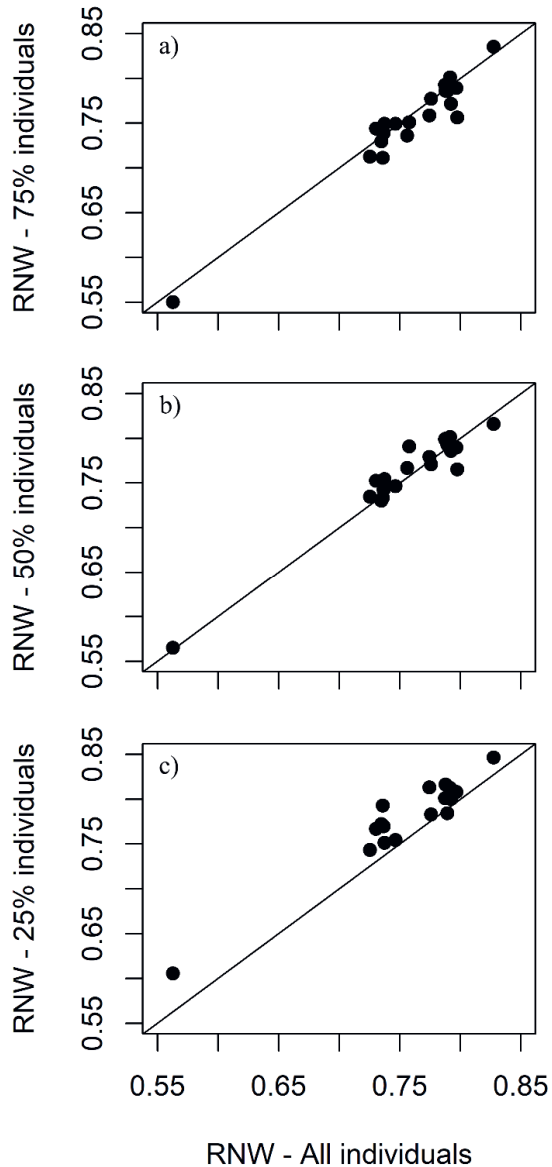
3.3.3 Sensitivity of the RNW to diverse scenarios

The selection of target taxa did leave out species that have the potential to be habitat specialists. *M. flavus* and *F. minor* had a smaller average Jaccard dissimilarity between sites (0.44 and 0.47, respectively) than that of *C. sphagnetorum* and another 8 species scored between *C. sphagnetorum* and *F. schmelzi*. Like *C. sphagnetorum*, *M. flavus* is found almost exclusively in acid forests (see above). We do not believe, however, that limiting target taxa to those observed in at least 8 sites created a bias, since non-target enchytraeids occurred on both sides of the niche width spectrum, reaching a maximum RNW of 0.95 in the case of *Achaeta eiseni* (2 sites). In fact Postma-Blaauw *et al.* (2012) found that *A. eiseni* persisted after land use conversion from grassland to arable land, and was found in both long term arable land and grassland. We suggest that further studies are conducted that include a more diverse set of habitats.

Subsets of the species matrix that contained 75, 50 and 25% of the individuals in the original dataset resulted in the loss of a maximum of 3 species per site (in the first two cases) and up to 7 species per site when only 25% of the individuals were included. Thirteen species were lost from the entire matrix. Our results indicate that the calculated RNW after random reduction in sampling effort did not lead to significant shifts in the RNW rank unless the sites were severely under sampled (Figure 3.2). The RNW calculated using the matrix containing 75% of the individuals differed from the original RNW by a maximum of 0.04, and a Spearman rank correlation of 0.86 (Figure 3.2a). However, a subset of 25% of individuals led to a variation in the RNW of 0.06, and decreased the spearman correlation between the original RNW and that calculated with the reduced matrix to 0.68 (Figure 3.2c). In this case, we also observed an overall shift towards specialism for all species. As we expected, rare species increase the Jaccard dissimilarity between sites, and by eliminating part of that random variation, the RNW becomes smaller. This effect, however seems to have an impact only with a significant reduction in the number of individuals sampled. In fact, the original RNW and the one calculated without these 13 rare species (but with all the other individuals included) have

a Spearman correlation coefficient of 0.97, with a maximum variation of 0.012, implying that the calculation of RNW is robust, and that only under strong under-sampling conditions does the index diverge from the original RNW values. While repeating this exercise with a larger set of sites might increase our understanding of the niche width of non-target species, this exercise proves the importance of sampling with a same effort, thus the use of datasets gathered from studies with different sampling methods and identifications methods are discouraged.

Figure 3.2. Effects of different sampling efforts on the calculation of the realised niche width. The realised niche width was calculated either using the whole species matrix (x-axis) or using a randomised subset that included 75%, 50% or 25% of the individuals observed in each site (a, b and c respectively).



3.3.4 Community level specialisation

We grouped the enchytraeid species into natural groups using Jenks natural breaks optimization and obtained 6 groups, where *C. sphagnetorum* and *E. parva* are the only enchytraeid species in their group (Goodness of variance fit = 0.99; Table 3.3). Opposite to our initial expectations regarding the effects of land use on the enchytraeid community, neither land use nor bio-climatic region explained the variation in the I_{CS} (Figure 3.3). In fact, according to the model with the lowest AIC the logarithm of organic matter content (which was strongly correlated to soil moisture) was the only factor that explained a portion of the variation in the I_{CS} (Beta regression, Pseudo $R^2 = 0.04$, log-likelihood of 23.8 on 3 degrees of freedom).

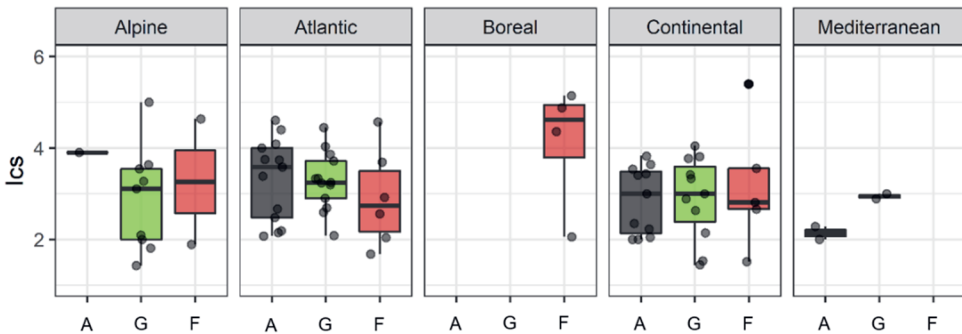


Figure 3.3. Index of community specialisation (I_{CS}) of the Enchytraeid community in 79 sites across five bio-climatic zones and three land use types: arable farming (A), grassland (G) and forestry (F).

However, this relationship was mainly driven by the high I_{CS} in boreal sites (Figure 3.3). Boreal sites had the highest organic matter content and lowest pH (Table 3.1), and had a high proportion of *C. sphagnetorum* (Table S1). In fact, repeating the model selection process but having excluded boreal sites led to an intercept only model, meaning that none of the explanatory variables included in the model could explain the variation in the I_{CS} . Few studies on soil communities have explored this aspect of the community. Amossé *et al.* (2016) found a negative relationship between soil age and the proportion of *r-strategist* enchytraeids, and suggest that the proportion of *r-strategists* might be a good indicator of urban soil disturbance. Using the data reported in their paper, we found no significant correlation between soil age and the I_{CS} , a result that supports our observation that RNW cannot solely be explained by life strategy. The level of community specialisation of the enchytraeid community is not a good indicator of land use type. There are several hypothesized mechanisms to explain the wide biodiversity of soil organisms (Wardle and Giller 1996; Bardgett 2002; Giller 1996). Amongst the more important are the diversity of food resources, habitat heterogeneity in both time and space, the spatial scale of habitats, which depends on each species mobility within

the soil and for example resource partitioning. Such variation has led to a broad range of strategies. Human disturbance acts like an environmental filter to specific enchytraeid species (Kapusta and Sobczyk 2015; Postma-Blaauw *et al.* 2010; Postma-Blaauw *et al.* 2012) and there is some evidence that supports the use of community weighted trait values (body mass in particular) as an assessment of environmental impact (Nowak 2004). However, a change in pH, might affect only some specialists, while others (for example species strongly limited by moisture content) will not. Therefore, a community weighted mean of niche width might not be a good indicator when the trait under study is the result of diverse environmental pressures.

3.4 Conclusion

We calculated the RNW of 23 enchytraeid species, and grouped them accordingly. *C. sphagnetorum* was the species with the smallest niche width, a classification supported by the literature. At the opposite end of the niche width spectrum from *C. sphagnetorum* we observed *E. parva* an enchytraeid species classified as stress tolerant and that can occur in communities typical of both neutral and acid soils. However, pH adaptations alone do not seem to explain the RNW of the rest of the target enchytraeid species. Some of the species with the broadest RNW (generalists) were in fact species complexes. These species being classified as generalists could be the result of combining the preferred habitats of several relative specialists, as is the case when combining *C. sphagnetorum* and *C. chlorofila*, two species that were until recently (Martinsson *et al.* 2019) combined under the umbrella of *C. sphagnetorum*. The calculation of RNW under different scenarios did not lead to any significant changes in the ranking of species in the generalist to specialist scale, meaning that this method is robust to diverse scenarios, but the simulations highlighted the importance of using datasets with equal sampling efforts, since excluding rare species can shift the RNW of all enchytraeids. Lastly, despite our expectations, the I_{CS} of the enchytraeid community was not a useful tool in measuring the impact of land use, which might be the result of a diversity of environmental constraints leading to the evolution of specific RNWs.

Supplementary information

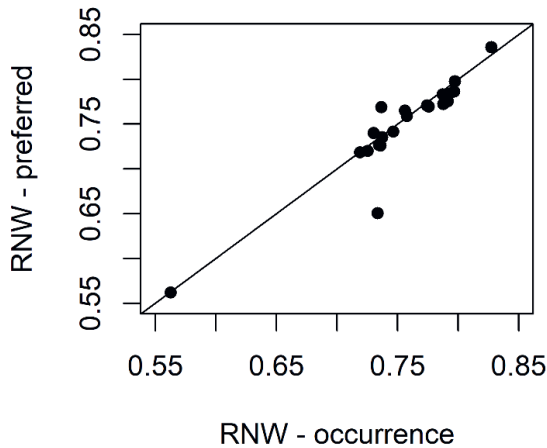


Figure S1. Realised niche width (RNW) of 23 target species calculated using the protocol proposed by Manthey and Fridley (2009), which results from the calculation of the average Jaccard dissimilarity between a random subset of the sites inhabited by the target species, averaged after 100 iterations. On the x-axis we present the RNW calculated using all sites in which the target species was present (hence, occurrence) and on the y-axis we present the RNW calculated using only the sites in which the target species occurred with an abundance higher than that of its lowest quartile (hence, “preferred” habitats).

Table S1. Species, author and code of the enchytraeid species found in the transect.

Genus name	Species epitheton	Author+year alternative	Code
<i>Achaeta</i>	<i>abulba</i>	Graefe, 1989	ACABU
<i>Achaeta</i>	<i>affinis</i>	Nielsen & Christensen, 1959	ACAFF
<i>Achaeta</i>	<i>bifollicula</i>	Chalupský, 1992	ACBIF
<i>Achaeta</i>	<i>bohemica</i>	Vejdovský, 1879	ACBOH
<i>Achaeta</i>	<i>eiseni</i>	Vejdovský, 1878	ACEIS
<i>Achaeta</i>	<i>sp. 1</i>		ACSP1
<i>Achaeta</i>	<i>sp. 2</i>		ACSP2
<i>Achaeta</i>	<i>sp. 3</i>		ACSP3
<i>Achaeta</i>	<i>sp. 4</i>		ACSP4
<i>Achaeta</i>	<i>sp. 5</i>		ACSP5
<i>Achaeta</i>	<i>sp. 6</i>		ACSP6
<i>Achaeta</i>	<i>pannonica</i>	Graefe, 1989	ACPAN
<i>Achaeta</i>	<i>unibulba</i>	Graefe, Christensen & Dózsa-Farkas, 2005	ACUNI
<i>Bryodrilus</i>	<i>ehlersi</i>	Ude, 1892	BREHL
<i>Buchholzia</i>	<i>appendiculata</i>	Buchholz, 1862	BUAPP
<i>Buchholzia</i>	<i>fallax</i>	Michaelsen, 1887	BUFAL
<i>Buchholzia</i>	<i>sp. 1</i>		BUSP1
<i>Buchholzia</i>	<i>subterranea</i>	Černosvitov, 1937	BUSUB
<i>Cernosvitoviella</i>	<i>atrata</i>	Bretscher, 1903	CEATR
<i>Cernosvitoviella</i>	<i>microtheca</i>	Rota & Healy, 1999	CEMIC
<i>Cernosvitoviella</i>	<i>minor</i>	Dózsa-Farkas, 1990	CEMIN
<i>Cernosvitoviella</i>	<i>sphaerotheca</i>	Healy, 1975	CESPH
<i>Cognettia</i>	<i>chlorophila</i>	Friend, 1913	COCHL
<i>Cognettia</i>	<i>cognettii</i>	Issel, 1905	COCOG
<i>Cognettia</i>	<i>varisetosa</i>	Martinsson, Rota & Erséus, 2015	COGLA
<i>Cognettia</i>	<i>sphagnetorum</i>	Vejdovský, 1878	COSPH
<i>Enchytronia</i>	<i>holochaeta</i>	Dózsa-Farkas, 2019	EOBAL
<i>Enchytronia</i>	<i>minor</i>	Möller, 1971	EOMIN
<i>Enchytronia</i>	<i>parva</i>	Nielsen & Christensen, 1959	EOPAR
<i>Enchytronia</i>	<i>pygmaea</i>	Graefe & Schmelz, 2017	EOPYGM
<i>Enchytronia</i>	<i>sp. 1</i>		EOSP1
<i>Enchytronia</i>	<i>sp. 2</i>		EOSP2
<i>Enchytronia</i>	<i>sp. 3</i>		EOSP3
<i>Enchytronia</i>	<i>sp. 4</i>		EOSP4
<i>Enchytraeus</i>	<i>buchholzi</i>	Vejdovský, 1878	ESBUC
<i>Enchytraeus</i>	<i>bulbosus</i>	Nielsen & Christensen, 1963	ESBUS

Genus name	Species epitheton	Author+year alternative	Code
<i>Enchytraeus</i>	<i>dichaetus</i>	Schmelz & Collado, 2010	ESDIC
<i>Enchytraeus</i>	<i>lacteus</i>	Nielsen & Christensen, 1961	ESLAC
<i>Enchytraeus</i>	<i>norvegicus</i>	Abrahamsen, 1969	ESNOR
<i>Enchytraeus</i>	<i>sp. 1</i>		ESSP1
<i>Fridericia</i>	<i>alata</i>	Nielsen & Christensen, 1959	FRALA
<i>Fridericia</i>	<i>auritoides</i>	Schmelz, 2003	FRAUI
<i>Fridericia</i>	<i>benti</i>	Schmelz, 2002	FRBEN
<i>Fridericia</i>	<i>bisetosa</i>	Levinsen, 1884	FRBIS
<i>Fridericia</i>	<i>bretscheri</i>	Southern, 1907	FRBRE
<i>Fridericia</i>	<i>brunensis</i>	Schlaghamerský, 2007	FRBRU
<i>Fridericia</i>	<i>bulboides</i>	Nielsen & Christensen, 1959	FRBUI
<i>Fridericia</i>	<i>christeri</i>	Rota & Healy, 1999	FRCHR
<i>Fridericia</i>	<i>connata</i>	Bretscher, 1902	FRCON
<i>Fridericia</i>	<i>cylindrica</i>	Springett, 1971	FRCYL
<i>Fridericia</i>	<i>deformis</i>	Möller, 1971	FRDEF
<i>Fridericia</i>	<i>discifera</i>	Healy, 1975	FRDIS
<i>Fridericia</i>	<i>dozsae</i>	Schmelz, 2003	FRDOZ
<i>Fridericia</i>	<i>dura</i>	Eisen, 1879	FRDUR
<i>Fridericia</i>	<i>galba</i>	Hoffmeister, 1843	FRGAL
<i>Fridericia</i>	<i>glandifera</i>	Friend, 1913	FRGLI
<i>Fridericia</i>	<i>healyae</i>	Schmelz, 2003	FRHEA
<i>Fridericia</i>	<i>hegemon</i>	Vejdovský, 1878	FRHEG
<i>Fridericia</i>	<i>isseli</i>	Rota , 1994	FRISS
<i>Fridericia</i>	<i>maculatiformis</i>	Dózsa-Farkas, 1972	FRMAF
<i>Fridericia</i>	<i>magna</i>	Friend, 1899	FRMAG
<i>Fridericia</i>	<i>minor</i>	Friend, 1913	FRMIN
<i>Fridericia</i>	<i>monochaeta</i>	Rota, 1995	FRMON
<i>Fridericia</i>	<i>paroniana</i>	Issel, 1904	FRPAR
<i>Fridericia</i>	<i>connatiformis</i>	Dózsa-Farkas, 2015	FRPCON
<i>Fridericia</i>	<i>perrieri</i>	Vejdovský, 1878	FRPER
<i>Fridericia</i>	<i>nemoralis</i>	Nurminen, 1970	FRPNEM
<i>Fridericia</i>	<i>ratzeli</i>	Eisen, 1872	FRRAT
<i>Fridericia</i>	<i>rendsinata</i>	Dózsa-Farkas, 1972	FRRES
<i>Fridericia</i>	<i>schmelzi</i>	Cech & Dózsa-Farkas, 2005	FRSCH
<i>Fridericia</i>	<i>semisetosa</i>	Dózsa-Farkas, 1970	FRSEM
<i>Fridericia</i>	<i>singula</i>	Nielsen & Christensen, 1961	FRSIN
<i>Fridericia</i>	<i>sp. 1</i>		FRSP1
<i>Fridericia</i>	<i>sp. 2</i>		FRSP2

Genus name	Species epitheton	Author+year alternative	Code
Fridericia	<i>sp. 3</i>		FRSP3
Fridericia	<i>sp. 4</i>		FRSP4
Fridericia	<i>sp. 5</i>		FRSP5
Fridericia	<i>sp. 6</i>		FRSP6
Fridericia	<i>sp. 7</i>		FRSP7
Fridericia	<i>sp. 9</i>		FRSP9
Fridericia	<i>sp. 10</i>		FRSP10
Fridericia	<i>sp. 11</i>		FRSP11
Fridericia	<i>sp. 12</i>		FRSP12
Fridericia	<i>sp. 13</i>		FRSP13
Fridericia	<i>sp. 14</i>		FRSP14
Fridericia	<i>sp. 15</i>		FRSP15
Fridericia	<i>sp. 16</i>		FRSP16
Fridericia	<i>sp. 17</i>		FRSP17
Fridericia	<i>sp. 18</i>		FRSP18
Fridericia	<i>sp. 19</i>		FRSP19
Fridericia	<i>sp. 20</i>		FRSP20
Fridericia	<i>sylvatica</i>	Healy, 1979	FRSYL
Fridericia	<i>tubulosa</i>	Dózsa-Farkas, 1972	FRTUL
Fridericia	<i>tuberosa</i>	Rota, 1995	FRTUS
Fridericia	<i>ulrikae</i>	Rota & Healy, 1999	FRULR
Henlea	<i>nasuta</i>	Eisen, 1878	HENAS
Henlea	<i>perpusilla</i>	Friend, 1911	HEPER
Henlea	<i>similis</i>	Nielsen & Christensen, 1959	HESIM
Henlea	<i>ventriculosa</i>	d'Udekem, 1854	HEVEN

Code	Alpine						Atlantic						Boreal		Continental				Mediterranean								
	Arable		Grass		Forestry		Arable		Grass		Forestry		Forestry		Arable		Grass		Forestry		Arable		Grass		Forestry		
	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a	± sd	a
BUSUB	-	-	-	-	43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
CEATR	-	-	-	-	-	-	33	-	2	-	-	-	-	-	1	-	3	-	-	-	-	-	-	-	-	-	
CEMIC	-	-	-	-	-	-	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
CEMIN	-	-	-	-	-	-	72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
CESPH	-	-	-	-	-	-	-	-	-	-	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
COCHL	-	-	114 ± 130.1	-	-	-	-	-	100.3 ± 183.9	-	84 ± 47.8	-	66 ± 35.4	81.7 ± 111.3	-	-	-	-	-	-	-	-	-	-	-	-	
COCOG	-	-	-	-	-	-	-	-	-	35	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	
COGLA	-	-	-	-	7	-	3 ± 1.4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
COSPH	-	-	-	-	399	-	-	-	677	-	108.8 ± 76.1	-	35 ± 25.5	-	-	-	-	-	-	-	-	-	-	-	-	-	
EOBAL	-	4 ± 4.4	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
EOMIN	-	-	-	-	-	-	21.2 ± 33.3	14.4 ± 17.8	7	-	-	-	4 ± 2.6	9.2 ± 7.2	9	-	-	-	-	-	-	-	1	-	-	-	
EOPAR	-	5.3 ± 3	5	-	-	-	6	56.8 ± 58	29.7 ± 42.8	-	-	-	-	172.7 ± 149.9	17 ± 19.8	-	-	-	-	-	-	-	-	-	-	-	-
EOPYGM	-	-	-	-	-	-	-	-	7	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
EOSP1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
EOSP2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
EOSP3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	
EOSP4	-	-	-	-	-	-	-	-	7.7 ± 4.2	-	-	-	79	-	-	-	-	-	-	-	-	-	-	-	-	-	
ESBUC	26	19.8 ± 15.8	-	-	-	-	45 ± 70.5	60.8 ± 105.1	13 ± 17	-	-	-	64.3 ± 90.9	14.1 ± 23.2	4	-	3.5 ± 2.1	1	-	-	-	-	-	-	-	-	
ESBUS	5	11 ± 16.2	9	-	-	-	17.1 ± 18.6	11 ± 8.9	13.7 ± 10.4	-	-	-	14.9 ± 12.6	17 ± 7.1	7	-	-	4	-	-	-	-	-	-	-	-	
ESDJC	-	1	-	-	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
ESLAC	-	-	-	-	-	-	-	4	-	-	-	-	4	3	1	-	-	-	-	-	-	-	-	-	-	-	

Code	Alpine			Atlantic			Boreal			Continental			Mediterranean		
	Arable	Grass	Forestry	Arable	Grass	Forestry	Arable	Grass	Forestry	Arable	Grass	Forestry	Arable	Grass	Forestry
	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd	a ± sd
FRMAF	-	15 ±	-	11	10.7 ± 9.3	-	-	-	-	180	-	-	-	-	-
FRMAG	-	1 ±	-	-	-	3 ± 1.4	-	-	-	-	-	-	-	-	-
FRMIN	-	-	-	-	9 ± 4.2	-	-	-	-	-	-	-	-	-	-
FRMON	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
FRPAR	-	10.7 ± 11.9	-	3.5 ±	0.7 16.3 ± 16.8	6.5 ± 5.5	-	21.3 ± 33.5	15 ± 12.7	-	-	-	-	-	-
FRPCON	-	-	-	4 ±	0	-	-	-	-	-	-	-	-	-	-
FRPER	-	6	-	-	32.3 ± 35.8	-	-	-	-	-	-	-	-	-	-
FRPNEM	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
FRRAT	-	7 ± 9.5	-	6 ±	7.1	-	-	15.5 ± 19.1	5	-	-	-	-	-	-
FRRES	-	-	-	-	-	-	-	-	35	-	-	-	-	-	-
FRSCH	-	9 ± 6.1	-	4	3 ± 1.4	-	-	5 ± 4.2	-	-	-	-	-	-	-
FRSEM	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-
FRSIN	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-
FRSP1	-	-	-	-	-	268	-	-	-	-	-	-	-	-	-
FRSP2	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-
FRSP3	-	6	-	-	8.5 ± 9.3	1	-	1	-	-	-	-	-	-	-
FRSP4	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
FRSP5	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-
FRSP6	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
FRSP7	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-
FRSP9	-	-	-	-	-	-	-	-	-	-	-	-	13	-	2

Chapter 4

The evolution of biological soil quality under long term agricultural management

C. Vazquez, R.G. M. de Goede, G.W. Korthals, M. Rutgers, R.E. Creamer

Abstract

Sustainable agricultural practices rely on the delivery of soil-based ecosystem services, which depend on processes mediated by the soil's fauna. However, agricultural practices often have negative impacts on soil biota. What is still unclear is whether the impacts associated with agriculture lead to a soil community that is adapted to said impacts or whether the community continues to evolve under agricultural management, since there is a gap in studies of soil biology that include time as a covariate. Using time-series analyses, we investigate how and whether biological soil quality (as measured by the nematode community) has developed through time in cropping systems situated on two contrasting soil textures and under different management regimes in The Netherlands. We sampled two experiments (the *Soil Health Experiment* in Vredepeel (SHE) and the *Broekmahoeve Applied Soil Innovation Systems* experiment in Lelystad (BASIS)) and 20 commercial farms at several points in time. We found changes in the nematode community through time in all three systems, albeit not consistent across the systems or treatments. All treatments at the SHE showed an increase in nematode richness, abundance as well as the structure and enrichment indices over time. The nematode community in BASIS also showed an increase in richness through time in all treatments except for conventional management combined with conventional tillage. In commercial farms, however, we observed a decrease in the structure index and an increase in community specialisation. While we expect that the trends observed at the SHE are a result of change in the intensity of management at the beginning of the experiment, the trends observed in the commercial farms indicate a widespread decrease in the soil's food-web structure, possibly associated with an increase in land use intensity. We observed that the nematode community continues to change over time, but whether the observed trend reflects an increase of the intensity of land management in time or that the effects of sustained agricultural land management continue to alter the nematode community over time remains uncertain.

4.1 Introduction

Soil-based ecosystem services (biomass production, biodiversity conservation, erosion control, pest and disease control, water quality and supply and climate regulation) are instrumental to human society (Kibblewhite *et al.* 2008; Adhikari and Hartemink 2016). The delivery of these ecosystem services depends on soil processes that rely strongly on the soil's living fraction, a part of soil quality that has often been neglected (Kibblewhite *et al.* 2008; Brussaard 2012; Bünemann *et al.* 2018). The cycling of nutrients, for example, as well as decomposition processes rely heavily on the microbial community, and both processes are facilitated by soil invertebrates, such as micro-arthropods, enchytraeids and nematodes (Coleman 2008; Buchan *et al.* 2013; Gebremikael *et al.* 2016; Jihua *et al.* 2010). Changes in the soil structure brought forth by ecosystem engineers can alter the rates at which such processes take place (Porre *et al.* 2016; Schon *et al.* 2017). Disease suppression (another soil mediated function) can also be affected by microbes and nematodes. These groups are often the source of plant disease, but can also lead to suppression by, for example, direct predation on parasites (Carrascosa *et al.* 2015; Sánchez-Moreno and Ferris 2007). Therefore, maintaining a functioning biological community in the soil is of utmost importance in sustainable soil management (Brussaard *et al.* 2007; Lavelle *et al.* 2006; Griffiths *et al.* 2018).

There is no denying that agriculture has had a significant impact on biodiversity (Tsiafouli *et al.* 2015; Brondizio *et al.* 2019). A strong disturbance, such as a change in land use from grassland to arable land, can reduce the soil's invertebrate richness, abundance and functional diversity (Tsiafouli *et al.* 2015; Postma-Blaauw *et al.* 2010; van Eekeren *et al.* 2008). The soil invertebrate community exists in a delicate equilibrium regulated via feed-back mechanisms between nearby organisms. Once such systems are disturbed it can take a long time until these mechanisms, and thus the entire soil community, recovers (Jouquet *et al.* 2006; Postma-Blaauw *et al.* 2012; Adl *et al.* 2006). Agricultural soils also present a great potential for biodiversity (Norris 2008). Organic management, for example, has been shown to promote biodiversity both above and below ground (Bengtsson *et al.* 2005; Tuck *et al.* 2014). Conservation tillage can positively impact the richness and abundance of several soil organisms (Sapkota 2012) and lead to soil communities similar to those found in undisturbed sites (Adl *et al.* 2006).

The relationship between soil organisms and soil functioning has led to the inclusion of biological indicators on soil quality assessments (Bünemann *et al.* 2018). Nematodes, for example, are often used as indicators of biological soil quality, for several reasons: (1) they are present in almost all soils and are easily extractable; (2) they exist at different levels of the soil's food-web, and as such, their functional diversity can represent the overall state of the soil's food-web; and (3) they respond quickly to changes in the

physico-chemical environment. For this reason, indicators have been developed to monitor different parts of the community and observe changes due to shifts in nutrient availability or recent disturbances (Bongers 1990; Ferris *et al.* 2001; Neher 2001).

Most studies on the effects of land use on soil biodiversity reflect a snapshot in time, but there is a lack of studies into soil biodiversity and the effects of land use that incorporate time as a covariate, rather than explore the before and after effects of a disturbance (Guerra *et al.* 2020; Cameron *et al.* 2018). Evidence suggests that continued disturbances (such as yearly tillage) might select for the organisms that can survive the stresses associated with such disturbances, either due to lack of sensitivity to the type of stress, or due to an ability to quickly reproduce and/or colonize the disturbed site (van Eekeren *et al.* 2008; Vazquez *et al.* 2019 (Chapter 2); Crittenden *et al.* 2014). This adaptation, however, can leave the community sensitive to other disturbances: (Van Der Wurff *et al.* 2007), for example observed that nematode communities adapted to heavy metal pollution (regardless of the initial pollutant levels in the soil) were not affected by Zinc additions, however, nematode communities from soils with larger pollutant concentrations were more sensitive to heat shock than those communities from soils with a lower pollution levels.

In this work we investigate how and whether biological soil quality as measured by the nematode community has changed over time, in arable farms situated on two contrasting soil textures and under different management regimes. We took soil samples from 20 commercial farms at three different time points over the course of twenty years and from two experimental farms (one on sandy soil and the other on a clayey soil) studying the effects of different management techniques at four time points spread over approximately 10 years. We also investigate which management practices promote an increase in the soil's biological quality, as measured by the selected nematode indicators. We expect to observe a trend in time in the nematode community related to agricultural management such that organic management and conservation tillage practices will have a positive impact on the nematode community, whilst conventional farming practices will either lead to a negative impact on the nematode community, or result in no observable trends over time if the nematode community of these arable fields is adapted to the impacts of farming. We aim to answer the following research questions:

1. Has agricultural management induced changes in the nematode community during the time of study?
2. Have the effects of agricultural management through time been more prevalent on the nematode communities of certain soil textures?
3. Have the effects of agricultural management through time been more prevalent on the nematode communities under different management strategies (namely

conventional vs. organic), and tillage (conventional tillage and minimum tillage) regimes?

4.2 Methods

To answer our research questions, we sampled soils from two experimental farms (4.2.1) and twenty commercial farms (4.2.2) a minimum of three times between 2007 and 2018 (experimental farms) or 2001-2018 (commercial farms). We identified and calculated several nematode indicators (4.2.3) and, to account for changes in the environment, we obtained weather data from local weather stations (4.2.4). Lastly, we carried out statistical analyses (4.2.5) to test the changes in time for the selected nematode indicators, as well as for the nematode community as a whole.

4.2.1 Experimental farms

Soil samples were taken from two long term experimental farms in the Netherlands, where the conditions and soil quality had been closely monitored: The Soil Health Experiment in Vredepeel (52° 32'N, 5° 52'E) and BASIS (*Broekemahoeve Applied Soil Innovation Systems*) situated in Lelystad (52° 32'N, 5° 34'E).

The SHE is situated on a reclaimed peatland, cultivated since 1955. The soil is a cultivated Gleyic Podzol with 1.1% clay, 3.7% silt and 94.9% fine sand (Boesten and Van der Pas 2000; Korthals *et al.* 2014). The experiment was set up in 2006 to study alternative pest control methods as well as to explore the effects of different farming systems (conventional versus organic) applied either with common practices or best practices with the focus on the control of plant parasitic nematodes (Korthals *et al.* 2014). In the top 20 cm of soil, the pH ranged between 5.3 and 5.9, and organic matter ranged between 2.5 and 4.3%. In 2006, the site was fertilised with liquid cattle manure and ploughed, after which wheat (*cv. Taifun*) was cultivated under conventional agricultural management. After the harvest (July 2006) the field was divided into four blocks, each consisting of 4 subplots that differed in management. Each subplot was managed either conventionally (using synthetic fertilizer and herbicides) or organically (using animal manures and manual weeding). In each subplot, the management of plant parasites was either conventional or what we refer to as “best practices”, which included for example the use of cover crops specifically suited for the prevention of *Pratylenchus penetrans* and *Ventricillium dahlia*. Each subplot was then divided into plots of 6x6 m where different soil health treatments were applied. In this study we focussed on the comparison of the effects of conventional management both in terms of fertilization and pest control (conventional management) versus the effects of organic management combined with best practices for the control of plant parasites (integrated management

from here onwards). Samples were taken in spring of 2007, 2009, 2011 and 2018 from the top 20 cm and used for chemical analysis and nematode identification.

The BASIS experiment is situated in a polder reclaimed in 1957 and consists of three organic fields and two conventional fields. The soil is composed of 61% sand, 22% silt and 17% clay (calcareous marine clay loam soil; Fluvisol WRB). On average in the 0-20 cm soil layer, the soil pH (KCl) ranges between 7.2 and 7.4, and soil organic matter ranges from 3.4-3.8% (in the organic fields) and 3.2-3.5% (in the conventional fields). Prior to 2009, all plots were annually ploughed with a mouldboard plough. Synthetic fertilizers and pesticides have not been used in the organic fields since 2002, and these fields were certified as organic in 2004. Each field is 95 by 183 m and is split into four blocks. Plots of 13 by 85 m were split into 4 controlled traffic lanes of 3.15 m wide. Each block has three tillage treatments: conventional tillage with mouldboard plough (CT; 23-25 cm), and two types of minimum tillage: non-inversion tillage with sub soiling (sub-soiling of 18-20 cm), and non-inversion tillage without subsoiling. The tillage treatments are arranged in a randomised complete block design. In this study we studied the CT and the non-inversion tillage without subsoiling treatments in two conventional fields and two organic fields (Table 4.1). In one of the organic fields non-inversion tillage without subsoiling switched to non-inversion tillage with subsoiling from 2011 onwards. In 2018 the non-inversion tillage treatment in one of the conventional fields was ploughed with a third variation, a shallow plough (15cm), which was included as a treatment from that year onwards. Therefore, we will compare conventional tillage to minimum tillage (MT) which included all alternative tillage treatments. Soil samples for chemical and biological analyses were taken in November of 2009 2012, 2014 and 2018 from the top 20 cm and sent to an external lab, where the samples were either dried for chemical analyses or used to assess the nematode community.

4.2.2 Commercial farms

As part of the National Soil Monitoring Network (NSMN; Rutgers *et al.* (2009)), in the Netherlands, soil quality was monitored from 1992 to 2014 in a range of land uses and across different soil texture classes: sand and marine clay. Every five years, the NSMN sampled 200 locations some of which were visited more than once for the duration of the NSMN campaign. For this study we selected arable farms that had been sampled on at least two occasions, with a minimum of 5 years interval between the samplings. From this subset of sites, we selected farms which were situated on two dominant soil texture classes in the Netherlands (sand and marine clay) (Siderius and De Bakker 2003) and with no reported changes in management style. This resulted in 20 farms which were sampled in 2001 or 2002 (first sampling event), 2008 or 2009 (second sampling

event) and in 2018 (third sampling event). The last sampling was done specifically for this project, as the NSMN is no longer in operation. Nine of these farms (6 organic and 3 conventional) were situated on sandy soils, while the rest (6 organic and 5 conventional) were situated on marine clay.

Table 4.1. Tillage treatments in the BASIS experiment per year* included in this study. Tillage treatments included conventional tillage (CT) applied with a mouldboard plough, and minimum tillage practices (MT) specifically non-inversion tillage without subsoiling (NIT), non-inversion tillage with subsoiling (NITS) and shallow tillage (ShT).

	Conventional				Organic			
	Field A		Field B		Field C		Field D	
	CT	MT	CT	MT	CT	MT	CT	MT
2009	CT	NIT	CT	NIT	CT	NIT	CT	NIT
2012	CT	NIT	CT	NIT	CT	NIT	CT	NITS
2014	CT	NIT	CT	NIT	CT	NIT	CT	NITS
2018	CT	ShT	CT	NIT	CT	NIT	CT	NITS
<i>* before the start of the experiment in 2009, conventional tillage by mouldboard ploughing was applied to all fields.</i>								

In the earlier sampling campaigns, 320 cores at 10 cm depth were taken from the entire farm. The number of cores taken in each field depended on the relative size of that field. Cores were taken from a zigzag shape in each field that stayed at least 10 meters away from ditches and roads. Samples were taken between the rows avoiding plant material. These cores were then bulked to form one sample per farm that was homogenised in the field. Three separate bags of approximately 500 ml of soil were then taken from the bulk, placed in coolers and sent to an external lab for analysis. While the original farming system mostly remained constant per farm, by the year 2018 some farms had acquired new parcels, and some had sold off some of their fields. In 2018, we selected only those fields that had belonged to the farm from the beginning of the NSMN and from those we selected a maximum of six random fields for resampling. To maintain the average core density taken in the previous sample campaigns we used the average size of arable farms in the Netherlands (close to 40 hectares) (CBS 2019) and estimated an average density of 8 cores per hectare. Separate bags for each sampled field were then weighed and bulked per farm. This bulk sample was then homogenised after which half of the soil was used to identify the nematode community, and the other half was used to analyse the physico-chemical parameters of the soil.

4.2.3 Nematode indicators

Nematodes were extracted from 100 ml of fresh soil using a modified Oostenbrink elutriator (Oostenbrink 1960; De Goede and Verschoor 2000). Nematodes were counted and identified to genus level (Bongers 1988). Identification was done at the same laboratory for all samples, except the 2018 sampling of BASIS. We calculated the nematode abundance (expressed as the number of nematodes per 100 g fresh weight) and richness (as the number of taxa present in a plot). Additionally, nematodes were classified according to their life strategy, from colonizers (nematode taxa with fast growth, numerous offspring, etc.) to persisters (those taxa characterised by a slower growth rate, a lower number of offspring and a longer life-span) in a scale from 1-5, respectively (Bongers 1990). In addition to this classification, nematodes were assigned to a feeding group, according to their mouth structure (Yeates *et al.* 1993). Different disturbances affect different parts of the nematode community, and consequently, several nematode indicators have been developed to capture these separate responses. Ferris *et al.* (2001) described three states of the nematode food-web: basal (stressed to the point where only the most resistant nematodes remain), structured (a food-web which sustains a higher number of trophic links), or enriched (where a flush of microbial activity supports an increase of bacterial feeding enrichment opportunists). They provided the formulas to calculate the enrichment (EI) and structure (SI) indices. In fact, by plotting these indices against each other, one can describe the soil food-web as stressed, enriched or structured, with stressed conditions represented by values closer to zero, while larger values of the EI and the SI indicative of increased nutrient enrichment and increased food-web structure, respectively. To calculate these indices, we used the Ninja webtool (Sieriebriennikov *et al.* 2014). Due to the impact of human activity on specialist species, community weighted specialism (that is the proportional abundance of specialist species) has been proposed as a measure of land use intensification (Clavel *et al.* 2011). A recent study found opposing trends in the nematode community, where Vazquez *et al.* (2019) (Chapter 2) found that the proportional abundance of specialist nematodes increased with increasing land use intensity. Therefore, in addition to the EI and the SI, we calculated the Index of community specialisation (I_{CS}). The I_{CS} was calculated such that: $I_{CS} = S_i / (S_i + G_i)$; where S_i is the abundance of specialist nematodes in site i , and G_i is the abundance of generalist nematodes in site i (as specified in Vazquez *et al.* (2019); Chapter 2).

4.2.4 Weather

Due to the large number of sites sampled in a year by the NSMN, farms situated in different soil texture classes were sampled in different years, possibly adding a bias to

the dataset. We took two measures to correct for this bias: firstly, all samples collected in 2018 were gathered during the same week. Secondly, we included weather variables as covariates in the analyses. For each individual sample we calculated the average daily temperature and cumulative rainfall the three months prior to the sampling date at the weather station nearest to the sampling point.

4.2.5 Statistical analyses

To study the effects of long-term farming on the nematode community over time we carried out several linear and generalised mixed models that included each nematode indicator as a response variable (i.e. taxon richness, abundance (in number of nematodes per 100 g fresh weight), structure index (SI), enrichment index (EI), and the Index of community specialisation (I_{CS})). All models included of a random effect that suited the experimental design, starting with a random slope and intercept model in time for each plot (nested within a field or block) or farm (formulas 1-3). To answer whether the observed trends were different under the two soil texture types, management and tillage regimes, these variables were included as covariates in the models. Additionally, the models also included cumulative rainfall and average daily temperature three months prior to sampling as covariates to account for differences that might relate to weather. To study the effects of farming on the nematode community we also carried out redundancy analyses with nematode genus abundance as response variable and the aforementioned covariates as predictors. All analyses were carried out in R (R Core Team 2019).

Richness (number of taxa) was analysed using either the Poisson distribution family as specified in the *glmer* function from package *lme4* (Bates *et al.* 2014) or a Conway-Maxwell Poisson as specified in the *glmmTMB* package (Brooks *et al.* 2017; Huang 2017), when the Poisson model was underdispersed as revealed by the *'dispersiontest'* function in the *AER* package (Kleiber and Zeileis 2008). To analyse the changes observed in nematode abundance, we log-transformed abundance prior to analysis, and used a linear mixed effect model (using the *lmer* function in *lme4*) or a linear model when the variance of the random part of the model was near zero (using the *lm* function in the *stats* package R Core Team (2019)). The values of the remaining indicators are limited from 0 to either 1 (I_{CS}) or 100 (EI and SI). The two latter were transformed such that they were restricted from 0-1, and we fitted generalized linear mixed models with a beta family using the *glmmTMB* function of the *glmmTMB* package (Cribari-Neto and Zeileis 2010; Ferrari and Cribari-Neto 2004; Brooks *et al.* 2017).

We started the modelling efforts for each indicator with a full model and considering all ecologically viable interactions, as well as a random structure that reflected the experimental set up such that:

- a) To analyse whether commercial farms showed a trend in time, we started with a free slope model for the sampling event and intercept for each individual farm such that:

$$\text{Index} \sim \text{Sampling event} * \text{Soil type} * \text{Management} * \text{Rainfall} * \text{Temperature} + (1 + \text{Sampling event} | \text{Farm ID}) \quad \text{Eq. 1}$$

Where sampling event is one of three (first, second or third sampling), soil type is either sand or marine clay, and management is either conventional or organic and Rainfall and Temperature are the standardized average daily temperature and the standardized cumulative rainfall three months prior to the sampling date.

- b) To study whether there had been a long term effect of agricultural management on the SHE at the Vredepeel experimental farm (i.e. on a sandy soil), and whether this effect was different under different management regimes, we started with a model that also included all ecologically relevant interactions, however, the interaction term between rainfall and temperature was significantly correlated with temperature and led to errors in the calculation of the estimates in the model. We therefore started the model selection procedure with the following model:

$$\text{Index} \sim \text{Management} * \text{Sampling event} + \text{Rainfall} + \text{Temperature} + (1 + \text{Sampling event} | \text{Block/plot}) \quad \text{Eq. 2}$$

Where management was either integrated or conventional, sampling event was the number of years passed since the first sampling, and Rainfall and Temperature are the standardized average daily temperature and the standardized cumulative rainfall three months prior to the sampling date. The random structure for the initial model included each individual plot nested within its block.

- c) To analyse whether there were any trends in the BASIS experimental site (i.e. on a marine clay soil) due to long term agricultural management, and to study which practices can lead to different trends we started with:

$$\text{Index} \sim \text{Tillage type} \times \text{Management} \times \text{Sampling event} + \text{Rainfall} + \text{Temperature} + (1 + \text{Sampling event} | \text{Field/plot}) \quad \text{Eq. 3}$$

Where tillage type can either be CT or MT, Management is either conventional or organic, Sampling event is the number of years that passed since the first sampling, and Rainfall and Temperature are the standardized average daily temperature and the standardized cumulative rainfall three months prior to the sampling date. In the random structure, we accounted for the relationship between each plot and the field in which it is contained.

For each model we first assessed the fit of the random factor. Starting with a fixed intercept and slope, we simplified the random structure in a stepwise manner whenever the variance was so close to zero that it led to convergence problems in the model, as suggested by the literature accompanying the R packages. We did this in a stepwise process by removing one random variable, then running the model. If convergence issues persisted and the variance of the remaining random effects was still zero, we removed the next variable, and so on.

The interactions expressed in formulas 1-3 are ecologically possible and might all explain variations in the nematode community. It is unlikely, however, that all factors and interactions therein equally explain the variation in the selected nematode indicators. Therefore, for each model we fitted an initial model (formulas 1-3) and calculated the AICc of all possible variants that included the random effects (Akaike's Information Criterion adjusted for small sample sizes) (Akaike 1973; Hurvich and Tsai 1989) and we present the model with the lowest AICc, a procedure carried out using function *dredge* from the *MuMIn* package (Barton 2019). In the supplementary material we also present the average model calculated from a subset of all plausible models (Burnham and Anderson 2002) (Appendix 1).

Lastly, we performed multivariate analysis to determine whether our descriptive variables explained the variation in the nematode community (Ter Braak 1986) using the *rda* function in the 'vegan' package (Oksanen *et al.* 2018). We calculated the gradient length of each axis (using the *decorana* function in the vegan package), which indicated that redundancy analysis was more appropriate for our data (all values were below 3). The variance inflation factor for the covariates in the model was explored using the *vif.cca* function. When a variable had a VIF higher than 10 it was considered as an indication of co-linearity, and said variable was eliminated from the model. This procedure was repeated until all variables in the model had a VIF value below 10 (Greenacre 1984; Gross 2003). We then used the *ordistep* function for variable selection by permutation tests (using the default 999 permutations). Finally, we tested the significance of the model, each individual variable, as well as each axis by analysing the differences in residual deviances in permutations of nested models using the *anova.cca* function (Legendre *et al.* 2011; Legendre and Legendre 2012). This procedure was

repeated for the nematode communities of the SHE and the BASIS experimental sites and the commercial farms. Experimental block was used as a descriptive variable in the experimental farms, and in the commercial farms we included each individual farm as a possible explanatory variable for the nematode community.

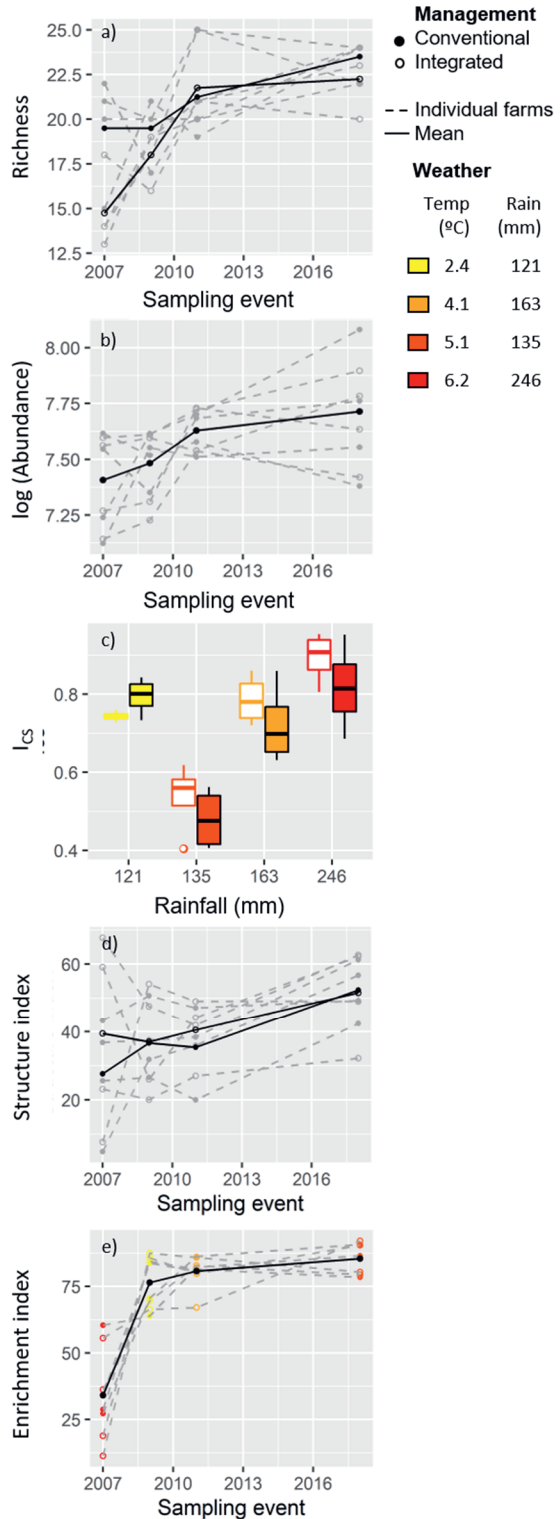
4.3 Results

4.3.1 *The nematode community in the Soil Health Experiment (SHE) through time*

In the SHE in Vredepeel we observed significant increases in time for taxon richness, the logarithm of abundance and the SI and EI (Figure 4.1; Table 4.2). Taxon richness was higher in conventional systems than under integrated management, but the opposite was true for the I_{CS} and the SI. There were, however, no significant interactions between management and sampling event in the best fitting models (Table 4.2). Some of the described effects change once other plausible models are used to calculate average estimates and 95% confidence intervals for the estimates (Table S1). However, the best fitting models were often superior to the next best ranking models (Figure S1).

We excluded rainfall from the possible explanatory variables prior to the calculation of the partial redundancy analysis. Rainfall had a variation inflation factor above 10 due to collinearity with both average temperature (positive correlation) and sampling event (negative correlation). Removing rainfall resulted in variation inflation factors below 10 for all other explanatory variables. After model selection procedures, we obtained a model ($F=16.5$, $p\text{-value}=0.001$) with an adjusted R^2 of 0.5 (Figure 4.2a) in which temperature and sampling event both explained a significant proportion of the variation in the nematode community ($F=11.5$, $p\text{-value} = 0.005$ and $F=22.2$, $p\text{-value}=0.005$ respectively). Neither management nor an interaction between management and sampling event were selected as explanatory variables explaining a significant variation in the community.

Figure 4.1. Shift in nematode indicators under integrated (empty) and conventional (filled) practices in the *Soil Health experiment* at the Vredepeel Experimental Farm in the Netherlands, established in 2006. Integrated management includes organic management as well as best practices regarding pest control. Plotted are nematode community indicators as a response variable against the variables that significantly explained the variation in the indices according several mixed effect models. When sampling event was not a significant factor, we plotted the indicator against a different predictor. The plots are coloured when temperature and/or rainfall were a significant factor explaining the variation in the indicator but where not the on the x-axis.



4.3.2 The nematode community in the Broekemahoeve Applied Soil Innovation Systems experiment (BASIS) through time

At the BASIS experimental site, we also observed a significant increase in taxon richness with time in all treatments except when conventional management was combined with conventional tillage (Table 4.3). There were more nematodes per 100 g fresh soil in the organic than the conventional fields, but in the latter, we observed an increase with time. We also observed a decrease of the EI in all treatments (Figure 4.3e). Sampling event did not explain a significant part of the variation in the I_{CS} or the SI (Table 4.3). The results regarding the effect of time obtained after averaging the most plausible models were not very different than those obtained in the best 2 models (Table S2; Figure S2). In a redundancy analysis that explained close to 25% of the variation in the nematode community (Adj. $R^2=0.26$), sampling event, rainfall and temperature each explained a small but significant portion of the variation in the nematode community at BASIS, together with an interaction between management (organic vs. conventional) and sampling event ($F=10$, p -value=0.001; Figure 4.2b).

Table 4.2. Variations in the soil community according to several nematode indicators in the Vredepeel experimental farm over a period of time. Presented in the table are the estimates, standard errors (std. errors), t or z values and p-values for the models with the lowest AICc. Explanatory variables are sampling event (as years from the first sampling), management, either conventional (Conv) or integrated (Int), standardised cumulative rainfall (Rain) and average temperature (Temp) three months prior to sampling and interactions therein. In bold are p-values below 0.05. Grey colouring of the cells indicates a significant effect of time.

Richness	<i>Fixed effects</i>		Estimate	Std. Error	z-value	p-value
	Sampling event		0.02	0.004	4.93	<0.001
	Management - Conv		2.94	0.04	80.3	<0.001
	Management - Int		2.85	0.04	75.8	<0.001
	<i>Mixed effects</i>		Variance	Std. Dev		
	Plot : Block		<0.001	<0.001		
Block		<0.001	<0.001			
Maxwell et Compois GLMM; Overdispersion parameter = 0.27						
Log(abundance)	<i>Fixed effects</i>		Estimate	Std. Error	t-value	p-value
	Intercept		7.43	0.05	153.34	<0.001
	Sampling event		0.03	0.008	3.39	0.003
	<i>Mixed effects</i>		Variance	Std. Dev		
	Plot		0.002	0.05		
	Residual		0.03	0.18		
Index of community specialisation	<i>Fixed effects</i>		Estimate	Std. Error	z-value	p-value
	Management - Conv		0.96	0.1	9.86	<0.001
	Management - Int		1.14	0.1	11.47	<0.001
	Rain		1.06	0.16	6.56	<0.001
	Temp		-0.9	0.16	-5.66	<0.001
	Management - Int : Temp		0.29	0.14	2.09	0.036
	<i>Mixed effects</i>		Variance	Std. Dev		
	Block		0.05	0.23		
Sampling event		0.001	0.04			
Beta GLMM; Theta = 40.1						
Structure index	<i>Fixed effects</i>		Estimate	Std. Error	z-value	p-value
	Sampling event		0.08	0.02	3.41	<0.001
	Management - Conv		-0.85	0.24	-3.57	<0.001
	Management - Int		-0.68	0.24	-2.88	0.004
	<i>Mixed effects</i>		Variance	Std. Dev		
	Plot : Block		<0.001	<0.001		
Block		0.12	0.34			
Beta GLMM; Theta = 14.3						
Enrichment index	<i>Fixed effects</i>		Estimate	Std. Error	z-value	p-value
	Sampling event		0.2	0.02	9.45	<0.001
	Temp		-0.59	0.1	-5.91	<0.001
	<i>Mixed effects</i>		Variance	Std. Dev		
	Plot : Block		<0.001	<0.001		
	Block		<0.001	<0.001		
Beta GLMM; Theta = 15.9						

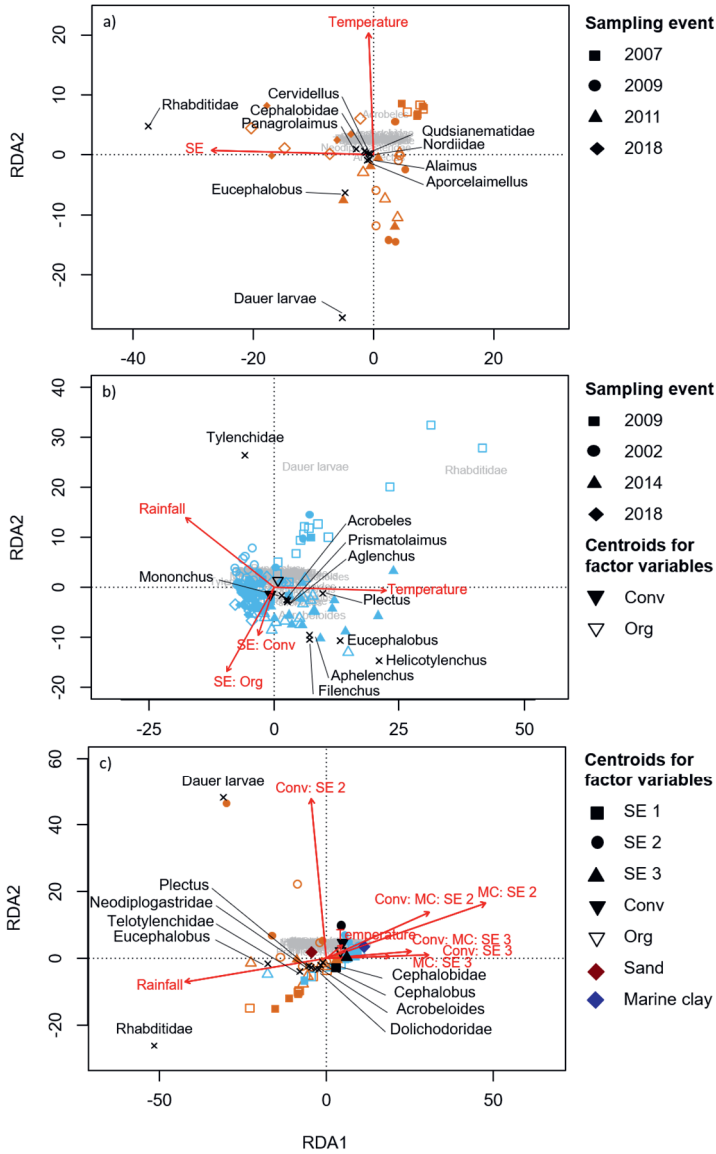


Figure 4.2. Biplot of the RDAs explaining the variation in the nematode communities at *the Soil Health Experiment in Vredepeel* (a), the *Broekmahoeve Applied Soil Innovation Systems Experiment BASIS* experiment (b) and in a representative set of commercial farms (c). Orange (sand) and light blue (marine clay) symbols represent each plot (a and b) or farm (c), and the shape of the symbol the sampling event (SE). Management is either conventional (filled) or organic/integrated (empty). In black symbols are the centroids for categorical variables. Crosses represent the position in the axes for 10 nematode taxa that account for the largest proportion of inertia on the first two axes, the remaining species are in grey. Scaling is done both on species and sites. We present variables that explain a significant portion of the variation selected via forward selection.

Table 4.3. Variations in the soil community according to several nematode indicators in the BASIS experimental field over a period of time. Presented in the table are model estimates, standard errors (std. errors), t- or z-values and p-values for the models with the lowest AICc. The intercept is calculated for minimum tillage (MT). Explanatory variables are sampling event (as years passed from the first sampling), either conventional (Conv) or organic (Org) management, conventional tillage (CT) and minimum tillage (MT) as tillage systems, and standardised cumulative rainfall and average temperature three months prior to sampling (Rain and Temp) and interactions therein. In bold p-values below 0.05. Grey colouring of the cells indicates a significant effect of time.

<i>Fixed effects</i>		Estimate	Std. Error	z-value	p-value
Richness	Management - Org	2.81	0.05	59.09	<0.001
	Management - Conv	2.81	0.02	59.13	<0.001
	Rain	0.10	0.02	5.84	<0.001
	Temp	0.23	0.02	12.04	<0.001
	Tillage - ST	-0.18	0.06	-3.00	0.003
	Sampling event (SE)	0.04	0.01	4.57	<0.001
	Management - Conv : Tillage - ST	0.29	0.08	3.48	<0.001
	Management - Conv : SE	-0.003	0.01	-0.25	0.79
	Tillage - ST : SE	0.02	0.01	1.47	0.14
	Management - Conv: Tillage - ST - SE	-0.04	0.02	-2.73	0.006
	<i>Mixed effects</i>		Variance	Std. Dev	
	Sample: Field	0.002	0.040		
	Field	<0.001	0.007		

Maxwell et Compois GLMM; Overdispersion parameter=0.33

<i>Fixed effects</i>		Estimate	Std. Error	t-value	p-value
Log(Abundance)	Management - Org	7.45	0.10	75.1	<0.001
	Management - Conv	7.07	0.10	71.31	<0.001
	Rain	-0.17	0.05	-3.59	<0.001
	Temp	0.35	0.06	6.24	<0.001
	Sampling event	0.01	0.02	0.69	0.49
	Management - Conv : SE	0.04	0.02	1.93	0.06

LMM

<i>Fixed effects</i>		Estimate	Std. Error	z-value	p-value	
Index of community	Management - Org	-0.07	0.06	-1.17	0.24	
	Management - Conv	0.18	0.07	2.8	0.005	
	Rain	0.06	0.04	1.51	0.13	
	<i>Mixed effects</i>		Variance	Std. Dev		
		Field	0.04	0.20		
	Sampling event	0.001	0.03			

Beta GLMM; Theta = 19.6

		Estimate	Std. Error	z-value	p-value	
<i>Fixed effects</i>						
	Management - Org	-0.19	0.52	-0.36	0.72	
	Management - Conv	-0.85	0.52	-1.62	0.11	
	Rain	0.43	0.10	4.54	<0.001	
Structure index	Temp	0.55	0.11	4.78	<0.001	
	Tillage - ST	-0.36	0.15	-2.39	0.02	
	Sampling event	0.11	0.08	1.28	0.20	
	<i>Mixed effects</i>		Variance	Std. Dev		
		Sample:Field	0.44	0.66		
	Sampling event	0.03	0.16			
	Field	0.77	0.88			
	Sampling event	0.02	0.14			
Beta GLMM; Theta = 6.76						
		Estimate	Std. Error	z-value	p-value	
<i>Fixed effects</i>						
	Rain	-0.35	0.09	-3.96	<0.001	
	Temp	-0.55	0.1	-5.37	<0.001	
	Tillage - ST	1.85	0.22	8.51	<0.001	
	Tillage - MT	2.14	0.22	9.69	<0.001	
Enrichment index	Sampling event	-0.2	0.04	-5.57	<0.001	
	<i>Mixed effects</i>		Variance	Std. Dev		
	Field	0.05	0.23			
Beta GLMM; Theta = 10.2						

4.3.3 The nematode community in commercial farms through time

We observed a general loss of structure in the nematode community in time in the commercial farms, and an increase in the I_{CS} , but the rest of the indicators did not show a consistent trend over time (Table 4.4). In fact, in conventional farms, the average SI went from 34 to 24% (sand), and 50 to 37% (clay), from the first to the last sampling (Table S4). Farms on sandy soils had a higher mean nematode richness and abundance (Figure 4.3a-b). Organic farms had a lower EI than conventional farms (Table 4.4). However, there were no significant interactions between sampling event and any of the variables included in the models, indicating that any effects in time were equal for all management and soil texture types. While the best models explaining the variation in the logarithm of abundance, the I_{CS} and the SI were superior to the next plausible models (Figure S1), the ranking of plausible models explaining variations in richness and the EI indicated that up to 35 and 37 models could be considered plausible, indicating a low explanatory power in both models. That being said, the conclusions regarding sampling event are not too different when using the best fitting models versus the averaged models (Table S3).

Table 4.4. Variations in the soil community according to several nematode indicators in commercial farms over a period of time. Presented in the table are model estimates, standard errors (Std. Error), z- or t- values and p-values for the models with the lowest AICc. Explanatory variables are sampling event, soil texture (either sand (S) or marine clay (MC)), either conventional (Conv) or organic (Org) management (M), standardised cumulative rainfall and average temperature three months prior to sampling and interactions therein. In bold are those variables with a confidence interval that does not include 0.

<i>Fixed effects</i>					
	Estimate	Std. Error	z-value	p-value	
Temp	0.05	0.03	1.8	0.07	
Soil type - S	2.92	0.04	70.2	<0.001	
Rain	-0.01	0.03	-0.4	0.7	
Soil type - MC	2.84	0.04	72.2	<0.001	
Rain : Soil type - MC	0.13	0.06	2.2	0.03	
<i>Mixed effects</i>		Variance	Std. Dev		
Farm ID (Intercept)		0.004	0.06		
Maxwell et Compois GLMM; Overdispersion parameter = 0.61					
<i>Fixed effects</i>					
	Estimate	Std. Error	t-value	p-value	
Soil type – S	8.12	0.09	93.1	<0.001	
Soil type – MC	6.88	0.08	87.2	<0.001	
<i>Mixed effects</i>		Variance	Std. Dev		
Farm ID (Intercept)		0.05	0.23		
Sampling event		0.04	0.19		
LMM					
<i>Fixed effects</i>					
	Estimate	Std. Error	z-value	p-value	
Sampling event	0.27	0.09	3.2	0.001	
Soil type – S	-0.96	0.21	-4.7	<0.001	
Soil type – MC	-1.53	0.21	-7.4	<0.001	
<i>Mixed effects</i>		Variance	Std. Dev		
Farm ID (Intercept)		0.1	0.31		
Sampling event		0.02	0.12		
Beta GLMM; Theta = 16.8					
<i>Fixed effects</i>					
	Estimate	Std. Error	z-value	p-value	
Sampling event	-0.25	0.09	-2.9	0.004	
Temp	0.29	0.09	3.2	0.001	
<i>Mixed effects</i>		Variance	Std. Dev		
Farm ID (Intercept)		0.38	0.62		
Sampling event		0.48	0.69		
Beta GLMM; Theta = 11.2					

	<i>Fixed effects</i>	Estimate	Std. Error	z-value	p-value
	Rain	0.17	0.09	1.8	0.07
	Temp	0.12	0.06	1.8	0.07
Enrichment index	Management - Org	1.45	0.14	10.5	< 0.001
	Management - Conv	2.16	0.21	10.1	< 0.001
	Soil type – MC	0.56	0.2	2.8	0.005
	Management -Conv: ST-MC	-0.76	0.31	-2.4	0.02
	<i>Mixed effects</i>	Variance	Std. Dev		
	Farm ID (Intercept)	0.73	0.85		
	Time point	0.23	0.48		
Beta GLMM; Theta = 54.7					

An RDA explaining the variation in the nematode community of commercial farms explained 73% of the variation (ANOVA, $F= 2.9$, p -value= 0.001). A large part of the variation was explained by the farm's identity (34%), followed by an interaction between soil type, management and sampling event (14%), and temperature (4%) and rainfall (4%). Farm identity and rainfall were not significant when tested using an ANOVA. Sampling event was entered not as a numeric variable but as a factor, and the results indicate a non-linear change in the nematode composition (Figure 4.2c).

4.3.4 The effect of temperature and rainfall on the nematode community

Although not part of our research questions, we did observe effects of both average temperature and cumulative rainfall three months prior to sampling on the nematode community (Tables 4.2-4.4). Rainfall was strongly correlated with sampling event in commercial farms (Pearson's $r= -0.75$), and less so in SHE (Pearson's $r= -0.55$) and BASIS (Pearson's $r= -0.37$). Temperature showed less strong correlations with sampling event, being the strongest correlation with BASIS (Pearson's $r= -0.62$), followed by commercial farms (Pearson's $r= 0.13$) and SHE (Pearson's $r= 0.07$). Increases in rainfall led to a significant increase of the I_{CS} in the SHE. Temperature was associated with decreases in the I_{CS} and the EI in this experimental site, although the effect on the I_{CS} was less strong under integrated management (Table 4.2). Temperature explained 18% of the variance in the nematode community at this site (ANOVA, $F=10.9$, p -value=0.001), and correlated negatively with the abundance of Dauer larvae and Eucephalobus (Figure 4.2a). At the BASIS experimental site, rainfall led to significant increases in richness and the SI, and decreases in the logarithm of abundance and the EI (Table 4.4). Temperature also led to increases of richness abundance and the SI, and a decrease in EI (Figure 4.3). Weather variables also led to changes in the nematode community composition at BASIS. Increased rainfall was correlated with increases in the abundance Tylenchidae, and a decrease of Helicotylenchus (Figure 4.2b). The latter also correlated with increases in temperature.

Figure 4.3. Shift in nematode indicators under conventional (filled) and organic (empty) practices in the in the *Broekemahoeve Applied Soil Innovation Systems* experiment in the Netherlands, established in 2009. Plotted are nematode community indicators as a response variable against the variables that significantly explained the variation in the indices according several mixed effect models. The I_{CS} is the Index of community specialisation. When sampling event was not a significant factor, we plotted the indicator against a different predictor. The plots are coloured when temperature and/or rainfall were a significant factor explaining the variation in the indicator but where not the on the x-axis.

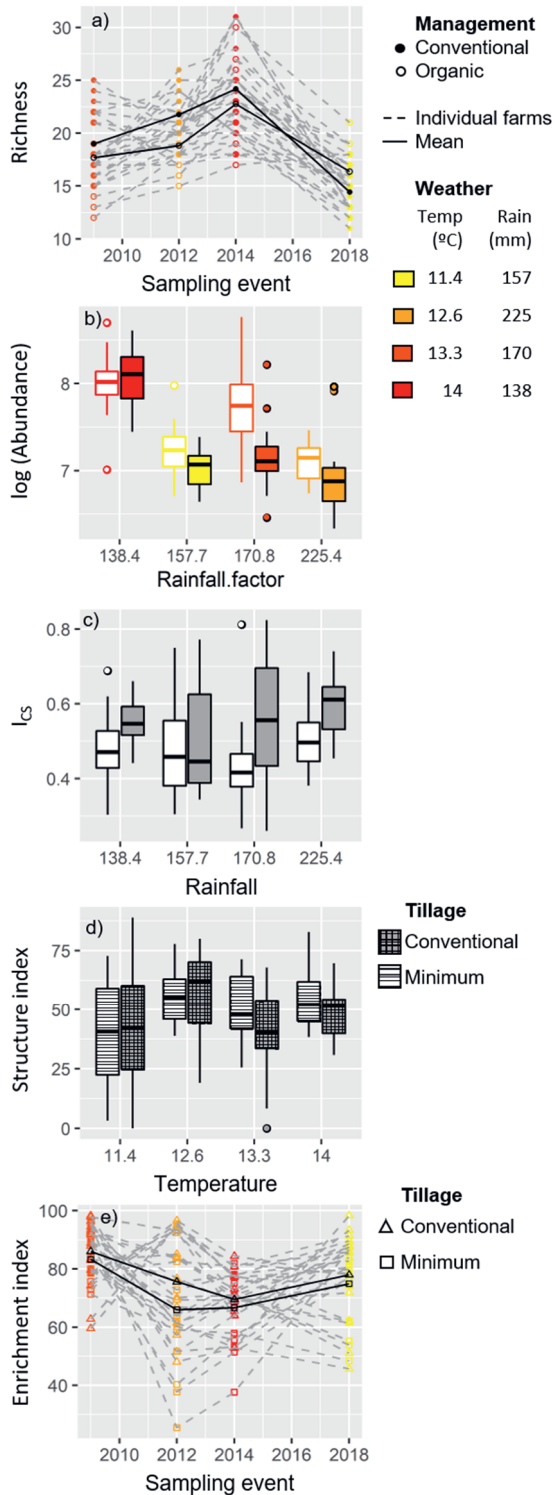
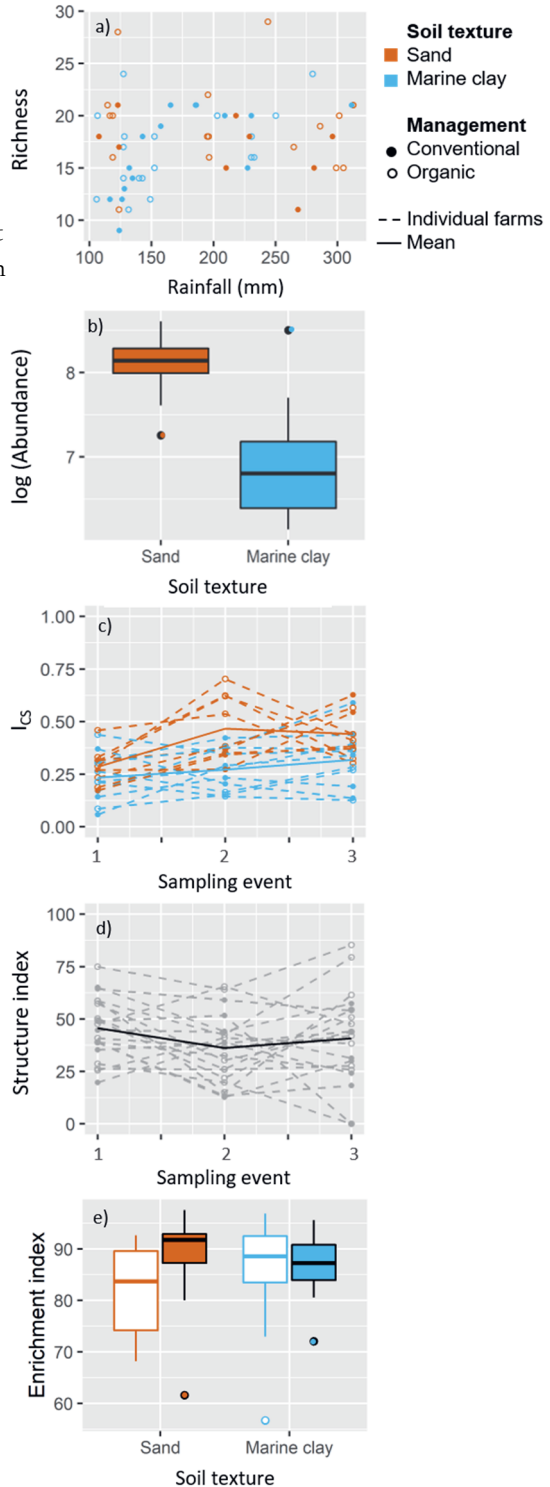


Figure 4.4. Shifts in the nematode community of commercial farms in the Netherlands on different soil textures and under conventional (filled) or organic (empty) management. Plotted are nematode community indicators as the response variable against the variables that explained a significant part of the variation in the indices according several mixed effect models. Figures are coloured whenever soil texture explained a significant part of the variation in the indicator. Not all significant variables are represented in each figure. See Table 4.4 for a complete overview.



4.4 Discussion

In this manuscript, we have studied the effects of agricultural management on biological soil quality over time, by studying the nematode community. Regarding our first research question, we found significant changes on the nematode communities in agricultural soils through time, albeit not always linear and sometimes distinct for each of the systems included in our study (which included two experimental farms, as well as 20 commercial farms on two different soil texture types). Taxon richness was affected by long term agricultural management at both experimental farms, and the effect was positive for all treatments except for conventional management combined with conventional tillage at BASIS, where we observed no significant trend in time. We found no trends in the taxon richness of commercial farms, where the trends were farm specific, and did not point to an overall loss of richness. The latter results are in line with studies of global richness trends, where (Dornelas *et al.* 2014) did not find a systematic loss of local richness in terrestrial or aquatic systems, but rather an increase in the species turnover driven by the spread of invasive species and changes in species distributions due to climate change. Regarding the second and third research questions in this study, the effect of time on the selected indicators was not dependent on soil texture class nor land management, except for the rate of richness increase at the BASIS experiment, which was close to zero when conventional tillage and management were combined, but increased otherwise.

Soil texture class was an important characteristic describing the nematode community of commercial farms, but the effects observed through time were not affected by the texture of the soil. Studies have highlighted the importance of parent material and soil texture in the taxonomic composition of nematodes (Quist *et al.* 2019). We also observed effects of tillage regime on some of the nematode indicators. Treatments with standard tillage had a lower taxon richness (although this difference was larger in the organic treatments), and fostered a less structured and enriched community (lower SI and EI) than those with minimum tillage practices at BASIS. While several studies have found significant effects of tillage on the nematode community (Zhong *et al.* 2017; Ito *et al.* 2015), studies conducted over time suggest that nematode communities are generally quick to recover from tillage related disturbances (Timper *et al.* 2012).

It is possible that some of the trends that we have observed, such as the increase in nematode abundance or the decrease of Ics at the SHE in the Vredepeel experimental site, are in fact due to the poor soil quality observed in this site in 2007. For example, nematode abundance was on average close to 1600 nematodes/100 g fresh weight (Table S5), while in our commercial farms on sandy soils, the average nematode abundance was almost always above 3000 (Table S4). Similarly, the I_{CS} (above 0.82) was

indicative of a very intensely managed system (Vazquez *et al.* 2019; Chapter 2) and the EI (below 38) indicated a very disturbed and depleted system with a very degraded food-web (Ferris *et al.* 2001). Previous studies have found increases in the richness, abundance, SI and EI after the addition of organic inputs in agricultural systems (Liu *et al.* 2016; Ugarte *et al.* 2013). However, according to our results, the rate of improvement as indicated by the nematode community indices was similar under conventional and organic land management, making it unlikely that this increase was due to the increase in organic amendments alone, but rather a consequence of changes in the crop rotation and overall management at the beginning of the SHE. A shift towards a less intensive arable system in this experimental farm is also supported by a decrease of the I_{CS} through time (Vazquez *et al.* 2019; Chapter 2).

The consistent decrease of the SI observed in commercial farms does not seem to be the result of an extreme starting point. During the first sampling event, we found SI values typical of arable farming (Ferris *et al.* 2001), with an average SI of 34 on sandy soils, and 50-58 on soils developed over marine clay (conventional and organic systems, respectively). While the model predicts a similar slope for the SI of organic and conventional farms, the average numbers reflect a larger decrease of the SI in conventional than in organic farms (Table S2). In fact, only under conventional management do we see a loss of predatory and omnivorous nematodes (both in relative and total abundance). These results suggest that either agricultural management in the Netherlands has become more intensive over the years or the nematode community has not completely adapted to arable farming but rather continues to change over time. We also found an increase in the I_{CS} , which has in the past been linked with increased land use intensity (Vazquez *et al.*, 2019; Chapter 2). While a recent survey of Dutch farmers found that overall, agricultural intensity has decreased in the Netherlands since 2006 (van der Sluis *et al.* 2016), the study focused on the amount of fertiliser and pesticides that were used by the farmers. It is possible that these have decreased on average, and have been substituted by other management strategies that could also result in negative impacts to the soil community. Acidification due to the use of synthetic fertilizers, the use of pesticides and the presence of heavy metals can all have detrimental effects on the soil food-web, and lead to decreases in the SI (Gutierrez *et al.* 2016; Liu *et al.* 2016; Korthals *et al.* 1996; Herren *et al.* 2020). Additionally, the use of heavy machinery can lead to compaction, which induces changes in the nematode composition, particularly an increase in the percentage of plant feeders (Bouwman and Arts 2000). In our study, there was a negative correlation between sampling event and rainfall and increased rainfall was associated with increases in the SI in BASIS, where sampling event and rainfall had the lowest correlation coefficient of all three systems. However, a strong correlation between rainfall and sampling event was also observed in the SHE where

biological soil quality in fact improved over time. A decrease in rainfall could explain part of the decrease in biological soil quality observed in commercial farms, but we do not think it is the only factor for consideration. In order to better answer this question, we suggest future studies that incorporate time as a covariate, but control for the effects of weather. Additionally, a better quantification of land use intensity by for example interviewing farmers would aid in the quantification of effects on land use.

Whatever the cause of the decline in the SI, it is important to address the significance of a degrading food-web structure, since changes to the SI reflect shifts in the entire soil food-web. Through top down control, predatory nematodes can have an impact on important processes such as nutrient cycling, soil suppressiveness and therefore primary productivity (Steel and Ferris 2016; Carrasco *et al.* 2014). A combination of management techniques can be explored to stop this trend. Reducing the repeated physical disturbance of the soil, such as the use of traffic lines within the fields, lighter machinery or the use of alternative tillage practices, combined with an increase in the use of organic inputs (fertilizers as well as cover crops) have positive effects on the soil organic matter and water holding capacity as well as the general soil structure that can cascade up the soil food-web to benefit predator and omnivorous nematodes (Zhong *et al.* 2017; Liu *et al.* 2016; Stubbs *et al.* 2004; Pires *et al.* 2017).

Our results also highlight the importance of weather variables in explaining the variation in the nematode community. Studies focused on global patterns of nematode distribution have recently highlighted the importance of annual rainfall and temperature as determinants of nematode assemblages (Nielsen *et al.* 2014). At a more local scale, studies have started to observe shifts (albeit small) in nematode communities due to the effects of climate warming. Siebert *et al.* (2020), for example, observed an increase in plant parasites and opportunistic nematodes under warming conditions. Our results also point to shifts in the community that might benefit specific plant parasitic nematodes and bacterial feeders: with increasing temperatures, coupled with increased rainfall, at the SHE we observed an increase of Rhabditidae (opportunistic bacterial feeders) over time, coinciding with a decrease in rainfall; in BASIS, increased rainfall led to an increase in Tylenchidae (small-sized root-hair and epidermal cell feeders), while increased temperatures led to increases in *Helicotylenchus* (plant parasitic) and *Eucephalobus*. Opposite to this observation we observed a decrease in the abundance of *Eucephalobus* due to increasing temperatures at the SHE. The experimental farms were sampled at different seasons, which could account for the differences in the trends that we observed, and sheds light on another issue to account for: seasonality. Both drought and flooding events, which are likely to become more common with climate change (IPCC 2013) have both been linked to decreases in the SI (Mueller *et al.* 2016; Ma *et al.*

2018). We do not think, however, that the general decrease we observed in the SI is a consequence only of drought, since all the measurements in the commercial farms were taken before the 2018 drought. The same cannot be said for some of the results we observed at BASIS, since we sampled BASIS in November, and the 2018 drought had caused an average rainfall deficit of 300mm by the month of August in the Netherlands (KNMI). With increasing evidence of the effects of local weather on the nematode community, we recommend that future studies take weather factors into account when carrying out modelling efforts or when monitoring the biological soil quality, so as not to confound the end conclusions.

4.5 Conclusion

While the effects observed in time are not consistent amongst the studied systems, the evidence suggests what the nematode community in soils under intensive agricultural management are in fact not fully adapted to the stresses associated with land management, but rather they continue to change under agricultural management beyond those changes expected due to changes in weather or climate. In order to stop this decline, measures should be taken that reduce the impact on soil communities and allow for the recovery of slower growing organisms. Our results indicate that there is not one management solution that will increase biological soil quality on its own. While general management recommendations can be made, such as adjustments to the use of machinery, fertilizers or pesticides, management choices outside of those commonly measured can lead to improvements in the biological soil quality as observed in the SHE experiment.

Supplementary Material

Appendix 1. Model selection and averaging

After calculation of the AICc (Akaike's Information Criterion adjusted for small sample sizes; Akaike (1973); Hurvich and Tsai (1989)) of each model within those in formulas 1-3, the models were ranked according to their AICc, with the lowest AICc indicating the best fitting model (Burnham and Anderson 2002). The best fitting models were then averaged to obtain an estimate and confidence interval for each variable in the models. To choose the best fitting models we calculated the relative plausibility of each model (compared to the best fitting model) using Akaike weights (Burnham and Anderson 2002). We then selected a confidence set of models including only those models with Akaike weights that were no smaller than 1/8th of the largest weight, which suggest a strong evidence of plausibility (Royall 1997). We calculated the average estimates as well as the 95% confidence intervals for all variables included in the confidence set, as well as ranked the importance of each variable as the sum of Akaike weights for candidate models in which each predictor occurred (Burnham and Anderson 2002). Multi-model inference was done with functions *dredge*, *get.model*, *model.avg*, *importance* and *confint* from the *MuMIn* package (Barton 2019).

The residuals from the best fitting models were visually checked against the fitted values, as well as every co-variate in the model, and those not included in the model (Zuur and Ieno 2016). For the models explaining the variation in richness we calculated the dispersion parameter using the *dispersion_glm* function in package *blmeco* (Korner-Nievergelt *et al.* 2015). If neither of these checks indicated a problem with the model fit, we continued with the averaging of the best models.

Appendix 2 – Supplementary Tables and Figures

Table S1. Variations in the soil community according to several nematode indicators in the Vredepeel experimental farm over a period of time. Presented in the table are average estimates, 95% confidence intervals (CI) and relative importance of explanatory variables for taxon richness, the index of community specialisation (ICs) and the Structure and Enrichment indices in the models with a delta AICc lower than 1/8. We present the estimates, standard errors, z-value and p-value for the best ranking model explaining the variation in the logarithm of nematode abundance since all other models had a delta AICc higher than 1/8. Explanatory variables are sampling event (as years from the first sampling), management (M), either conventional (C) or integrated (I), standardised cumulative rainfall (Rain) and average temperature (Temp) three months prior to sampling and interactions therein. In bold are those variables with a confidence interval that does not include 0.

	Richness	Abundance	ICs	Structure index	Enrichment index
	Average estimate ± CI	Estimate ± SE	Average estimate ± CI	Average estimate ± CI	Average estimate ± CI
	Relative importance	t value	Relative importance	Relative importance	Relative importance
		Pr(> t)			
Intercept	-	7.43 ± 0.05	-	-	-
Sampling event	-	0.03 ± 0.01	-	-	0.27 ± 0.38
Temperature	-	3.391	-	0.51 ± 0.83	-1.08 ± 2.25
Rainfall	-	-	0.93 ± 0.65	-0.63 ± 0.96	1.38 ± 3.62
Management - Conv	2.73 ± 0.52	-	1.4 ± 0.69	-0.82 ± 0.54	-2.07 ± 1.99
MC : Sampling event	0.07 ± 0.12	-	-0.15 ± 0.05	0.04 ± 0.15	-
MC : Temperature	-0.51 ± 0.63	-	-0.31 ± 0.95	-	-
MC : Rain	0.65 ± 0.81	-	-	-	-
Management - Int	2.62 ± 0.53	-	1.58 ± 0.68	-0.64 ± 0.53	-2.2 ± 1.98
MI : Sampling event	0.01 ± 0.02	-	0 ± 0.07	-0.04 ± 0.09	-
MI : Temperature	-0.05 ± 0.09	-	0.29 ± 0.29	-	-
MI : Rain	-0.08 ± NA	-	-	-	-
Random effects	1 + 1 Block/plot	1 + 1 Plot	1 Block/Plot	1 Block/Plot	1 Block
Number of models	11	1	6	11	3
Model family	Conway-Maxwell Poisson GLMM	Linear mixed model	Beta GLMM	Beta GLMM	Beta GLMM

Table S2. Variations in the soil community according to several nematode indicators in the BASIS experimental field over a period of time. Presented in the table are average estimates, 95% confidence intervals (CI) and relative importance of variables explaining the variation in taxon richness, the logarithm of nematode abundance, the index of community specialisation (Ics) and the enrichment index in in the models with a delta AICc lower than 1/8. We present the estimates, standard errors, z-value and p-value for the best ranking model explaining the variation in the structure index since all other models had a delta AICc higher than 1/8. The intercept is calculated for organic non-inversion tillage without subsoiling (NIT). Explanatory variables are sampling event (as years passed from the first sampling), either conventional (Conv) or organic (Org) management (M), tillage system (either conventional (CI) or minimum (MT)), standardised cumulative rainfall and average temperature three months prior to sampling and interactions therein. In bold are those variables with a confidence interval that does not include 0.

	Richness		Abundance		Ics		Structure Index		Enrichment index	
	Estimate ± SE	P(> t)	Average estimate ± CI	Relative importance	Average estimate ± CI	Relative importance	Average estimate ± CI	Relative importance	Average estimate ± CI	Relative importance
Fixed effects										
Rainfall	0.1 ± 0.03	1	-0.17 ± 0.10	1	0.07 ± 0.09	0.62	0.42 ± 0.19	1	-0.35 ± 0.18	1
Temperature	0.23 ± 0.04	1	0.34 ± 0.12	1	0.05 ± 0.11	0.41	0.54 ± 0.23	1	-0.56 ± 0.20	1
Management - Org	2.80 ± 0.10	1	7.44 ± 0.21	1	-0.08 ± 0.16	0.84	-0.34 ± 1.37	0.73	1.75 ± 0.48	0.39
Management - Org : Sampling event	0.04 ± 0.02	1	0.02 ± 0.04	0.92	0.01 ± 0.05	0.14	0.15 ± 0.20	0.67	-0.2 ± 0.070	1
Management - Org : Tillage: MT	-	-	-	-	-	-	-0.26 ± 0.70	0.66	1.84 ± 0.44	1
Management - Org : Tillage: ST	-0.17 ± 0.13	1	-0.05 ± 0.16	0.35	0.01 ± 0.19	0.13	-0.52 ± 0.69	0.66	1.43 ± 1.80	1
Management - Org: Tillage ST: Sampling event	0.02 ± 0.02	0.8								
Management - Conv	2.82 ± 0.10	1	7.12 ± 0.23	1	0.18 ± 0.18	0.84	-0.53 ± 1.04	0.73	1.95 ± 0.52	0.39
Management - Conv: Sampling event	-0.007 ± 0.03	1	0.04 ± 0.04	0.66	-0.04 ± 0.07	0.04	-0.25 ± 0.21	0.23	0.005 ± 0.08	0.06
Management - Conv: Tillage ST	0.26 ± 0.21	1	0.03 ± 0.27	0.05	0.15 ± 0.31	0.04	0.29 ± 1.15	0.19	-0.2 ± 0.47	0.08
Management - Conv : Tillage - ST : Sampling event	-0.04 ± 0.03	0.8	-	-	-	-	-0.22 ± 0.26	0.06	-	-
TillagesT:Sampling event			0.0003 ± 0.04	0.05	-	-	0.08 ± 0.19	0.14	-0.01 ± 0.08	0.22
Random effects	1 + Year Field/Sample		-		1 + Year Field		1 + Year Field/Sample		1 + 1 Field	
Number of models	2		7		12		12		6	
Model family	Conway-Maxwell Poisson GLMM		Linear mixed model		Beta GLMM		Beta GLMM		Beta GLMM	

Table S4. Average values and standard deviation of nematode community indicator measured for conventional (Conv) and organic (Org) farms in the Netherlands at three different sampling campaigns (SE1, SE2 and SE3). These sampling campaigns took place at least 5 years apart from one another.

	Sand						Marine clay						
	SE 1		SE 2		SE 3		SE 1		SE 2		SE 3		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Ecological indicators	Richness	18 ± 3	15 ± 4	19 ± 4	19 ± 2	2	19 ± 3	15 ± 3	15 ± 3	16 ± 5	5	5	
	Abundance (n per 100g)	19 ± 3	20 ± 5	19 ± 5	6	6	19 ± 3	14 ± 3	14 ± 3	18 ± 4	4	4	
	Index of community specialisation	Conventional	3851 ± 292	3832 ± 1731	2643 ± 684	553	553	1087 ± 553	835 ± 372	835 ± 372	867 ± 486	486	486
		Organic	3377 ± 1035	3577 ± 782	3438 ± 1183	419	419	1287 ± 419	876 ± 415	876 ± 415	1702 ± 1701	1701	1701
	Structure Index	Conventional	0.25 ± 0.07	0.33 ± 0.05	0.52 ± 0.12	0.12	0.12	0.21 ± 0.13	0.31 ± 0.09	0.31 ± 0.09	0.34 ± 0.18	0.18	0.18
		Organic	0.30 ± 0.09	0.54 ± 0.14	0.40 ± 0.10	0.10	0.10	0.25 ± 0.12	0.24 ± 0.10	0.24 ± 0.10	0.30 ± 0.10	0.10	0.10
	Enrichment Index	Conventional	34.4 ± 13.1	32.1 ± 16.2	24.6 ± 6.5	13.9	13.9	50.5 ± 13.9	41.2 ± 17.7	41.2 ± 17.7	37.1 ± 23.3	23.3	23.3
		Organic	34.9 ± 9.6	29.6 ± 8.5	30.7 ± 17.7	9.6	9.6	57.9 ± 9.6	40.6 ± 20.7	40.6 ± 20.7	62.0 ± 17.0	17.0	17.0
	Herbivores	Conventional	90.6 ± 2.9	94.3 ± 2.9	77.8 ± 15.2	3	3	91.3 ± 3	82.6 ± 6.4	82.6 ± 6.4	86.8 ± 4.9	4.9	4.9
		Organic	82.5 ± 7.6	81.4 ± 8.5	81.4 ± 9.4	8.4	8.4	88.4 ± 8.4	85.4 ± 3.1	85.4 ± 3.1	85.2 ± 14.6	14.6	14.6
Feeding Groups (%)	Fungivores	Conventional	21.2 ± 6.0	9.7 ± 5.8	35.4 ± 9.0	23.2	23.2	13.6	29.6 ± 10.4	29.6 ± 10.4	29.9 ± 8.5	8.5	8.5
		Organic	24.9 ± 2.9	24.1 ± 8.8	25.8 ± 3.5	12.0	12.0	42.1 ± 12.0	39.9 ± 12.5	39.9 ± 12.5	47.4 ± 9.4	9.4	9.4
	Bacterivores	Conventional	1.8 ± 0.7	1.2 ± 0.4	3.6 ± 1.5	1.5	1.5	5.2 ± 3.5	10.8 ± 4.3	10.8 ± 4.3	4.3 ± 3.0	3.0	3.0
		Organic	2.1 ± 2.5	1.3 ± 1.2	3.8 ± 2.6	1.3	1.3	3.8 ± 1.3	10.0 ± 3.8	10.0 ± 3.8	4.6 ± 4.0	4.0	4.0
	Predators	Conventional	67.5 ± 6.8	83.7 ± 8.2	57.9 ± 9.6	65.1	65.1	12.9	54.9 ± 9.9	54.9 ± 9.9	62.9 ± 9.2	9.2	9.2
		Organic	68.5 ± 4.5	70.2 ± 9.3	66.1 ± 6.5	14.7	14.7	50.3 ± 14.7	48.6 ± 14.4	48.6 ± 14.4	44.1 ± 12.9	12.9	12.9
	Omnivores	Conventional	0.9 ± 0.8	0.2 ± 0.4	0.0 ± 0.0	0.9	0.9	0.4 ± 0.4	0.5 ± 0.8	0.5 ± 0.8	1.1 ± 1.2	1.2	1.2
		Organic	0.6 ± 0.8	1.8 ± 0.8	0.8 ± 1.2	0.4	0.4	0.4 ± 0.4	0.6 ± 1.1	0.6 ± 1.1	0.5 ± 0.8	0.8	0.8
	Organic	Conventional	8.7 ± 5.2	5.2 ± 2.2	3.0 ± 1.9	6.1	6.1	6.1 ± 6.1	4.2 ± 3.8	4.2 ± 3.8	1.9 ± 1.4	1.4	1.4
		Organic	4.0 ± 2.1	2.8 ± 2.0	3.5 ± 2.0	2.5	2.5	3.5 ± 2.5	1.0 ± 1.6	1.0 ± 1.6	3.4 ± 2.4	2.4	2.4

	Sand			Marine clay			
	SE 1	SE 2	SE 3	SE 1	SE 2	SE 3	
Herbivory strategy (% from herbivores)	Conventional	4.8 ± 1.8	8.3 ± 5.4	7.5 ± 9.8	9.8 ± 9.8	1.9 ± 3.4	4.1 ± 4.1
	Organic	1.6 ± 2.9	4.8 ± 5.2	9.2 ± 19.0	0.2 ± 0.5	8.4 ± 18.5	3.9 ± 5.4
	Conventional	17.1 ± 15.1	16.4 ± 12.2	21.0 ± 14.8	14.4 ± 13.5	3.2 ± 4.5	5.2 ± 7.8
	Organic	17.0 ± 7.1	17.3 ± 9.9	20.3 ± 13.5	25.6 ± 21.1	25.2 ± 15.3	24.6 ± 12.1
	Conventional	1.1 ± 1.8	2.9 ± 5.1	1.8 ± 2.1	3.5 ± 7.9	9.7 ± 18.4	2.9 ± 6.4
	Organic	2.4 ± 5.8	1.5 ± 2.5	12.4 ± 16.1	3.9 ± 4.3	4.3 ± 7.1	4.5 ± 4.6
	Conventional	52.5 ± 22.7	33.7 ± 16.7	41.0 ± 10.1	16.6 ± 13.4	17.8 ± 14.9	22.3 ± 32.4
	Organic	49.2 ± 16.0	42.1 ± 7.7	33.7 ± 18.8	21.3 ± 10.5	19.1 ± 15.9	16.1 ± 16.2
	Conventional	28.3 ± 13.5	42.2 ± 31.2	30.9 ± 24.6	47.7 ± 19.5	67.9 ± 21.5	65.6 ± 29.7
	Organic	29.4 ± 12.2	34.4 ± 10.6	24.5 ± 10.2	41.4 ± 13.2	43.0 ± 19.8	51.0 ± 13.1
	Conventional	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	7.9 ± 7.3	0.6 ± 1.3	0.6 ± 1.3
	Organic	0.5 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	7.6 ± 8.6	0.0 ± 0.0	0.0 ± 0.0
C-P group (% of Free living)	Conventional	67.4 ± 8.4	79 ± 9.4	48.1 ± 22.9	66.6 ± 11.4	49.4 ± 11.8	58.3 ± 12.3
	Organic	52.0 ± 13.1	51.1 ± 13.5	50.9 ± 17.4	60.8 ± 17.4	52.8 ± 6.6	53.9 ± 24.2
	Conventional	27.7 ± 6.0	18.7 ± 8.4	47.3 ± 20.6	25.3 ± 6.4	42.6 ± 9.2	34.6 ± 8.5
	Organic	41.8 ± 12.0	43.4 ± 11.8	42.5 ± 13.9	28.9 ± 13.9	39.3 ± 7.2	29.9 ± 18.1
	Conventional	2.0 ± 1.8	0.5 ± 0.9	1.9 ± 1.6	0.6 ± 0.9	1.6 ± 1.8	1.1 ± 1.0
	Organic	1.3 ± 2.1	2.0 ± 1.5	1.9 ± 1.9	0.5 ± 0.9	0.5 ± 1.1	2.8 ± 3.3
	Conventional	2.9 ± 2.2	1.5 ± 2.1	2.0 ± 1.2	7.3 ± 4.8	3.8 ± 3.5	5.7 ± 4.9
	Organic	4.9 ± 2.5	3.3 ± 1.5	4.1 ± 3.9	9.8 ± 5.2	7.0 ± 5.8	13.0 ± 10.6
	Conventional	0.0 ± 0.0	0.2 ± 0.4	0.7 ± 0.6	0.2 ± 0.5	2.6 ± 3.1	0.2 ± 0.5
	Organic	0.0 ± 0.0	0.2 ± 0.4	0.6 ± 0.7	0.0 ± 0.0	0.4 ± 1.0	0.5 ± 0.8

Table S5. Average and standard deviations values of nematode community indicators measured for conventional (Conv) and integrated land management practices (Int) at the Vredepeel experimental farm taken on different years.

		2007		2009		2011		2018	
Ecological indicators	Richness	Conventional	20 ± 3	3	20 ± 2	2	21 ± 3	3	24 ± 1
		Integrated	15 ± 2	2	18 ± 1	1	22 ± 2	2	22 ± 2
	Abundance (n per 100g)	Conventional	1639 ± 382	382	1834 ± 199	199	2041 ± 182	182	2273 ± 709
		Integrated	1654 ± 359	359	1720 ± 332	332	2073 ± 226	226	2206 ± 439
	Index of community specialisation	Conventional	0.82 ± 0.11	0.11	0.79 ± 0.05	0.05	0.72 ± 0.10	0.10	0.48 ± 0.08
		Integrated	0.89 ± 0.07	0.07	0.74 ± 0.01	0.01	0.79 ± 0.06	0.06	0.54 ± 0.09
	Structure Index	Conventional	27.7 ± 16.8	16.8	36.7 ± 10.4	10.4	35.4 ± 11.3	11.3	52.4 ± 8.3
		Integrated	39.4 ± 28.6	28.6	37 ± 16.4	16.4	40.5 ± 9.5	9.5	51.5 ± 14.3
	Enrichment Index	Conventional	37.7 ± 15.5	15.5	75.7 ± 10.1	10.1	82.6 ± 2.5	2.5	85.3 ± 5.1
		Integrated	30.5 ± 19.7	19.7	77.3 ± 10.9	10.9	78.9 ± 8.2	8.2	85.6 ± 6.6
Feeding Groups (%)	Herbivores	Conventional	52.5 ± 18.1	18.1	27.0 ± 5.4	5.4	23.9 ± 8.2	8.2	15.3 ± 4.2
		Integrated	63.0 ± 22.8	22.8	26.5 ± 4.9	4.9	25.2 ± 5.4	5.4	17.3 ± 8.0
	Fungivores	Conventional	2.4 ± 2.6	2.6	3.3 ± 1.1	1.1	3.7 ± 1.6	1.6	7.1 ± 4.7
		Integrated	1.0 ± 0.4	0.4	4.7 ± 2.4	2.4	3.4 ± 1.9	1.9	3.8 ± 2.3
	Bacterivores	Conventional	42.2 ± 17.0	17.0	63.6 ± 8.1	8.1	67.2 ± 7.2	7.2	69.8 ± 4.6
		Integrated	31.5 ± 23.1	23.1	64.7 ± 4.7	4.7	62.4 ± 6.2	6.2	70.6 ± 7.8
	Predators	Conventional	1.7 ± 1.1	1.1	1.4 ± 0.5	0.5	1.2 ± 1.0	1.0	1.3 ± 1.1
		Integrated	3.9 ± 2.8	2.8	2.1 ± 2.8	2.8	2.5 ± 2.3	2.3	1.0 ± 0.8
	Omnivores	Conventional	1.3 ± 0.0	0.0	4.7 ± 4.0	4.0	4.1 ± 2.0	2.0	6.6 ± 2.3
		Integrated	0.7 ± 0.9	0.9	2.1 ± 0.8	0.8	6.6 ± 3.4	3.4	7.3 ± 3.3

		2007	2009	2011	2018
Herbivory strategy (% from herbivores)	Sedentary parasites				
	Conventional	0.6 ± 1.1	0.0 ± 0.0	15.3 ± 17.7	8.1 ± 10.9
	Integrated	0.5 ± 0.9	0.6 ± 1.1	0.0 ± 0.0	2.7 ± 3.4
	Migratory endoparasites				
	Conventional	23.0 ± 22.2	43.9 ± 10.7	8.9 ± 3.7	16.7 ± 5.8
	Integrated	6.0 ± 4.2	8.7 ± 7.8	4.2 ± 1.1	3.4 ± 5.3
	Semi endoparasites				
	Conventional	5.5 ± 6.4	11.7 ± 14.2	4.7 ± 6.6	6.0 ± 9.5
	Integrated	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.9 ± 3.6
	Ectoparasites				
	Conventional	61.7 ± 33.1	18.4 ± 16.8	29.8 ± 23.0	27.7 ± 7.3
	Integrated	86.5 ± 6.8	48.8 ± 15.5	50.7 ± 11.2	52.7 ± 13.2
	Epidermal root hair feeders				
	Conventional	9.2 ± 7.9	26.1 ± 6.2	41.4 ± 3.2	41.7 ± 6.3
Integrated	7.1 ± 11.0	42 ± 10.8	45.1 ± 11.5	38.3 ± 18.8	
Algal lichen moss feeders					
Conventional	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
Integrated	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
C-P group (% of Free living)	CP1				
	Conventional	12.2 ± 8.5	42.1 ± 14.1	51.1 ± 3.9	53.5 ± 9.8
	Integrated	8.6 ± 7.5	44.1 ± 14.2	45.4 ± 10.6	55.2 ± 11.5
	CP2				
	Conventional	79.2 ± 9.3	51.1 ± 11.3	43.8 ± 5.0	37.1 ± 9.4
	Integrated	76.0 ± 16.4	49.5 ± 16.3	47.0 ± 9.8	36.3 ± 13
	CP3				
	Conventional	2.7 ± 3.9	0.5 ± 0.5	0.4 ± 0.5	0.6 ± 0.7
	Integrated	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.9	0.0 ± 0.0
	CP4				
Conventional	4.7 ± 4.4	3.4 ± 1.1	2.7 ± 1.0	6.7 ± 0.9	
Integrated	14.5 ± 14.3	5.2 ± 3.1	5.4 ± 2.0	7.2 ± 2.8	
CP5					
Conventional	1.3 ± 1.0	2.9 ± 2.7	2.1 ± 1.4	2.1 ± 1.7	
Integrated	0.9 ± 1.3	1.2 ± 1.8	1.7 ± 1.9	1.4 ± 0.8	

Table S6. Average nematode community indicator values measured for conventional and organic land management practices under different tillage (T) regimes at the BASIS experimental farm four different years: conventional tillage (CT) and minimum tillage (MT).

	Standard Tillage						Minimum tillage					
	2009	2012	2014	2018	2009	2012	2014	2018	2009	2012	2014	2018
Ecological indicators	Richness	21 ± 3	22 ± 2	24 ± 4	14 ± 2	18 ± 3	22 ± 2	14 ± 2	18 ± 3	22 ± 2	25 ± 5	15 ± 2
	Abundance (n per 100g)	16 ± 3	18 ± 2	21 ± 4	16 ± 2	20 ± 2	20 ± 1	16 ± 2	20 ± 2	20 ± 1	24 ± 4	17 ± 3
	Index of community specialisation	1446 ± 364	895 ± 200	3229 ± 1258	1223 ± 288	1390 ± 983	1398 ± 886	1084 ± 222	1452 ± 691	2696 ± 1674	3214 ± 793	1442 ± 302
	Structure Index	2430 ± 1106	1223 ± 241	3026 ± 1390	1452 ± 691	2696 ± 1674	1240 ± 323	1442 ± 302	0.52 ± 0.15	0.45 ± 0.11	0.64 ± 0.07	0.48 ± 0.12
		0.67 ± 0.14	0.55 ± 0.06	0.53 ± 0.07	0.52 ± 0.15	0.45 ± 0.11	0.64 ± 0.07	0.48 ± 0.12	0.47 ± 0.15	0.49 ± 0.15	0.5 ± 0.11	0.48 ± 0.1
		0.4 ± 0.07	0.51 ± 0.06	0.51 ± 0.12	0.47 ± 0.15	0.49 ± 0.15	0.5 ± 0.11	0.48 ± 0.1	22.8 ± 24.1	50.6 ± 17.4	57.7 ± 11.8	25.9 ± 22.9
		41.2 ± 19.2	58.4 ± 13.1	53 ± 9.9	22.8 ± 24.1	50.6 ± 17.4	57.7 ± 11.8	25.9 ± 22.9	57.4 ± 17.1	50.5 ± 11.9	51.2 ± 11.1	54.3 ± 16.6
		35.6 ± 24	54.4 ± 22.2	42.9 ± 8.7	57.4 ± 17.1	50.5 ± 11.9	51.2 ± 11.1	54.3 ± 16.6	78.8 ± 12	78.3 ± 5.6	79 ± 16.3	71.2 ± 14.3
		79.3 ± 12.6	81 ± 14.4	74.2 ± 6.7	78.8 ± 12	78.3 ± 5.6	79 ± 16.3	71.2 ± 14.3	77.2 ± 19.4	88.3 ± 5.8	60.3 ± 14.3	78.6 ± 10.9
		92.8 ± 5.9	70.2 ± 16.1	64.8 ± 8.1	77.2 ± 19.4	88.3 ± 5.8	52.9 ± 18	78.6 ± 10.9	28.1 ± 11.7	33 ± 8.3	45.7 ± 16.2	25.9 ± 12.5
Feeding Groups (%)	Herbivores	47.8 ± 12.6	44.4 ± 13.7	30.1 ± 6.3	28.1 ± 11.7	33 ± 8.3	45.7 ± 16.2	25.9 ± 12.5	38.9 ± 15.4	36.2 ± 15.9	49 ± 7.1	37.8 ± 11.2
	Fungivores	16.4 ± 13.7	54.6 ± 5.6	29.6 ± 14.3	38.9 ± 15.4	36.2 ± 15.9	49 ± 7.1	37.8 ± 11.2	14 ± 4.8	12.1 ± 7.3	7.6 ± 4.6	14.6 ± 9.6
	Bacterivores	10.6 ± 4	6.8 ± 3.6	16.3 ± 4.9	14 ± 4.8	12.1 ± 7.3	7.6 ± 4.6	14.6 ± 9.6	14.5 ± 10.3	8 ± 5.5	11.4 ± 4.8	10.6 ± 3.9
		5.5 ± 6	11.4 ± 4.5	20.2 ± 8.6	14.5 ± 10.3	8 ± 5.5	11.4 ± 4.8	10.6 ± 3.9	56.4 ± 11.5	50.9 ± 7.4	43.4 ± 19.5	58.3 ± 7.9
		38 ± 10.2	44.7 ± 18	46.6 ± 12.6	56.4 ± 11.5	50.9 ± 7.4	43.4 ± 19.5	58.3 ± 7.9	41 ± 19.5	52.2 ± 16.5	37.4 ± 7.5	46.5 ± 13.5
		75.3 ± 19.7	31.8 ± 9.4	46.3 ± 11.3	41 ± 19.5	52.2 ± 16.5	37.4 ± 7.5	46.5 ± 13.5	0.3 ± 0.7	1.2 ± 1.2	1.5 ± 0.9	0.1 ± 0.4
		0.8 ± 1.6	2 ± 1.5	3.1 ± 2.9	0.3 ± 0.7	1.2 ± 1.2	1.5 ± 0.9	0.1 ± 0.4	0.3 ± 0.5	0.7 ± 0.6	1.4 ± 1.1	0.3 ± 0.7
		0.5 ± 1.3	0.4 ± 0.5	0.7 ± 0.8	0.3 ± 0.5	0.7 ± 0.6	1.4 ± 1.1	0.3 ± 0.7	1.3 ± 2.1	2.8 ± 2.2	2 ± 1.3	1.1 ± 1.5
		2.9 ± 1.4	2.2 ± 1.9	4 ± 3.3	1.3 ± 2.1	2.8 ± 2.2	2 ± 1.3	1.1 ± 1.5	5.4 ± 2.5	2.9 ± 2.3	4.4 ± 4.5	4.9 ± 2.1
		2.3 ± 1.9	1.9 ± 1.5	3.3 ± 2	5.4 ± 2.5	2.9 ± 2.3	0.9 ± 1.2	4.9 ± 2.1				

		Standard Tillage				Minimum tillage			
		2009	2012	2014	2018	2009	2012	2014	2018
Herbivory strategy (% from herbivores)	Sedentary parasites	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.9	0.0 ± 0.0	0.4 ± 0.8	0.9 ± 1.3	0.5 ± 1.5
	Organic	0.2 ± 0.7	0.0 ± 0.0	2.7 ± 4.3	3.1 ± 6.0	0.7 ± 1.4	0.0 ± 0.0	0.7 ± 2.1	0.5 ± 1.4
	Conventional	7.2 ± 3.8	4.9 ± 2.0	13.9 ± 9.8	9.6 ± 12.8	0.8 ± 1.5	6.7 ± 5.7	9.6 ± 9.7	18.8 ± 12.8
	Organic	3.0 ± 4.0	0.0 ± 0.0	3.0 ± 3.5	0.5 ± 1.5	2.3 ± 2.2	0.0 ± 0.0	4.4 ± 11.3	2.8 ± 2.6
	Conventional	51.7 ± 22.4	26.4 ± 12.9	38.9 ± 15.9	23.1 ± 20.3	16.9 ± 20.7	29.6 ± 14.6	53.3 ± 14.9	6.2 ± 7.9
	Organic	10.9 ± 9.9	12.1 ± 15	43.1 ± 28.3	20 ± 18.3	37.6 ± 18.1	8.6 ± 7.6	35.3 ± 21.2	19.6 ± 12.8
	Conventional	12.0 ± 6.7	8.8 ± 8.5	13.1 ± 12.5	37.4 ± 22.7	1.2 ± 2.7	14.7 ± 7.2	16.6 ± 10.4	45.1 ± 17.3
	Organic	3.0 ± 3.1	8.6 ± 7.0	8.8 ± 11.7	34.5 ± 28.3	5.1 ± 3.1	13.0 ± 9.2	10.3 ± 8.0	23.1 ± 15.8
	Conventional	20.5 ± 13.5	59.3 ± 11.9	23.6 ± 17.8	24.0 ± 6.1	64.5 ± 19.4	48.5 ± 13.5	15.9 ± 13.9	16.3 ± 12.8
	Organic	57.4 ± 17.2	79.4 ± 12.3	21.3 ± 17.8	15.9 ± 8.2	38.6 ± 14.6	78.4 ± 9.6	31.3 ± 26.7	32.9 ± 13.8
	Conventional	8.4 ± 7.2	0.7 ± 1.0	10.6 ± 15.0	5.0 ± 6.6	16.6 ± 12.8	0.2 ± 0.6	3.8 ± 4.8	13.1 ± 9.6
	Organic	25.5 ± 14.3	0.0 ± 0.0	21.1 ± 19.6	26.0 ± 6.9	15.7 ± 11.9	0.0 ± 0.0	18.0 ± 12.0	21.2 ± 9.5
C-P group (% of Free living)	CP1	46.2 ± 21.7	49 ± 26.5	33 ± 11.1	46.3 ± 17.4	40.3 ± 10.4	47.3 ± 28.6	31.4 ± 9.6	35.5 ± 15.1
	Organic	74.4 ± 16.6	31 ± 18.3	24.3 ± 7.1	42.6 ± 27.7	60.9 ± 15.2	18.3 ± 13.7	21.8 ± 12.7	40.4 ± 14.2
	Conventional	43.8 ± 15	36.8 ± 19.9	50.7 ± 6.9	48.9 ± 19	46.2 ± 8.5	37.5 ± 20.2	50.5 ± 9.9	58.6 ± 19.3
	Organic	21.3 ± 13.9	51.8 ± 21.5	62.7 ± 5.1	44 ± 26.6	31.2 ± 11.2	63.4 ± 11.2	59.6 ± 8.1	43.5 ± 9.7
	Conventional	1.1 ± 1.6	3.6 ± 2.8	3.9 ± 2.7	2.1 ± 4.0	1.6 ± 1.6	4.0 ± 4.4	4.1 ± 3.7	2.1 ± 2.0
CP4	0.3 ± 0.6	3.5 ± 2.6	2.2 ± 2.1	1.4 ± 1.6	0.3 ± 0.5	2.8 ± 1.8	3.1 ± 3.0	2.1 ± 3.1	
Conventional	7.0 ± 5.7	8.9 ± 6.1	10.8 ± 4.5	2.5 ± 2.8	9.9 ± 6.3	9.1 ± 5.8	10.9 ± 4.0	3.4 ± 4.8	
Organic	3.4 ± 3.8	11.5 ± 6.7	10.4 ± 4.9	12 ± 2.5	4.8 ± 2.7	14 ± 5.7	14.1 ± 6.0	14.1 ± 9.7	
Conventional	1.8 ± 1.4	1.7 ± 2.2	1.5 ± 1.9	0.2 ± 0.5	2.1 ± 1.6	2.1 ± 3.3	3.1 ± 3.4	0.4 ± 1.2	
Organic	0.6 ± 1.1	2.2 ± 2.5	0.4 ± 1.0	0.0 ± 0.0	2.8 ± 3.4	1.5 ± 2.4	1.4 ± 2.1	0.0 ± 0.0	

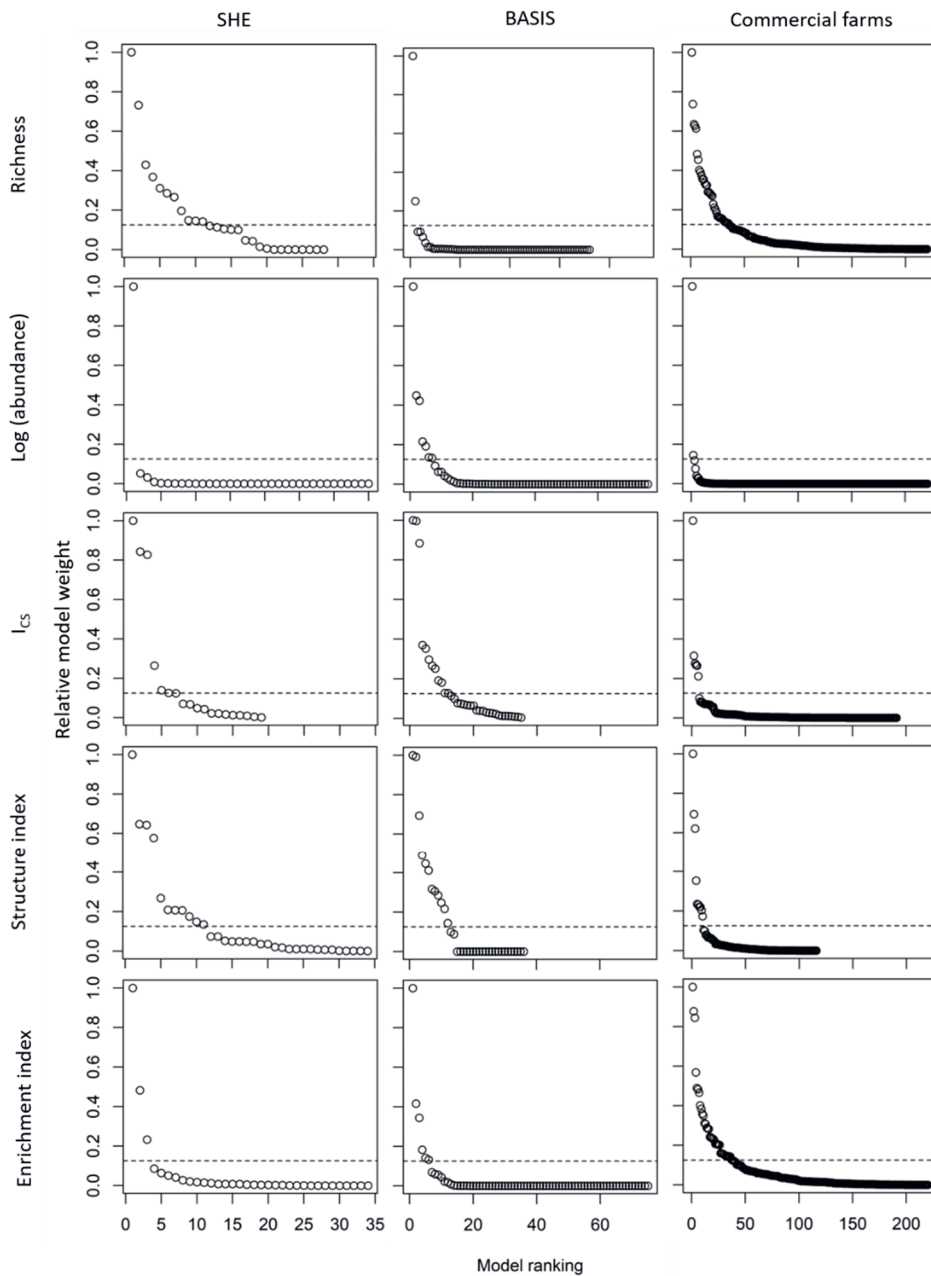


Figure S1. Relative plausibility of each model compared to the best fitting model for the Soil ealth experiment (SHE) on the left column, the *Broekemahoeve Applied Soil Innovation Systems experiment* (BASIS) on the middle column and Commercial farms on the panel on the right; and each indicator analysed as a response variable in the models (rows). The best model is number 1 on the x-axis, with the highest relative model weight on the y-axis.

Chapter 5

Assessing multifunctionality of agricultural soils: Reducing the biodiversity trade-off

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Abstract

Soils are indispensable for the provision of several functions. Agricultural intensification and its focus on increasing primary productivity (PP) poses a threat to soil quality, due to increases in nutrient loads, greenhouse gas emissions and declining biodiversity. The EU Horizon 2020 Landmark project has developed multi-criteria decision models to assess five soil functions: PP, nutrient cycling (NC), soil biodiversity and habitat provision (B-HP), climate mitigation and water regulation, simultaneously in agricultural fields. Using these algorithms, we evaluated the supply of PP, NC and B-HP of 31 grasslands and 21 croplands as low, medium or high. The multi-criteria decision models showed that 38% of the farms had a medium to high supply of all three soil functions, whereas only one cropland had a high supply for all three. Forty-eight per cent of the farms were characterized by a high supply of PP and NC. We observed a clear trade-off between these two functions and B-HP. Multivariate statistical analyses indicated that higher organic inputs combined with a lower mineral fertilization concur with higher biodiversity scores while maintaining a medium delivery of PP and NC. Additionally, we compared the outputs of the model predictions to independent variables that served as proxies for the soil functions and found: (a) croplands (but not grasslands) with high PP had a higher standardized yield than those with medium PP; (b) grasslands (but not croplands) with high NC had a significantly lower fungal to bacterial biomass ratio, suggesting faster decomposition channels; and (c) a positive though non-significant trend between B-HP score and rank according to soil invertebrate biodiversity. These comparisons suggest a successful upscaling of the models from field to farm level. Our study highlights the need for systematic collection of management-related data for the assessment of soil functions. Multifunctionality can be achieved in agricultural soils; however, without specifically managing for it, biodiversity might come at a loss.

Highlights:

- We study how well soils can provide primary productivity, nutrient cycling and biodiversity.
- We study trade-offs and synergies among soil functions, as well as the drivers of these relationships.
- Soil biodiversity is largely sacrificed for primary productivity and nutrient cycling
- Changes in pesticide and fertilizer management can increase soil multifunctionality.

Keywords: cropland, grassland, land management, nutrient cycling, primary productivity, soil biodiversity, soil functions, yield

5.1 Introduction

Humans derive multiple benefits from the soil system. Soils provide us with feed, fibre and fuel, cycle and mobilise nutrients, control diseases, regulate the climate, maintain water quality and provide a habitat to support biodiversity amongst other things (Dominati *et al.* 2010). The continued delivery of these soil functions is at risk, due to the effects of economic growth, population growth and climate change (Montanarella *et al.* 2016). These threats result not only in the loss of functional soils, but also reduce the capacity of the soils to function optimally. To face this challenge, we need to change land management and extend our focus away from the delivery of only primary productivity (PP) (Tilman *et al.* 2011; Rutgers *et al.* 2012; Schulte *et al.* 2014; Clay *et al.* 2019).

Agricultural soils are particularly complex to manage. Farmers face a continuous challenge to maintain high productivity whilst considering the resilience of their soil to ensure that the land can support continued productivity for future generations. Additionally, maintaining or even increasing agricultural yield is imperative to sustain a growing population, but to do so, farmers have relied heavily on external inputs and intensive crop rotations, which result in increased greenhouse gas emissions, eutrophication, pollution, loss of organic carbon and loss of above and belowground biodiversity (Matson *et al.* 1997; Tilman *et al.* 2001; Foley *et al.* 2005; Foley *et al.* 2011; Tsiafouli *et al.* 2015). These effects clash with societal goals regarding nature conservation and climate change (e.g. Common European Agricultural Policy, which intends to contribute to sustainability and climate change mitigation; <https://ec.europa.eu/info/food-farming-fisheries/key-policies/common-agricultural-policy>).

Since the introduction of the ecosystem service concept (Costanza *et al.* 1997) many studies have attempted to quantify ecosystem services, but only a few have studied several simultaneously, and even less have explored the synergies and trade-offs therein (Seppelt *et al.* 2011). Understanding the trade-offs and synergies between soil functions as well as recognising which variables might be important in swaying the system, is a step forward in providing management solutions to farmers and policy makers alike (O'Sullivan *et al.* 2015). Schulte *et al.* (2014) proposed such a framework (the functional land management framework) to assess the supply of soil functions in order to simultaneously meet agronomic and environmental objectives. It is based upon the principle that the suitability of soils to provide a given function, for example, PP depends on soil composition, environmental conditions, as well as the choices made by the land manager, and it has steered the development of tools to aid land management,

from farmer decision making (Soil Navigator; <http://soilnavigator.eu>) to European policy assessment (Schulte *et al.* 2019) and monitoring (Zwetsloot *et al.* 2020).

The functional land management framework allows for the simultaneous assessment of five predominant soil functions related to agricultural soils (O'Sullivan *et al.* 2015). These are: (a) PP defined as the capacity of a soil to supply nutrients and water and to produce plant biomass for human use, providing food, feed, fibre, and fuel (Sandén *et al.* 2019); (b) water purification and regulation, as the capacity of a soil to remove harmful compounds from the water that it holds and to receive, store and conduct water for subsequent use and the prevention of both prolonged droughts and flooding and erosion; (c) climate mitigation as the capacity of a soil to reduce the negative impact of increased greenhouse gas emissions on climate; (d) nutrient cycling (NC) as the capacity of a soil to receive nutrients in the form of by-products, to provide nutrients from intrinsic resources or to support the acquisition of nutrients from air or water, and to effectively carry over these nutrients into harvested crops (Schulte *et al.* 2015; Schröder *et al.* 2016) and (e) soil biodiversity and habitat provision (B-HP) as the multitude of soil organisms and processes, interacting in an ecosystem, providing society with a rich biodiversity source and contributing to a habitat for aboveground organisms (van Leeuwen *et al.* 2019).

In this study, we evaluated the performance of three out of the five soil functions (PP, NC and B-HP) in 52 Dutch farms using the multi-criteria decision models (MCDMs) developed by Debeljak *et al.* (2019) after adjustment for use at the farm level. We explore the synergies and trade-offs between these three functions as well as the drivers of high functionality. The following research questions were answered:

- Were the soils under study capable of delivering a medium to high supply of more than one function simultaneously?
- Do synergies and trade-offs exist between the soil functions?
- Which input variables in the model lead to high supply of soil functions and soil multifunctionality?

5.2 Methods

5.2.1 Assessing Soil Functions

To answer all the research questions, we first calculated soil function scores. In order to do this, we first (a) gathered the necessary data to calculate soil functions using (b) a decision support system. Since the data we obtained was mostly at the farm level, but the decision support system was designed to be used at the field level, we (c) had to

adjust the model from field to farm level. We checked the success of this adjustment to the model by selecting proxy indicators of each soil function and comparing them to the scores obtained using the adjusted decision support system. All statistical analyses were carried out using R version 3.6.1 (R Core Team 2019)

a) Data acquisition

The first step to answer all research questions was gathering farm data on soil quality and management. Throughout the years 1993 and 2014, biotic and abiotic measurements of soil quality were assessed as part of the Netherlands Soil Monitoring Network (Rutgers *et al.* 2009). The assessment included new sites over the years, covering a range of agricultural and natural ecosystems. Simultaneously data were collected by Wageningen Economic Research from commercial farms through the Minerals Policy Monitoring Programme (RIVM 2019-0026 2019), aimed at monitoring the effectiveness of the Nitrates Directive (EU, 1991) and the National Minerals Policy (MANFQ & MIWM, 2017). These data provide data on farm nutrient balances including information on the type and amount of manure and mineral fertilization as well as the yield and area cover for each crop (Table S1-S2). We used these sources of information to find farms that had been sampled by both institutions in the same year leading to a total 52 farms: 31 dairy farms (referred to as grasslands from here onwards, although they often reserve some land for maize) and 21 arable farms (or croplands) sampled between 2006 and 2014. Weather variables (Table S3) were extracted from the weather station closest to each farm and are specific to the year that samples were taken from said farm using data from the Royal Dutch Meteorological Agency. Bulk density, cation exchange capacity, groundwater depth and salinity were not measured in the field but were calculated using published transfer functions or extracted from local maps (Tables S2-S3) (Helling *et al.* 1964; Rawls 1983; Dufour 2000; De Vries 2007; De Vos 2011).

b) Decision Support System

In order to calculate the performance of soil functions in the selected farms, we used MCDMs designed by the Landmark project as part of the EU Horizon 2020 (Debeljak *et al.* 2019). The initial models were developed by experts in each of the soil functions and were further evaluated and validated (Sandén *et al.* 2019; Trajanov *et al.* 2019; Van de Broek *et al.* 2019; van Leeuwen *et al.* 2019). The experts established a hierarchical model that builds up to a final function score (of low, medium or high supply) starting from qualitative input variables related to the management, environment, and soil properties. Discretization of these variables is carried out prior to running the model in accordance to a set of thresholds suited to different land uses and climatic zones. The input variables aggregate to the upper level of the model structure through if-then

functions and aggregation continues until the top node is reached and an assessment of the performance of a specific function is made. For example, in order to assess B-HP (top node), the model is fitted with input variables such as 'Soil pH' and 'Liming' at the lowest nodes. These two variables aggregate through if-then functions: if they are both low, then 'pH condition' is also low. 'pH condition' when aggregated with 'Nutrient content' and 'Nutrient input' (which each are assessed by their own input variables) leads to a score in the super-attribute 'Nutrients'. Lastly four super-attributes (*nutrients, biology, structure and hydrology*) are aggregated to a final B-HP score. The Decision Expert integrative methodology was used to build these decision models (Bohanec and Rajkovič 1990; Bohanec 2008; Bohanec *et al.* 2013).

The combination of the two datasets did not include all of the variables necessary to compute the five main soil functions, even after we complemented the dataset by analysing dried soil samples taken by the Netherlands Soil Monitoring Network (Rutgers *et al.* 2009) for total nitrogen, carbon to nitrogen ratio, pH, organic carbon (%), organic matter content (%), clay content (%), phosphorus, and magnesium (Table S2-S3). We therefore chose to focus on PP, NC and B-HP. Additionally, we had to make some assumptions about the farms. Slope was considered low ($< 3^\circ$) for all farms. Because organic arable farms did not use chemical pesticides, we assumed they used some form of biological pest management. Crop failure risk, defined as the number of times in the past 20 years that a field provided no yield in the MCDMs (<http://landmark2020.eu/landmark-glossary/>), was assumed to be low in all farms. In the Netherlands, drought can have a strong effect on crop yield, but it rarely leads to complete crop failure. A study into the most extreme droughts since 1970 found that the most affected crop (onions) can experience up to a 50% decrease in yield when compared to years with average precipitation. Such drop in yield was documented only once since 1976 (Prins *et al.* 2018). Soil phosphorus status was assessed according to the Dutch Nitrates Directive (2014-2017) (Table S3). Due to a number of missing variables while calculating PP and NC (biological-pest management, rooting depth, grass cutting frequency and share of legumes) some of the farms did not receive a full score, but rather were classified between two performance scores. In such cases, we downgraded the farm to the lower of the two suggested scores. In total, this measure affected three grasslands which scored medium to high in NC and where downgraded to medium NC; one cropland that was re-classified as having medium PP; and all the grasslands performing at medium PP, which were initially performing at medium to high PP.

c) From field to farm level

The MCDMs were intended to be used at field scale, however, the data within the Netherlands Soil Monitoring Network on soil quality as well as some of the information collected in the Minerals Policy Monitoring Programme, were collected at farm level. Consequently, parts of the models had to be adjusted to account for farm level variability. The input variable ‘number of crops in rotation’ was changed to ‘crop diversity’ to reflect the spatial variability in crops within a farm using the Shannon-Weaver index, calculated using the ‘*diversity*’ function of the ‘*vegan*’ package for R (Oksanen *et al.* 2018). The threshold values for low or high crop diversity were the lower and higher quartiles of the calculated indices: 1.10 and 1.52 respectively, with an average crop diversity of 1.35. The input variable ‘loss of ammonia’ from manure per farm was calculated within the Minerals Policy Monitoring Programme (RIVM 2019-0026 (2019); Appendix 2), and as with crop diversity, the thresholds for low, medium and high were derived from the distribution of the data, such that losses below 11 kg of nitrogen per hectare were considered low, between 11-30.3 kg they were considered medium, and above 30.3 kg they were considered high.

To assess the success of upscaling the MCDMs from field to farm level, we compared the results obtained from the farm level MCDMs with selected proxies for soil multifunctionality. Choosing appropriate proxies was complicated since the chosen variables had to be independent from those required by the MCDMs (Table S2-S3) and the MCDMs encompass many aspects of each soil function.

We compared the results from the PP MCDM with standardised yield. The Minerals Policy Monitoring Programme contains information on the crop, the area devoted to said crop, and the crop’s yield for each farm. Using this information, we calculated standardised yield using the following formula:

$$SY = \sum_{i=1}^n \left(\frac{100 \times MY_i}{PY_i} \right) \times PA_i$$

where n is the number of crops in farm i , MY_i is the measured yield for crop i , PY_i is the country wide potential yield for that crop (Silva *et al.* 2020) and PA_i is the proportional area that that crop occupies in the farm. This approach had three limitations: (i) potential yield was only known for the most common crops grown in the Netherlands, which means fields with uncommon crops were not included in the calculation; (ii) the grass yield was not measured, but rather calculated from information on the farm’s overall performance using the amount of external feed bought, the number of cows in each dairy farm, the energetic requirements of cows, and the total

milk production of the farm (RIVM 2019-0026 (2019); Appendix 2); and (iii) there was no potential yield estimate for grass, therefore, we used the distribution of grass yield to establish the low medium and high categories, using the first and third quartiles as the threshold values. Although potential yield is likely to be different for different soil types, we chose to use a national level potential yield to be able to compare the standardized yield with the result from the PP MCDM, which includes soil type in the calculation of the PP score.

Due to the fact that there are two distinct decomposition paths in the soil (fungal and bacterial) we compared the NC MCDM scores with two biological indicators: the fungal to bacterial (F:B) biomass ratio and the ratio between the maturity of the plant parasitic nematode community (PPI) (Bongers 1990) and the maturity of the free-living nematode community (MI) (Bongers 1990). Sites with readily decomposable materials tend to be bacterial dominated (Bloem and Breure 2003). Nematode composition can also provide insight into the efficiency of the nutrient cycle. Bongers *et al.* (1997), based on empirical observations, proposed that the PPI:MI ratio can indicate a nutrient enrichment effect. With increasing fertilization, an increase in opportunistic species causes the MI to decrease, and the PPI to increase due to an increase in carrying capacity for plant parasitic nematodes. The microbial and nematode communities were sampled as part of the Netherlands Soil Monitoring Network. The MI and PPI were calculated using the online tool Ninja (Sieriebriennikov *et al.* (2014); accessed on May 2019). Neither of the two selected proxy indicators (PPI:MI ratio and F:B biomass ratio) can fully compare to the full NC model since both proxy indicators can only represent variations in the capacity of a soil to mineralize materials (*'Mineralization'* in the NC MCDM) and, to a certain degree, the ability of a soil to make nutrients available to plants (*'Nutrient recovery'* in the NC MCDM), but neither can capture the variation within the *'Harvest index'*, the third upper node in the NC model.

Lastly, we compared the results from the B-HP model, with the farms' biodiversity rank, calculated by using principal component analyses (PCA) (Ter Braak 1986; Legendre and Legendre 2012). The PCAs explained the variation in the invertebrate diversity, including earthworm, micro-arthropod and enchytraeid species and nematode genera. As input variables for the PCA we used the Shannon-Weaver diversity: $H' = \sum_{i=1}^n p_i \times \ln(p_i)$, where p_i is the proportional abundance of species i ; and Pielou's evenness indices: $J' = H'/H'_{max}$, where H'_{max} is the Shannon-Weaver diversity if all species present in a site were present with equal proportional abundance (Shannon 1948; Pielou 1966) of the afore-mentioned faunal groups, scaling the input variables to unit variance.

We performed either one-way ANOVAs or Kruskal Wallis' test (when the assumptions of homogeneity of variance and normality of the residuals were not met) using each of the selected indicators as the dependent variable and the MCDM scores for each soil function as the grouping variable (Chambers and Hastie 1992; Hollander and Wolfe 1999). PCA was calculated with the *'rda'* function in the *'vegan'* package (Oksanen *et al.* 2018).

5.2.2 Soil multifunctionality

To answer our first research question, we created frequency plots for the low, medium and high scores in each soil function (PP, NC and B-HP) and assessed the number of farms with a high and low supply of all three, two, one or none of the studied functions.

5.2.3 Synergies and trade-offs

To understand the synergies and trade-offs that occurred between PP, NC and B-HP we used frequency plots and observed the overlap between sites with low, medium or high supply scores. We also studied how often functions tended to be supplied at a high or low level together.

5.2.4 Variables associated with soil multifunctionality

Studying the variables that associate with multifunctionality was a two-step process. Firstly, we looked into the scores of the highest tier of each of the MCDMs. Each of these super-attributes is a result of several aggregations of discretized input variables into several layers of tiers. The upper tiers represent distinct parts of each soil function. They are *'Soil'*, *'Management'*, *'Land use related management'*, and *'Environment'* for PP; *'Nutrient recovery'*, *'Mineralization'* and *'Harvest index'* for NC; and *'Structure'*, *'Nutrients'*, *'Hydrology'* and *'Biology'* for B-HP. The distribution of high, medium and low scores for these super-attributes can help us understand the drivers behind the MCDM scores. Secondly, we performed redundancy analyses (RDA) (Ter Braak 1986; Legendre and Legendre 2012) to understand which variables better explained the capacity of grasslands and croplands to provide high PP, NC and B-HP. We calculated 6 separate RDAs, specifically, 3 for grasslands and 3 for croplands: using either the soil, the environmental or the management related variables that served as inputs for the MCDMs. To reduce the number of explanatory variables and avoid over-parameterisation, we eliminated redundant variables, variables with very little variation, and those that were co-linear when they had a variation inflation factor higher than 10 (Greenacre 1984; Gross 2003). Cation exchange capacity and bulk density were not

independent from soil organic matter and clay content, hence, these parameters were not included in the RDA models. Yearly average temperature and precipitation correlated strongly with the number of days with an average temperature above 5°C, and precipitation during the first month of the growing season, respectively. Only the latter two were included into the RDA models. Soil pH, phosphorus and potassium content had high variation inflation factors and were excluded from the RDA model. We used the ‘*ordistep*’ function for variable selection. We calculated the adjusted R² of each model using Peres-Neto *et al.* (2006) permutation approach and an ANOVA-like permutation test for Redundancy Analysis of Principal Coordinates for each of the covariates, axes and for the whole RDA model (Legendre *et al.* 2011). All calculations were performed using the ‘*vegan*’ package for R (Oksanen *et al.* 2018).

5.3 Results

5.3.1 From field to farm level

There was a significantly lower standardised yield in croplands with medium PP than those with high PP (Kruskal Wallis $\chi^2 = 4.9$, p -value = 0.03). For the grassland farms we found no significant differences between the standardised yield for farms categorised into the low, medium or high supply of PP (ANOVA, $F = 2.13$, p -value = 0.15).

We observed no significant differences in the PPI:MI ratio of grasslands (Kruskal Wallis $\chi^2 = 0.39$, p -value = 0.22; with a mean PPI:MI of 1.54) or croplands (Kruskal Wallis $\chi^2 = 1.79$, p -value = 0.23; with a mean PPI:MI ratio of 1.57) with low, medium or high NC. The F:B ratio was significantly lower in grasslands with high NC compared to those farms with medium NC, but neither differed significantly from the low NC supply (Kruskal Wallis $\chi^2 = 8.31$, p -value = 0.02). There was no significant relationship in the croplands, where overall the F:B ratio was very low (Kruskal Wallis $\chi^2 = 2.95$, p -value = 0.23; with an average value of 0.56).

The first two axes of the PCAs explaining the variation in diversity indicators in grasslands and croplands explained 54% and 56% of the total variation for grassland and cropland farms, respectively (Figure S1). In both cases, enchytraeid evenness was strongly positively correlated with the first axis, and nematode diversity with the second, as can be observed by the direction of increase of both indices along the axes of the biplot (Figure S1).

General positive trends were apparent between the scores of the first PCA axes and the B-HP scores, but none of the differences were statistically significant (ANOVA, $F=2.34$, p -value = 0.12 for grasslands and ANOVA, $F=1.57$, p -value = 0.23 for the croplands). The rank of the sites along the second RDA was also not significantly related

to the score received by the MCDM (ANOVA, $F=0.04$, p -value = 0.96 for grasslands and ANOVA, $F=0.95$, p -value = 0.41 for the croplands).

5.3.2 Soil multifunctionality

Only two grasslands performed poorly in two functions simultaneously (Figure 1c), in other words from the 31 grasslands included in our study, 29 of them delivered two or more functions at a medium or high level simultaneously, while all the croplands delivered two or more functions (Figure 5.1d). Additionally, none of the farms had a low supply of all three functions (Figure 5.1c-d). The MCDM, however, predicted that only one cropland soil could score high in all three functions (Figure 5.1b). PP was high in 18 grasslands and 18 croplands (58% and 86% respectively; Figure 5. 1a-b), and only three out of the 52 farms (6%) had a low supply of PP. Seven out of the 52 sites (13%) showed a low NC while 46% of the farms had a low B-HP (Figure 5.1c-d). The proportion of farms with low, medium and high NC was similar in both grasslands and croplands (Figure 5.2 a-b).

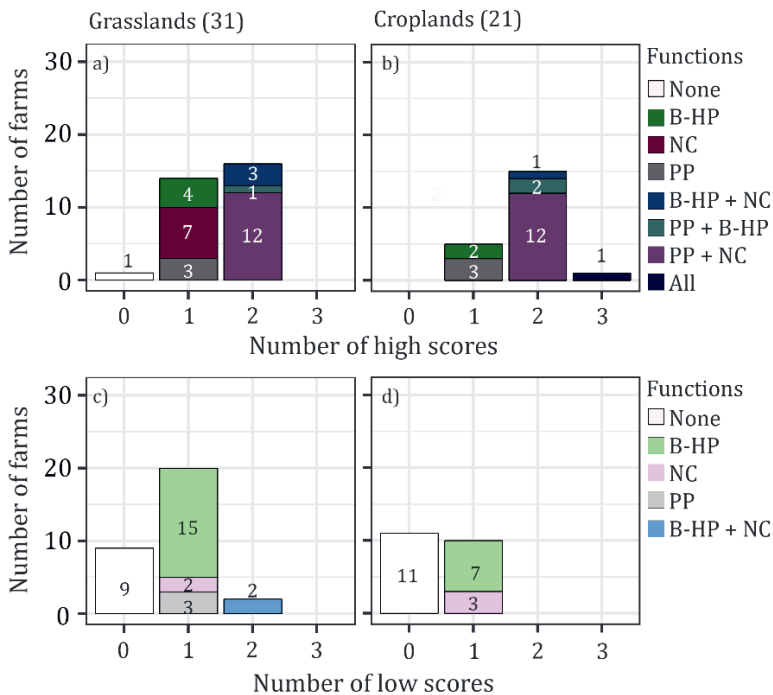


Figure 5.1. Number of grasslands (left panels) and croplands (right panels) with a high (a-b) or low (c-d) supply of none, one, two or three soil functions. Scores were obtained from multi-criteria decision models for primary productivity, nutrient cycling and biodiversity and habitat provision. Colours denote which soil function/s had a high or low score. B-HP, biodiversity and habitat provision; NC, nutrient cycling; PP, primary productivity

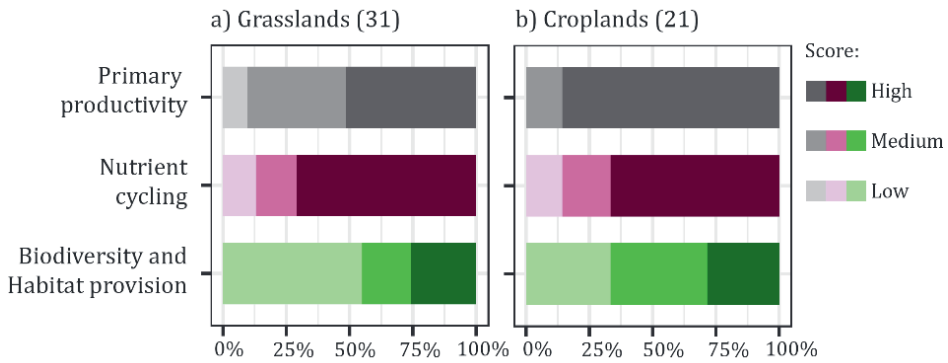


Figure 5.2. Frequency of functionality scores derived from multi-criteria decision models for primary productivity (grey), nutrient cycling (pink) and biodiversity and habitat provision (green) on grasslands (a) or croplands (b) in the Netherlands

5.3.3 Synergies and trade-offs

As the MCDM model provided the supply of the three functions for any given farm, it enabled further assessment of potential synergies and trade-offs between the soil functions. High PP scores co-occurred with high NC in 12 grasslands (39%) and 13 croplands (62%), but only one grassland and three croplands had both high B-HP and high PP simultaneously (Figure 5.1a-b). 20 farms did not receive a low score for any of the functions and sites with medium PP did not necessary get a medium B-HP score which implies that the relationship between the provision of PP and B-HP is not strictly linear (Figure 5.3).

5.3.4 Variables associated with soil multifunctionality

The analysis of the top tier of the MCDM (or super-attributes) that adds up to the PP score gave us insight into which parts of the soil system might be driving the score. Out of the four super-attributes related to PP, ‘*Environment*’ always scored high (Figure 4a-b), because the input variables related to ‘*Environment*’ (slope, annual precipitation and annual temperature) were considered suitable for high PP by the MCDM. ‘*Soil*’, which is an aggregate from input variables such as pH, soil organic matter, carbon to nitrogen ratio, abundance of macro-elements, cation exchange capacity or salinity, was often either medium or low (Figure 5.4a-b). ‘*Grassland management*’ (which results from the evaluation of variables such as stocking rate and legume presence; Figure 5.4a) showed a lower score than ‘*cropland management*’ (crop diversity, legume cover and type of crops; Figure 5.4b).

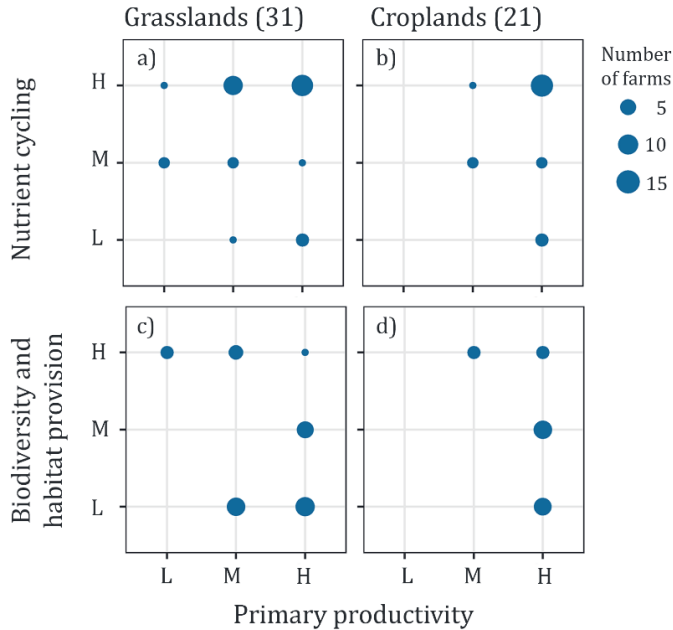


Figure 5.3. Frequency of grasslands (left panels) and croplands (right panels) with low (L), medium (M) or high (H) capacity to provide primary productivity and nutrient cycling (a-b) and primary productivity and biodiversity and habitat provision (c-d). Soil function scores were obtained using multi-criteria decision models.

The super-attributes determining the NC supply all indicate mostly medium to high supply capacity (Figure 5.4c-d). The ‘*Harvest index*’ was high on all farms, which is the result of a combination of an assumption of low residues left in the field and as well as low crop failure risk (both input variables into the MCDM). ‘*Mineralization*’, which in the MCDM is a result of the aggregation of temperature, precipitation and pH, was medium on only 13% of the farms, and for the remainder was either high or low. No croplands scored low in terms of nutrient recovery (Figure 5.4d).

According to the scores of the B-HP super-attributes, the soil ‘*Structure*’ is more suited to B-HP in croplands than grasslands. ‘*Nutrient status*’ and ‘*Hydrology*’ of the studied soils, however, show a higher potential for high B-HP in grasslands than croplands (Figure 5.4e-f). The ‘*Biology*’ super-attribute, however, received either a medium or a low score in all sites, except one cropland (Figure 5.4f). The variables that determine the ‘*Biology*’ super-attribute were the use of pesticides, the presence of grassland in the arable rotation, crop diversity, and legume presence, variables which were low in most of the studied farms.

According to the results from the RDAs, management variables (Figure 5.5a) and environmental (Figure 5.5c) explained an important part of the variation in the distribution of high supply scores for different soil functions in grasslands (with an adjusted R^2 of 0.52 and 0.28 respectively; Table 5.1). Grasslands with a high capacity to support NC and PP tended to a low ground water table, high clay content (above 25%; Figure 5.5c) and were measured in years where the number of days with an average temperature above 5°C, was medium i.e. 180-230 days. In grasslands, clay content was positively correlated with magnesium content (Pearson $\rho=0.7$). According to the RDA, these sites also show a positive correlation with high mineral nitrogen fertilization (more than 150 kg N/ha per year) combined with cows spending a high amount of time in the fields. High mineral nitrogen fertilization showed collinearity with the use of pesticides and only the first was included in the models. In contrast, grasslands with a high capacity to provide B-HP tended to have a high groundwater table and were sampled in years with a higher number of days with an average temperature above 5°C (above 230 days; Figure 5c) as well as a mineral nitrogen fertilization below 50 kg N/ha per year (Figure 5.5a).

For croplands, important parts of the variation in the high supply of PP, NC and B-HP were explained by soil and management variables (Table 5.1). Croplands with high PP and NC scores were characterized by very low soil organic matter (below 2%; Figure 5.5d), and high levels of mineral nitrogen fertilization (>150 kg N/ha per year) combined with low levels of organic nitrogen fertilization (Figure 5.5b; <50 kg N/ha per year). Soil organic matter was strongly and negatively correlated with bulk density (Pearson, $\rho=-0.75$), but high fertilization rates were not strongly correlated with any of the variables not included in the RDAs, that is with a correlation coefficient equal to or larger than 0.7. High B-HP scores were correlated with a medium to high nitrogen to phosphorus ratio (above 20) and a high carbon to nitrogen ratio, and more than 8% soil organic matter, but correlated negatively with the use of cover crops, and crop diversity. The nitrogen to phosphorus ratio was negatively correlated with pH (Pearson, $\rho = -0.71$), meaning that this observation could in fact be a result of lower pH values in sites with high B-HP than those with a low score. Similarly, the carbon to nitrogen ratio of croplands was negatively correlated with clay content, bulk density and pH (Pearson, $\rho = -0.73$, $\rho = -0.74$ and $\rho = -0.97$ respectively) and positively with the phosphorus and potassium load (Pearson, $\rho = 0.82$ and $\rho = 0.73$ respectively).

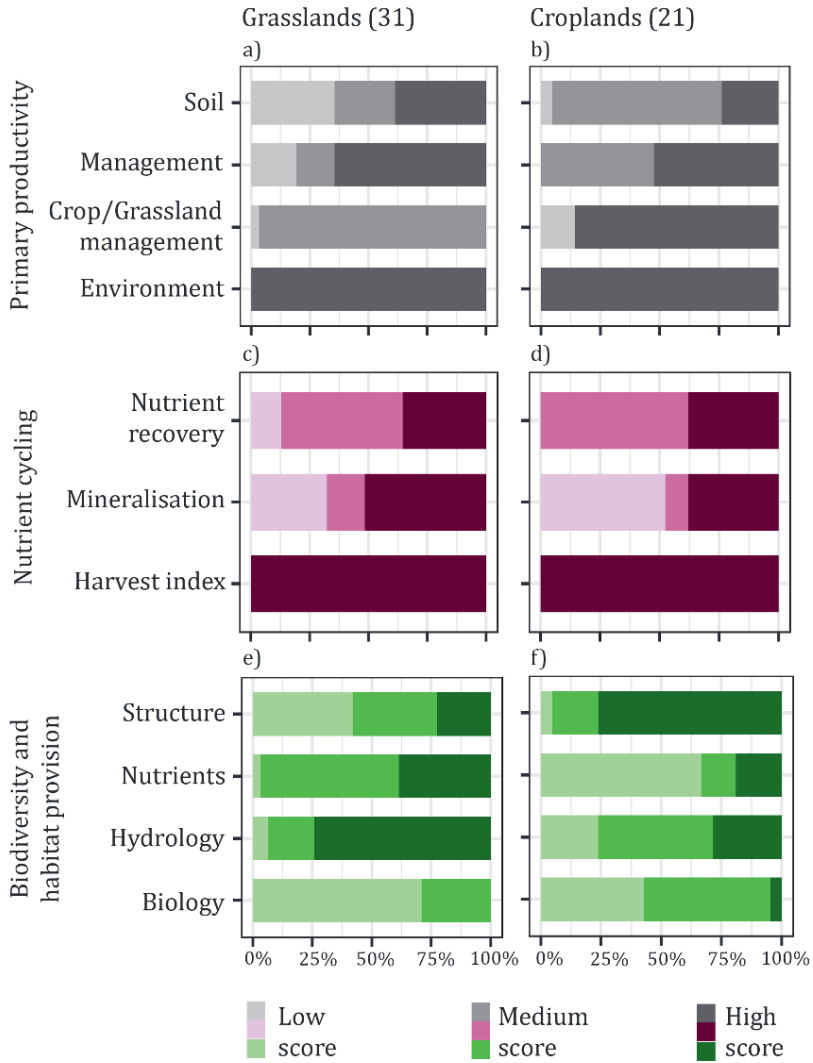


Figure 5.4. Frequency of scores for the top-tier of multi-criteria decision models for primary productivity (grey), nutrient cycling (pink) and biodiversity and habitat provision (green) on grasslands (a) or croplands (b) in the Netherlands. Each tier represents the suitability of part of the system to sustain a high, medium or low supply of each function. ‘Soil’ stands for soil properties in primary productivity. In biodiversity and habitat provision ‘Structure’ stands for soil structure and ‘Nutrients’ for nutrient status.

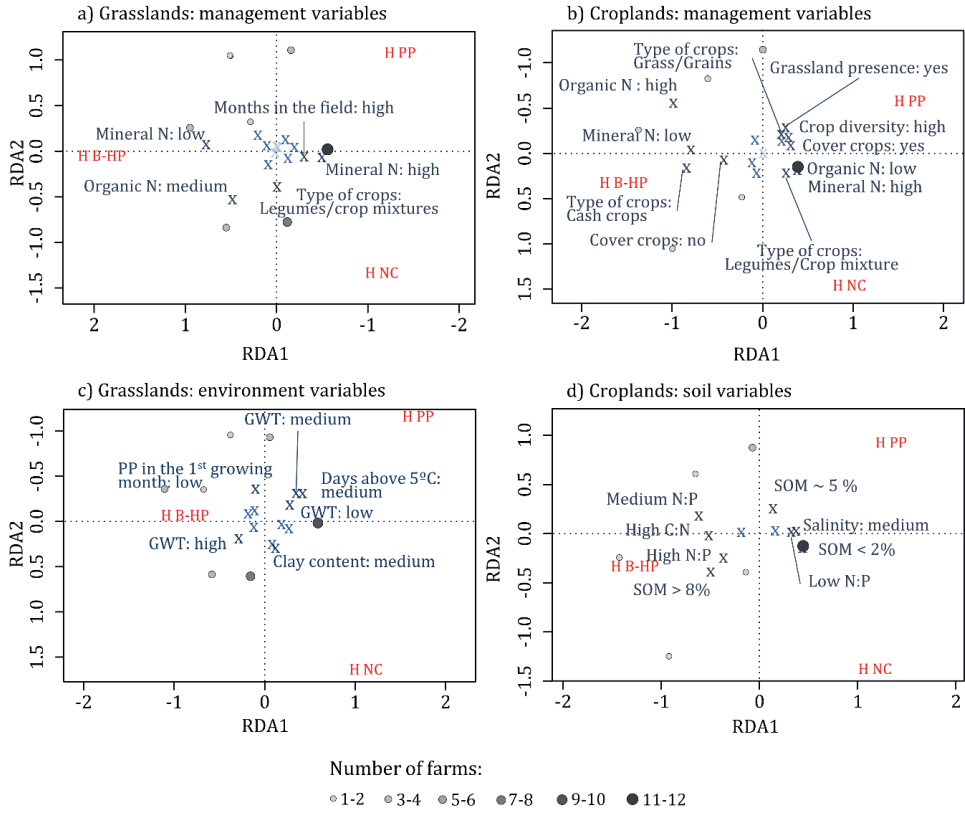


Figure 5.5. Redundancy analyses of the effect of management (a and b), environmental (c) and soil related variables (d) on the high (H) capacity of the soil to provide primary productivity (PP), nutrient cycling (NC) and biodiversity and habitat provision (B-HP) in grasslands (left panels) and croplands (right panels). Circles denote the position of the farms, and the colour the number of farms in that position. Only RDAs where variables (indicated by crosses) explained a significant variation in distribution of the farms’ high scores have been included in this figure, leaving out soil variables in grasslands, and environmental variables in croplands. SOM stands for soil organic matter and GWT for ground water table.

Table 5.1. Statistics from six redundancy analyses of the distribution of high supply of primary productivity, nutrient cycling and biodiversity and habitat provision in grasslands and croplands, as explained by either environmental (Env), soil or management (Mng) variables. In bold are variables with an adjusted R² above 0.3 or *p*-values below 0.05. df stands for degrees of freedom.

			Grasslands			Croplands		
			Env	Soil	Mng	Env	Soil	Mng
RDA results	Total Inertia (=3)	Constrained (%)	47.7	57.7	72.7	47.7	54.3	75.7
	Eigenvalues for constrained axes (%)	RDA1	55.9	61.3	72.9	76.9	69.9	77.5
		RDA2	38.5	30.6	21.6	22.4	26.4	15.9
		RDA3	5.6	8.1	5.5	1.4	3.7	7.0
RDA - Diagnostics	Adjusted R ²		0.28	0.16	0.52	0.20	0.35	0.45
	ANOVA	df - model	7	15	10	7	6	10
		df - res	19	15	13	13	14	8
		Variance - model	1.43	1.73	2.18	1.43	1.63	2.27
		Variance - residual	1.57	1.27	0.82	1.57	1.37	0.73
		F	2.48	1.37	3.46	1.69	2.79	2.48
		Pr (>F)	0.004	0.18	0.001	0.12	0.01	0.03
ANOVA – RDA AXES	RDA 1	Variance	0.8	1.06	1.53	1.1	1.14	1.76
		F	11.73	22.62	65.98	11.88	14.24	36.07
		Pr (>F)	0.03	0.15	0.008	0.11	0.03	0.03
	RDA 2	Variance	0.55	0.53	0.53	0.32	0.43	0.36
		F	8.13	11.26	22.77	3.46	5.3	7.29
		Pr (>F)	0.07	0.71	0.72	0.75	0.22	0.77
	Residual	Variance	1.57	1.27	0.82	1.57	1.37	0.73

5.4 Discussion

We upscaled the MCDMs developed by the Landmark project from field to farm scale to assess the soil's ability to provide PP, NC and B-HP in 31 grasslands and 21 croplands in the Netherlands. The soils often delivered more than one function at a time with a medium or high score, but it was often a combination of PP and NC. B-HP received a low score on 24 out of 53 farms (46%), while NC and PP were low on 7 (13%) and 3 (6%) of the farms respectively. Management variables, namely the source of nitrogen

fertilization and the type of crops in rotation, play an important role in explaining the trade-offs and synergies between B-HP, NC and PP.

5.4.1 Multi-criteria decision models: the issue of data collection

The MCDM were originally created as a tool for farmers or advisors to assess the multifunctionality of their soils. As such, they were designed to utilise soil management information alongside basic soil property and environment data to derive the multifunctionality of soils at field scale. These models have provided a framework for the assessment of soil functions across Europe and they are being utilised to support scientific assessment of multifunctionality, but in this context the models often suffer from the lack of management data, which is essential for each model (Schröder *et al.* 2016, van Leeuwen *et al.* 2019). This proved a disadvantage in our research. The soil data and environment data were readily available at a farm level, and when not directly measured, we could access soil maps that helped in filling in the gaps. But overall, few existing research projects or organisations have field or farm level information that includes details about soil/land management. This resulted in 20% of the input variables, particularly management related variables, being based either proxy values derived from literature research (such as the depth of the organic matter layer) or on expert assumptions of common agricultural practices in the Netherlands (such as a low amount of crop residues left in the fields). We accept that these assumptions could introduce a potential error into the calculation of the final function score within the model and reduce the precision of the predictions, but the final effect of this on our results is difficult to assess without comparing them to results gathered with a full set of input variables. More importantly, because not one single variable is solely responsible for the score obtained but rather a result of a tiered aggregation process, we do not expect the resulting score to be inaccurate. We suggest that future studies that use these MCDMs carry out a sensitivity analysis. The importance of soil management variables within this study emphasises the need for future research projects to collect data additional to soil based measurements to really be able to define the functional capacity of a soil. We suggest, specifically that studies exploring soil quality in European systems collect at least the information on farm management collected by the Landmark project, or a variation of these that includes more categories for each variable (Creamer *et al.* 2019).

5.4.2 From field to farm level: challenges and opportunities

Comparison of the MCDM with proxy variables was performed to see if the upscaling field to farm level had been successful. We did not, however, expect a perfect relationship between the proxy variables and the functioning scores according to the MCDM. The proxy variables serve as a representation of one part of a soil function, whereas the MCDMs attempt to encompass all the main processes that affect the same soil function.

We observed a clear difference between the average standardised yield observed in croplands with medium and high PP scores. These results are in line with those obtained during the model's validation process (Trajanov *et al.* 2019). However, comparison of the standardised yield with the functional capacity of PP in the grassland sites did not result in a significant relationship. The grassland PP model had not previously been validated (unlike the PP crop model), and might require adjustment to better represent potential PP. The processes underlying the PP MCDM, however, are very similar in grasslands and croplands and should represent the relationship between soil properties and PP even after being adjusted to farm level. In our dataset, an average 18 % of a dairy farm were dedicated to maize. The upscaling to farm scale led us to include these areas in the calculation of standardised yield but the PP MCDM, even after adjustment for farm level, did not include these maize fields. Although this could have meant a small overestimation of standardized yield in farms with a successful crop maize it should affect farms with a medium and high PP equally. More importantly, all the grasslands with a medium score where in fact scored between a high and a medium PP by the MCDM. We believe that the difference in grass yield between farms with medium to high PP and that of farms with high PP is simply not large enough to be detected within our dataset. Either a larger sample size, or a more diverse set of grasslands (with a broader PP spectrum) could shed further light on the relationship between standardised yield and the PP MCDM. Another approach would be to increase the number of final scores from three to five and increase the overall precision of the model without compromising interpretability.

Selecting proxy variables for NC was difficult, due to the holistic nature of the NC MCDM model, which encompasses the process of NC and recovery from the application of residues/manure to the off-take of nutrients in the plant yield. The F:B ratio provided some assessment of the NC MCDM potentially representing the mineralisation process within the model, which also contributes to the nutrient recovery (Schröder *et al.* 2016). We found a lower F:B biomass ratio in grasslands with high NC when compared to those with medium NC (although neither group was significantly different than grasslands with low NC capacity, which was probably because one of the

four grasslands with low NC had a F:B ratio of 0.46, a value close to the mean F:B ratio of sites with high NC). Lower F:B ratios are typical in more intensively managed system, with higher nitrogen inputs, and readily decomposable materials (Ruess and Ferris 2004). We observed similarly low F:B values in all the cropland farms, regardless of their capacity to supply NC according to the MCDM, indicating a bacterial dominated decomposition pathway for all the croplands.

The comparison of the B-HP function with the diversity of soil invertebrates resulted in promising trends that were, however, not significant, indicating a high level of overlap and variability. A significant but weak correlation was observed when the B-HP MCDM scores were compared with those assigned by experts in the soil biodiversity field (van Leeuwen *et al.* 2019). Due to the discrete nature of the MCDMs, much of the variation in the data is discarded. The use of pesticides, irrigation or the use of drainage, are all variables that can be either positive or negative as input data in the MCDMs, yet we know that pesticide load or the duration and timing of droughts (and therefore the resilience to water shortages) can both have an effect on the belowground biodiversity (Korthals *et al.* 1996; Cycoń and Piotrowska-Seget 2009; Chelinho *et al.* 2011). We recommend to either increase the number of categories, or to use a continuous scale for these variables to further refine the B-HP MCDM, and although it can be costly, we recommend the inclusion of at least some of the soil fauna into the assessment of this soil function.

5.4.3 Drivers of soil multifunctionality

The MCDMs indicated that up to 38% of the farms in our study had either a medium or a high supply of all three soil functions, which answers our first research question: a proportion of the farms under study were delivering three functions at a medium or high level simultaneously. Similar patterns have been observed recently in Europe, where more than a third of the visited fields achieved a supply of five functions at either medium or high levels (Zwetsloot *et al.* 2020). Only one farm in our study (a cropland) had high levels of all three functions. What is also significant is that only two farms (both grasslands) had a medium to high supply of only one soil function, which was PP in both cases. Indicating that a vast majority of sites could supply at least two functions at a higher level simultaneously.

In answer to our second research question, the MCDM predicted clear synergies between PP and NC, but a trade-off between the two functions and B-HP was clearly evident (Figure 3). Only 27% of the farms in the study were expected to have a high level for B-HP. These results are in line with many studies before which have found

strong negative relationships between agricultural intensification and biodiversity losses (Tilman *et al.* 2001; Tsiafouli *et al.* 2015; Geisen *et al.* 2019). Soil fauna are paramount for the delivery of soil functions and ensuring the delivery of adequate B-HP should be a priority (Kibblewhite *et al.* 2008; Brussaard 2012). Increases in organic fertilization, over time, increase the organic matter content in the soil, and provide a more suitable habitat for soil biota by providing food for the microbial community and more stable soil moisture conditions (Zsolnay and Görlitz 1994; Birkhofer *et al.* 2008). In our study, high soil organic matter was a common attribute amongst the croplands with a high B-HP. Additionally, lack of chemical pest management and high input of organic fertilisers were highlighted in our results as common input variables in farms with high B-HP. In fact, 80% of the farms with a high B-HP supply were organic farms. The use of insecticides and pesticides has been linked to a widespread loss of biodiversity in European farmland (Geiger *et al.* 2010). Conversion to organic farming can lead to more erratic yields, but measures such as the use of green manure or increased fertilization can be taken to reduce the variation (Knapp and van der Heijden 2018). In fact, a meta-analysis showed that biodiversity increases on average by 30% under organic farming management, and this effect is larger in intensively managed landscapes (Tuck *et al.* 2014).

The type and amount of fertiliser were important variables in explaining multifunctionality: moderate values of each could lead to multifunctionality of grasslands and croplands. In grasslands weather variables explained a significant proportion of the prevalence of high functioning. In croplands, however, moderate values of soil variables, such as soil organic matter, nitrogen to phosphorus ratio, carbon to nitrogen ratio and soil salinity could lead to improvements in multifunctionality. Our results suggest that implementing changes in fertilization and pest management practices could have a big impact in the multifunctionality of soils even without a complete switch to organic management. Specifically, we found the type and amount of fertiliser to be an important variable explaining soil multifunctionality. Farms with a high capacity for B-HP were characterized by low mineral nitrogen fertilization, and high organic fertilization, and in the case of croplands a soil organic matter content above 8%. The effects of mineral nitrogen fertilization on soil biota are often not direct. Their application can lead to shifts in the microbial composition by inducing changes in the PP, crop residue and soil organic matter content, but can also lead to soil acidification, which in turn has a negative effect on earthworms and nematodes (Bünemann *et al.* 2006; Treseder 2008; Eisenhauer *et al.* 2012; Chen *et al.* 2015), in fact, in our study, sites with the highest carbon to nitrogen ration (with correlated strongly with low pH values) had the highest B-HP scores. There are measures to improve biodiversity that we have not tackled through the redundancy analysis (Figure 5). The

amount of residues left in the field, which we assumed to be low in our study sites, can positively impact the abundance, and increase the functional diversity of the earthworm community (Frazão *et al.* 2019). Reduced tillage practices can lead to an increase in the richness and abundance of soil organisms (Kladivko 2001; Sapkota 2012). Moreover, these two practices combined can have a larger positive impact on the soil biodiversity than organic management (Henneron *et al.* 2015). The number and identity of crops in rotation can also have a positive impact on total organic carbon and microbial biomass, particularly when combined with the use of cover crops (McDaniel *et al.* 2014).

Although our results have uncovered important relationships between PP, NC and B-HP, we could not include water or climate regulation functions, thus, some of the synergies and trade-offs that occur in these soils remain unknown. High nitrogen fertilization rates are associated to increases in productivity, but also to increased residual soil nitrate and increasing the risk of nitrous oxide (N₂O) emissions (Snyder *et al.* 2009). In fact, Zwetsloot *et al.* (2020) found a trade-off between PP and climate regulation in their study into European farms. The use of organic fertilizers has been linked with increased soil carbon stocks (Kukal *et al.* 2009), and might therefore indicate a positive link between B-HP and climate mitigation. While such a relationship was observed by Zwetsloot *et al.* (2020) in Pannonian climatic soils, it became negative in the Atlantic climate zone, showing that there is still much to learn regarding how these soil functions interact with one another.

The functional land management framework was designed to promote soil multifunctionality by addressing soil function needs and delivery at different scales, such that local, regional and national objectives for multifunctionality can co-exist (Schulte *et al.* 2014; Schulte *et al.* 2015). Our results reflect a need to include the improvement of B-HP as a regional and national goal, to partially off-set the effects of agricultural land management, but at the local scale sustainable agricultural practices should also be introduced to safe-guard soil biodiversity. It is a step in the right direction that the Common Agricultural Policy in the European Union now includes “Effective Soil Management” as an objective, with specific recommendations regarding soil biodiversity, its role in soil functions, and management practices that are less impactful or even beneficial to the soil system. However, each member state can implement these measures at a different level, and there is evidence to suggest that including environmental concern into the Common Agricultural Policy is not enough to protect the environment (Alons 2017; Pe'er *et al.* 2019). Better incentives for sustainable farming practices could lead to decreases in biodiversity loss, particularly because some of these practices are not without risk, and farmers might not want to make changes that put their income at risk (Lefebvre *et al.* 2020).

5.5 Conclusions

Our study shows the potential for multifunctionality in soils in farms of the Netherlands, with most farms having a moderate or high capacity in two of three functions. The models predict trade-offs between biodiversity and habitat provision and both primary productivity and nutrient cycling. Management related variables regarding the use of pesticides, and the amounts of mineral and organic nitrogen fertilization were considered very important within the model predictions and explained part of the synergies and trade-offs in both croplands and grasslands, and soil parameters that can be changed via improved soil management (such as soil organic matter) are also important to increase the delivery of B-HP. Providing incentives that make the adoption of sustainable practices less risky to farmers might be a step forward in providing multiple soil functions simultaneously while maintaining the provision of B-HP.

5.6 Acknowledgements

Special thanks to Jaap Schröder and Taru Sanden for their support in running the primary productivity and nutrient cycling multi-criteria decision models, and to Vladimir Kuzmanovski, for his technical help in running all the models. We would also like to thank Auke Greijden and Ruud van der Meer for their help in navigating the Minerals Policy Monitoring Programme dataset. Lastly, we would like to thank Sylvana Harmsen, for her aid during data analysis.

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5.8 Data accessibility

The data that support this work were collected by the Netherlands National Institute for Public Health and the Environment and by Wageningen Economic Research and are protected by confidentiality agreements.

5.9 Authors' contributions

C. V., R.E.C. and R. G. M. G. conceived the presented idea and received important feedback from all co-authors. M. R. and T. K provided the data necessary to perform the analyses. C.V. led the writing of the manuscript and the analysis of the data. All authors discussed the methods and results and contributed to the final manuscript.

Supplementary information

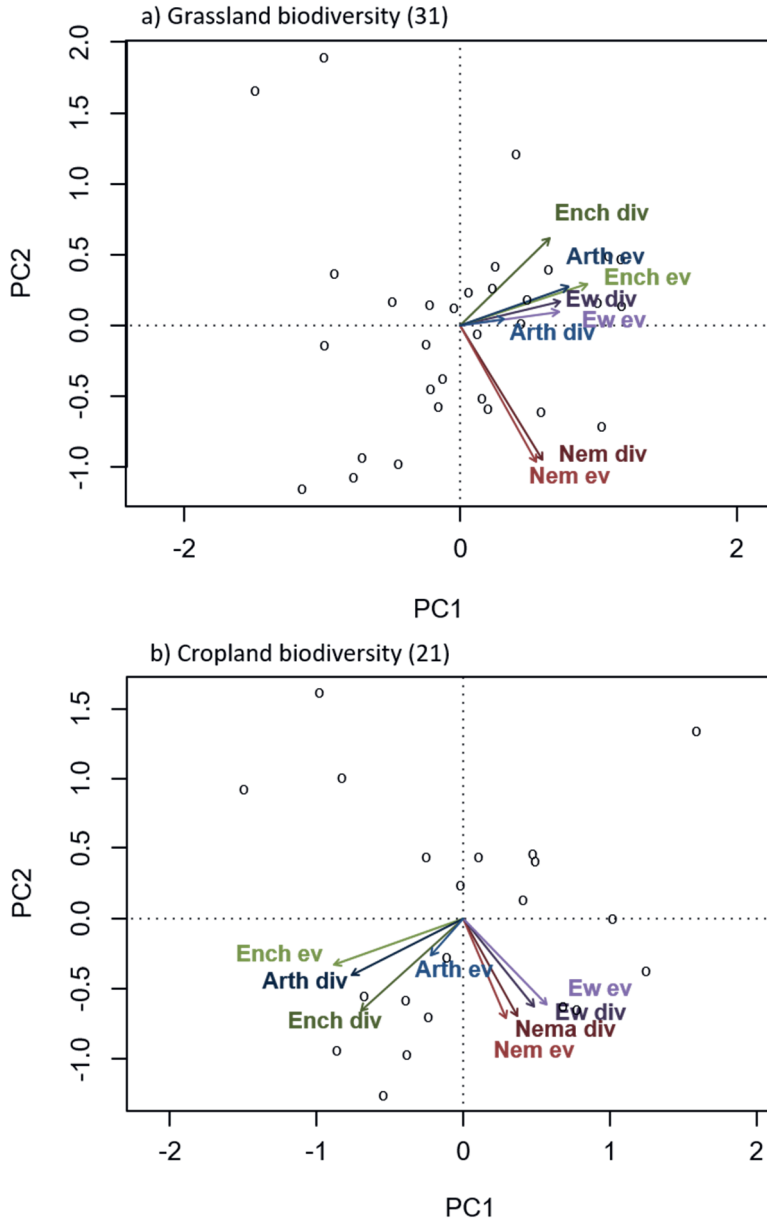


Figure S1. Biplot of the PCA model explaining the variation in soil invertebrate evenness and diversity. Open circles represent fields. Different invertebrate groups are presented in different colours.

Table S1. Environmental input variables for the decision support models developed by Landmark (Debaljek 2019), status regarding their inclusion in our model, measurement units, soil function they relate to, threshold values in the models as established by Landmark, as well as the data's source for this particular study. The multi-criteria decision models used to assess primary productivity (PP), nutrient cycling (NC) and biodiversity and habitat provision (B-HP) each had a different requirement in terms of input variables. If we assumed all sites to have the same value, we have underlined it.

Input variable	Status*	Unit	Related soil function	Threshold values			Source and method**
				Low	Medium	High	
Altitude	D	M.a.s.l	PP	<u>≤150</u>	150-300	>300	De Vries (2007)
Annual cumulative precipitation	M	Mm	NC PP; B-HP	<745 <600	745-992 600-900	>992 >900	KNMI
Average annual temperature	M	°C	B-HP	<9	9-11	>11	KNMI
Average daily temperature in first month of growing season	M	°C	NC	<5	-	>5	KNMI
Clay content	M	%	PP	<15	15-25	>25	NSMN NIRS (TSC®)
Crop failure risks	A	n/ 20 years	NC	<1	1-4	>4	-
Drainage class	D	Clay %	NC	>40	40-27	<27	NSMN NIRS (TSC®)
Groundwater Table Depth	D	M from surface	PP; NC; B-HP	<0.4	0.4-2	>2	Dufour (2000)
Number of days with average temperature above 5 degrees C	M	Days	NC PP	<247 <180	247-271 180-230	>271 >230	KNMI
Precipitation in first month of growing season	M	Mm	NC	<30	-	>30	KNMI
Slope	A	Degree s	PP	<u>≤3</u>	3-10	>10	
Soil texture	M	-	NC; B-HP	Sand	Loam	Clay	NSMN; (Siderius and De Bakker 2003) NIRS (TSC®)
Thickness of organic layer	A	cm	B-HP	<10	10-30	>30	

* A = Assumed; D = Derived from available data; M = measured; U = Unknown

** NSMN = National soil monitoring network (Rutgers et al 2009); KNMI = Royal Dutch Meteorological Agency

Table S2. Management related input variables for the decision support models developed by Landmark (Debaljek 2019), status regarding their inclusion in our model, measurement units, soil function they relate to, threshold values in the models as established by Landmark, as well as the data's source for this particular study. The multi-criteria decision models used to assess primary productivity (PP), nutrient cycling (NC) and biodiversity and habitat provision (B-HP) each had a different requirement in terms of input variables. If we assumed all sites to have the same value, we have underlined it.

Input variable	Status*	Unit	Related soil function	Threshold values			Source **	Formula (if applicable)
				Low	Medium	High		
% legumes	K/A	%	PP; NC; B-HP	<10	-	>10	LMM	
Ammonia losses	D	<u>Kg N/h</u> a	NC	<11	11-30.27	>30.27	LMM	Details on the calculation of ammonia losses can be found on Appendix 2 of (RIVM 2019-0026)
Artificial drainage	A	Yes/no	PP; NC; B-HP	-	-	-	-	
Biological pest management	U	Yes/no	PP	-	-	-	-	
Catch crops, cover crops and green manure	M	Yes/no	PP; NC; B-HP	-	-	-	LMM	
Chemical pest management	M	Yes/no	PP; B-HP	-	-	-	LMM	
Grassland in rotation	M	Yes/no	PP; B-HP	-	-	-	LMM	
Grassland type	A		B-HP	<u>reseeded</u>	<u>permanent</u>			
Grazing Frequency	D		PP	-	-	-	LMM	When animals spend a low, medium or high amount outdoors, grazing frequency was considered low, medium or high respectively
Irrigation	M	Yes/no	PP; NC				LMM	
Liming	M/U	Yes/no	B-HP				LMM	
Manure type	M		B-HP	No manure	Sludge or slurry	Solid manure or compost	LMM	
			NC	Solid manure/compost	Cattle slurry	Pig slurry & liquid fractions		

Input variable	Status*	Unit	Related soil function	Threshold values			Source **	Formula (if applicable)
				Low	Medium	High		
Mineral nitrogen fertilisation	M	kg N/h a/ye ar)	PP; B-HP NC	<50 <20	50-150 20 - 50	>150 >50	LMM	
Crop diversity	M		PP; B-HP	<1.10	1.10- 1.52	>1.52	LMM	$\sum_{i=1}^n p_i \times \ln(p_i)$ <p>where p_i is the proportional cover of the i^{th} crop</p>
Number of months in the field	K	mont hs	B-HP	<4	4-8	>8	LMM	
Organic nitrogen fertilisation	M	kg N/h a/ye ar)	PP	<50	50-170	>170	LMM	
Mechanical pest management	U/A	Yes/ no	PP; B-HP	-	-	-	-	
Share of crop residues left on the field	A	%	NC	<10	10-50	>50	-	
Stocking Rate	M	$\frac{LSU}{ha}$	PP; B-HP	<1 <1	1-2 1-2.5	>2 >2.5	LMM	$LSU = \text{Livestock unit}$
Tillage	U/A	Yes/ /no	B-HP	-	-	-		
Type of crops	K		B-HP	Cash crops	Grass/ grains	Legumes/ Crop mixture	LMM	

* A = Assumed; D = Derived from available data; M = measured; U = Unknown

** LMM = Minerals Policy Monitoring programme

Table S3. Soil related input variables for the decision support models developed by Landmark (Debaljek 2019), status regarding their inclusion in our model, measurement units, soil function they relate to, threshold values in the models as established by Landmark, as well as the data's source for this particular study. The multi-criteria decision models used to assess primary productivity (PP), nutrient cycling (NC) and biodiversity and habitat provision (B-HP) each had a different requirement in terms of input variables. If we assumed all sites to have the same value, we have underlined it.

Input variable	S*	Unit	Related soil function	Threshold values			Source**	Method
				Low	Medium	High		
C:N ratio	M	-	PP B-HP	<8 <10	8-12 10-30	>12 >30	NSMN	(NEN-ISO 12099 2010)
Cation exchange capacity (CEC)	D	$\frac{mmol\ IE}{Kg}$	PP	<10	10-30	>30	(Helling <i>et al.</i> 1964)	$CEC_{clay} = 400 \frac{mmol(+)}{kg}$ $CEC_{SOM} = 250 \times pH - 250$ $CEC = CEC_{clay} \times Clay + CEC_{SOM} \times SOM$ <p>Where Clay is the percentage of clay in the soil, and SOM is the percentage of organic matter in the soil.</p>
Magnesium	M	$\frac{mg\ Mg}{Kg}$	PP	<30	30-170	>70	NSMN	Houba <i>et al.</i> (1998)
N:P ratio	M	-	B-HP	<10	10-20	>20	NSMN	-
Phosphorus (P)	M	$P_w: \frac{mg\ P_2O_5}{L}$ $PAL: \frac{mg\ P_2}{100g}$	PP; NC	CL: <36 GL: <27	36-55 27-50	>55 >50	NSMN	Pw according to Sissingh (1971), and PAL according to NEN-ISO 5793 (2010)
Plant available potassium (K)	M	$\frac{mg\ K}{Kg}$	PP	<80	80-160	>160	NSMN	Houba <i>et al.</i> (1998)
Rooting depth	U	<i>Cm</i>	PP	-	-	-	-	-
Salinity	D	$\frac{ECe\ dS}{m}$	PP	<2	2-8	>8	(De Vos 2011)	-
Soil bulk density	D	$\frac{Kg}{dm^3}$	PP; NC; B-HP	<1.1	1.1-1.5	>1.5	(Rawls 1983)	$BD = \frac{100}{\frac{SOM}{BD_{SOM}} + \frac{100 - SOM}{BD_{Mineral}}}$
Soil organic matter (SOM)	M	%	PP; B-HP	<2	2-5	>5	NSMN	(NEN-ISO 12099 2010)
Soil pH (pH-CaCl2)	M	-	PP NC B-HP	<5.5 <4.5 <4	5.5-7.2 5-6; 4-5; 5-6; 6-7	>7.2 >4.5 >7	NSMN	(NEN-ISO 12099 2010)

* S-Status: A = Assumed; D = Derived from available data; M = measured; U = Unknown

** NSMN = National soil monitoring network (Rutgers *et al.* 2009)

Chapter 6

General discussion

C. Vazquez

Soil biota are indispensable for the delivery of soil functions, but agricultural management has been shown to cause harm to soil biodiversity (de Graaff *et al.* 2019; Emmerson *et al.* 2016; Geiger *et al.* 2010; Pothula *et al.* 2019). This has been highlighted by the recent communication of a new Soil Strategy as part of the new European Union Biodiversity Strategy for 2030

(<https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/12634-New-EU-Soil-Strategy-healthy-soil-for-a-healthy-life> and

https://ec.europa.eu/environment/nature/biodiversity/strategy/index_en.htm

respectively).

The Soil Strategy includes objectives such as the protection of soil fertility and soil biodiversity or the reduction of soil erosion and land degradation, these objectives are quite broad and there is no information yet on how these objectives will be tackled. The Biodiversity strategy, however, includes goals related to agricultural management that could impact soil biodiversity. For example, this strategy proposes the increase in the surface dedicated to organic farming, and a decrease in agricultural inputs (fertilizers and pesticides alike). These goals provide a great starting point in the protection of soil biodiversity, as research has shown that organic pesticides can be detrimental to biodiversity not just below but also aboveground (Geiger *et al.* 2010), excess fertilization has also been associated with decreases in biodiversity in addition to the more commonly known multiple environmental problems (de Graaff *et al.* 2019), and organic agriculture has a positive effect on biodiversity, particularly in areas dominated by agricultural land (Bengtsson *et al.* 2005; Tuck *et al.* 2014). We should not just focus on reducing the negative effects of farming. Sustainable farming practices should adopt practices that promote not just primary productivity, but multiple soil functions, and rely more strongly on the delivery of soil functions that are mediated by soil biota, such as disease suppression, water regulation or nutrient cycling (Bommarco *et al.* 2013; Kleijn *et al.* 2019). To this end, tools that provide specific advice regarding soil-based functions such as the Soil Navigator (<http://www.soilnavigator.eu/>) or the SQAPP (<http://www.isqaper-project.eu/news/232-soil-quality-app-launched>) could positively impact the multifunctionality of European farmland.

In this thesis I explored the effects of land use intensity and land use management on an aspect of the soil community that had been previously unexplored: community level specialisation, using an index of community specialisation (I_{CS}). I aimed to answer: whether land use intensity affects specialist soil organisms disproportionately (1), whether agricultural practices affected the soil communities through time (2), and whether there occurred synergies and trade-offs in agricultural soils between three soil

functions: primary productivity, nutrient cycling and biodiversity and habitat provision (3). In doing so, this thesis has provided estimates for the realised niche width (RNW) of soil nematodes (Chapter 2) and enchytraeids (Chapter 3), and noted the difference between the response of the specialist nematodes to increased land use intensity when compared to that reported for above-ground organisms. Moreover, I provide evidence that biological soil quality, as measured by nematode indicators is in a constant state of change even under controlled experimental field conditions and after accounting for variations in weather (Chapter 4). Lastly, using decision support models designed as part of the Landmark project (Sandén *et al.* 2019; Schröder *et al.* 2016; van Leeuwen *et al.* 2019) I qualified the performance of three soil functions in 52 farms across the Netherlands, and observed a common synergy between primary productivity and nutrient cycling, and a common trade-off, between these two functions and biodiversity and habitat provision. In the following sections, I discuss soil multifunctionality, and the intricacies associated with the models I used to estimate the performance of soil functions (section 6.1); I then discuss the effects of arable farming on biological soil quality (section 6.2) and the role of the index of community specialisation (I_{CS}) in understanding these impacts (section 6.3); lastly, I discuss the realised niche width (RNW) of nematodes and enchytraeids (section 6.4).

6.1 Multifunctionality

The projected rise in the world's population is linked to a growing demand of agricultural products in the coming years. This is bound to lead to increased land use intensity, and it is of utmost importance that future agricultural intensification is paired with a decrease in the environmental impacts associated with farming (Bommarco *et al.* 2013; Bommarco *et al.* 2018). A way to do this is to lean on supportive ecosystem services and design agricultural systems that are less reliant on external inputs (Bommarco *et al.* 2018). To this end, decision support models such as the Soil Navigator (Debeljak *et al.* 2019) can aid in the adoption of more sustainable management measures. This is because they are specifically aimed at helping the end user visualize the effects of management decisions on long term sustainability by including an overview of the performance of multiple soil functions as well as specific management recommendations that promote multifunctionality. There is, however, little knowledge about the perception that farmers have regarding ecosystem services, and a recent study on this topic suggests that while scientists focus on the effects on processes (such as pollination), farmers are more interested in what new management would mean in terms of profit. This divide might lead to a lack of adoption of new management techniques by farmers (Kleijn *et al.* 2019). The Soil Navigator, the tool that incorporates the models

that we used in Chapter 5, was built in collaboration with farmers, taking into account their needs and wishes, and experts to help build the multi-criteria decision models that predict soil functions (Bampa *et al.* 2019).

The Soil Navigator is based on the concept of Functional Land Management, which introduces a landscape or regional aspect to multifunctionality such that rather than expecting single fields to supply all five functions, the supply and demand of soil functions should be balanced across a landscape (Schulte *et al.* 2015; Schulte *et al.* 2014). The results in Chapter 5, although limited to three out of the five functions available in the soil navigator indicate that in the Netherlands the provision of soil functions is not balanced, since most low performance scores were in fact for biodiversity and habitat provision. The low score for biodiversity and habitat provision was often accompanied by high scores in nutrient cycling and primary productivity. This result is in line with similar studies, where authors found negative relationships between primary productivity and for example climate regulation and carbon storage (O'Sullivan *et al.* 2015; Zwetsloot *et al.* 2020). Zwetsloot *et al.* (2020) highlighted that synergies and trade-offs between soil functions are sometimes dependent on the climatic region in which they occur. Acquiring more knowledge regarding the effects of agricultural management on soil functions, and estimating the potential of specific regions to deliver soil functions is of paramount importance to the achievement of functional land management.

In Chapter 5 I applied models created at a European level to evaluate the soil functions within one country (The Netherlands). To better assess soil multifunctionality, these functions should be tuned to national conditions, using expert knowledge to adjust the thresholds that define the input variables, and a mix of expert knowledge and machine learning algorithms to weight the importance of different parts of each model to the final function score (Sandén *et al.* 2019; Trajanov *et al.* 2019). These models could be used to establish priority areas within the country for the delivery of different ecosystem services, since without a set of specific priorities biodiversity could come at a loss in the Netherlands.

Further improvements to these models could be the inclusion of more input and output categories. As the models stand, each input variable is evaluated according to a pre-set threshold, usually into three or two categories. Due to the hierarchical nature of these models, the evaluation of several input variables is then aggregated through if-then functions and provides the score of the next tier. This renders the models insensitive to small changes in the input and output variables, and only when enough thresholds are passed for several input variables does the outcome change to a different performance

level (Debeljak *et al.* 2019). Additionally, one could explore the possibility of adding management variables such as conservation tillage practices.

Another issue to discuss is the inclusion of biodiversity in the functionality assessments provided by the Soil Navigator. Throughout this thesis I have advocated for the importance of biodiversity in the provision of soil functions, and yet only one of the five soil functions assessed by the Soil Navigator (biodiversity and habitat provision) allows for the user to include actual measures of biodiversity into the calculation of soil functions (such as richness and abundance of diverse groups of soil organisms). The reason behind it is clear: first, while scientists understand the importance of biota to soil functioning, their actual contribution is difficult to measure (Behan-Pelletier and Newton 1999; Bradford and Newington 2002; Briones 2014; Coleman and Whitman 2005); secondly, the Soil Navigator was designed to be used by farmers, who rarely have detailed information on the biota inhabiting their soils and obtaining such information incurs costs to the farmers; thirdly in some cases (such as in the case of microbes and nutrient cycling) the size of the role of biota on soil functioning depends on environmental and climatic restrictions; data that are much easier to obtain than bacterial biomass (Schröder *et al.* 2016).

These issues are reflected in the '*Biodiversity and habitat provision*' model. This model was built to accept input on the actual abundance and richness of earthworms, enchytraeids, nematodes and micro-arthropods. However, since it is unlikely that a farmer would have information on all of these soil fauna groups, the model will provide a score even when no information on biota is included. The model will base its score on information related to the nutrient status, structure, hydrology and biology of the soil to provide a final score, but in the case that actual data on the soil biota is unknown, there are assumptions made using the physico-chemical data in conjunction with the management and climate input data (van Leeuwen *et al.* 2019). However, using the data presented in Chapter 5, I observed a divergence in some of the scores obtained for Biodiversity and habitat provision of the farms when including or excluding information on the richness and abundance of soil organisms (Figure 6.1). In grasslands, the exclusion of biodiversity led to lower biodiversity scores, while in cropland it led to higher scores. This disparity should be further explored, and could result in the recalibration of some of the threshold values used in the biodiversity and habitat provision model.

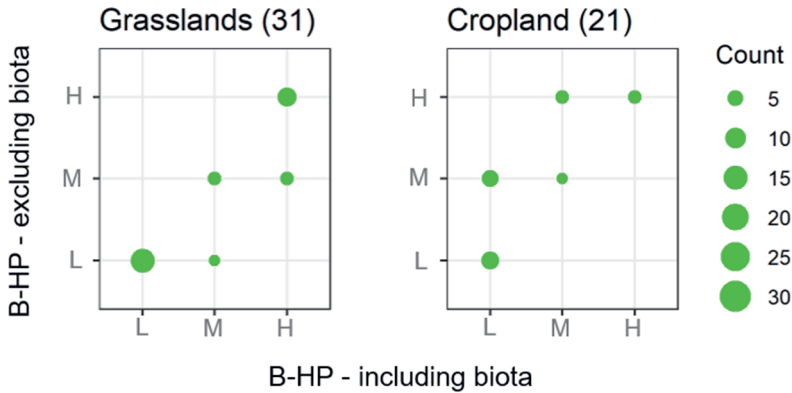


Figure 6.1. Performance of the Biodiversity and Habitat provision function in 31 grasslands and 21 croplands in the Netherlands assessed including (x-axis) or excluding (y-axis) actual measurements of the richness and abundance of soil biota.

Finally, one of the more difficult aspects regarding the assessment of soil multifunctionality is the collection of adequate data. Even thorough monitoring efforts such as that carried out in the Netherlands (Rutgers *et al.* 2009) are lacking in information regarding management practices. In our study we collected data from two institutions regarding soil parameters (from The National Institute for Public Health and the Environment; RIVM) and farm management data (from Wageningen Economic Research), but even then, we could not calculate all five soil functions, meaning there is a lot we still do not know about the synergies and trade-offs that occur within e.g. Dutch agricultural soils. In order to successfully do so, research institutes need to cooperate not just during data collection but also in later stages of data sharing, and projects need to promote collaboration across disciplines, to capture all aspects of the agricultural system.

6.2 Arable farming and biological soil quality

Biological indicators based on relative abundances of soil organisms are useful for understanding the effects of agricultural practices on changes to functional and taxonomic diversity (Bongers and Ferris 1999; Carrascosa *et al.* 2014; Ferris 2010a; Ferris *et al.* 2001; Sanchez-Moreno *et al.* 2009). I found clear differences in the indicators of biological soil quality and the composition of the soil community that were due to land use type and land management in Chapters 2, 3 and 4. I also found an overall decrease in time of the food web structure (measured by the nematode structure index)

combined with an increase in the nematode I_{CS} in farms across the Netherlands. These observations, however, were not always consistent across the chapters, nor across the different systems studied in Chapter 4, in fact in the experimental farms. *Broekemahoeve Applied Soil Innovation Systems experiment* (BASIS) and *Soil Health Experiment in Vredepeel* (SHE) we observed increased taxon richness over time

In Chapter 2 I observed an increase in the nematode I_{CS} with increasing land use intensity, with the highest proportions of specialist nematodes found in productive grasslands, followed by (and not significantly different from) arable fields. In Chapter 4, I observed higher levels of I_{CS} in arable fields under conventional management in (BASIS) as well as in the commercial farms, in line with the conclusions of Chapter 2 that increasing land use intensity leads to increases in the I_{CS} . These results are in line with recent studies that show the potential of organic amendments to increase nematode richness and community structure (Liu *et al.* 2016), and the use of inorganic fertilizers, as well as the use of pesticides to changes in the nematode community (Liu *et al.* 2016; Carrascosa *et al.* 2015). In the SHE, however, integrated management had a higher I_{CS} than conventional practices, and both treatments had a much higher I_{CS} (0.8 on average) than treatments at BASIS (between 0.4 and 0.6) or values found in commercial farms (between 0.2 and 0.4). We do not have enough information to explain this disparity. Future studies that include the calculation of the nematode I_{CS} in controlled experimental conditions could aid in understanding the factors that drive underlying values and changes in the I_{CS} .

Although there is variability in the results regarding specific indicators of biological soil quality, recent meta-analyses have observed overall negative effects of agricultural management on the nematode community (Pothula *et al.* 2019; Liu *et al.* 2016) in line with the decrease in the nematode Structure index and the increase in the nematode I_{CS} , reported in Chapter 4. The examples of literature presented above are a great start in understanding the effects of specific management decisions on biological soil quality, but it is necessary that these changes are adopted by farmers, which touches upon subjects discussed before and in the discussion of Chapter 5: providing incentives that make the adoption of sustainable practices less risky to farmers as well as tools that provide insight into management decisions not just to maximise primary productivity, but to do so sustainably.

6.3 Index of community specialisation in soils

The level of community specialisation has been proposed as a powerful measure of the community's response to spatial and temporal disturbance as well as to the effects of agricultural intensification (Devictor and Robert 2009; Fried *et al.* 2010). These studies,

as well as those mentioned throughout this thesis, found a generalised decline in the number of specialist species when human impact increased. I, however, found the opposite trend in the nematode community (Chapter 2) and no trends related to land use class in the enchytraeid community (Chapter 3). In Chapter 2, I argue, amongst others, that specialism can be a result of stable conditions during a life cycle (Levins 1968; Clavel *et al.* 2011), and given the difference in life span between nematodes, those with a short life cycle might experience agricultural intensity as a stable environment where pH, moisture content and nutrient availability are continuously kept at values suited to support plant growth. In fact, opportunist nematodes often have short life cycles and can quickly utilize the plentiful resources available in some agricultural soils (DuPont *et al.* 2009; Ettema and Bongers 1993). In Chapter 3, where the I_{CS} of soil enchytraeids was studied, I observed no such pattern, and hypothesized that due to the diversity of environmental constraints in soils, specialism might have evolved as a response to varied constraints. In other words: the environmental constraints that define the preferred habitat of one species could be very different than those that define the niche of another. Hypothetically, increasing land use intensity could lead to the increase of specialists that have short lives and benefit from the stable conditions found in arable land (such as I hypothesized for the nematodes), and simultaneously to the decrease of specialist enchytraeid species that are sensitive to physical disturbances and are therefore negatively affected by practices such as tillage. In such a scenario, an increase in land use intensity would result in no differences in the calculated I_{CS} .

I cannot be sure about the difference in response between these two groups, but I can highlight three possibilities. I used different datasets to calculate RNW of each: nematodes and enchytraeids. These datasets were different in terms of the number of habitats included and the scope at which sampling was done. The National soil monitoring network (NSMN) (Rutgers *et al.* 2009) included a more diverse set of habitats sampled at a much larger spatial scale within one country while the dataset used in Chapter 3 included several bio-climatic zones across Europe, but only three land use classes, and the sampling of EcoFinders was done at the field level. Therefore, the divergence in response to land use could be linked to the differences between these two datasets. By using the same (or a similar) training dataset to calculate the RNW of Enchytraeids and nematodes one could control part of the variation in our study (i.e. the habitat diversity). Both the NSMN and EcoFinders projects sampled the enchytraeid and the nematode communities (Rutgers *et al.* 2009; Stone *et al.* 2016), however, they did so at different taxonomic levels. The analysis of enchytraeids for the NSMN was done effectively at genus level (with the exception of *Fridericia*, which was mostly identified to species level), while the nematode identifications carried out by the

EcoFinders project across Europe were done at the trophic level. Therefore, the data available currently in these projects did not allow for comparison of both taxonomic groups for the same range of habitat diversity.

There is also a large difference between the ecology of nematodes and enchytraeids. Firstly, nematodes are smaller in size ranging from 0.3 mm to 5 mm where enchytraeids range from 1-30 mm (Orgiazzi *et al.* 2016b), secondly nematodes can take the role of plant parasites and predators. This means that nematodes and enchytraeids will have different responses to similar disturbances, due to their difference in size (for example impacts that directly or indirectly affect pore size) or feeding strategy. Some specialist plant parasitic nematodes such as *Meloidogyne* (RNW= 0.56) could contribute to the increase of the I_{CS} in agricultural land. Additionally, none of the target nematodes in Chapter 2 showed an association with acidic soils, whereas the enchytraeid species *C. sphagnetorum* was associated with very acidic soils and contributed to the increase of the I_{CS} in forest soils. A similar behaviour has been observed in the nematode *Halicephalobus minutus*, which shows an exceptional preference for low pH soils (Alkemade and van Esbroek, 1994). Calculating the RNW of *H. minutus* could shed light on the behaviour of the I_{CS} if calculated at species level also for the nematodes.

The aim of Chapters 2 and 3 was to study the effect of agricultural impacts on soil specialists. However, one of the disadvantages of calculating community weighted trait is the loss of resolution. In Chapter 2, we tracked the increase in I_{CS} with an increase in specialist nematodes. We did not, however check the proportional contribution of each species to the overall pattern. This is a difficult feat, since not all species would be present in all habitats. Future studies that wish to further our understanding of the effects of human impacts on the niche width of the soil community should consider the calculation of the I_{CS} from pre-existing or new experiments that control the nature and intensity of the impact applied and that have a common starting community (such as a field experiment). In such a controlled environment, one could study the contribution of individual species to the RNW. Experiments like these could shed light on the evolution mechanisms that lead to differing RNWs in soil organisms. The nature of the disturbance might be an important factor to consider: positive disturbances introduce nutrients to the system, and disproportionately benefit one part of the community, while negative disturbances lead to the death of one or several species in the community. The responses of specialists to these two disturbance types could also shed light on the response of community weighted niche width of soil organisms to disturbance.

6.4 Realised niche width

In this thesis I calculated the realised niche width (RNW) of nematodes (Chapter 2) and enchytraeids (Chapter 3). To do so, we used distance metrics that compared the taxonomic composition between sites, and classified target taxa (genera or families in the case of nematodes and species in the case of enchytraeids) in a spectrum from habitat specialist to habitat generalists. This method provides a measure of the realised niche width of a species, first, because it captures the response of the target taxon to the environmental conditions and resource availability and secondly because it incorporates the impacts of each species on the others (Devictor *et al.* 2010). This method provides a great opportunity to advance our understanding of soil ecology, by providing ecological information without the burden of exhaustive and expensive analyses of environmental variables and could be applied to a broad number of soil fauna groups, such as earthworms and collembolans.

The one limitation to the calculation of RNW through the use of co-existence matrices is that if there is no linear relationship between size of the regional species pool (gamma diversity) and the local (or alpha) species diversity, the resulting scores are biased by the size of the species pool, such that species that inhabit species rich habitats are considered by the protocol to have a larger RNW than expected (Manthey and Fridley 2009). In order for the relationship between regional and local species pools to exist, there needs to be migration and dispersal within the studied habitats. While some soil organisms can actively travel short distances and colonize new areas (Rantalainen *et al.* 2006), the most common dispersal strategy amongst soil organisms is passive dispersal via anemochory (transport by wind), water or zoochory (transport by attachment to larger animal) (Morris and Blackwood 2007). Smaller soil animals are more likely to be wind- or water-dispersed than larger ones (Ptatscheck *et al.* 2018). In fact, we expected smaller nematodes to be generalists, but found no such relationship between RNW and body size (Chapter 2). Nevertheless, the success of this dispersal method at larger distances might be dependent on the evolution of mechanisms such as anhydrobiosis (Nkem *et al.* 2006). Similar effects can be observed in passive transport via water: while body size can determine the mechanism of dispersal, it is by no means the only determining factors in the success of dispersal through water (Schuppenhauer *et al.* 2019). Much in this regard is still to discover, and in fact we do not know much about the relative importance of each mechanism in the dispersal of nematodes or enchytraeids. However, there is no reason to suspect that migration and dispersal are limited in soils.

Due to the choice of metric in our study (Jaccard dissimilarity; (Jaccard 1912)), the RNW was contained between 0 (which would describe the RNW of a taxon present always in communities of identical composition) to almost 1 (that of a species that occurs in communities with no species overlap). Both of these scenarios are unlikely in real life. If we could identify every species present in a community, the first case would indicate either the sampling of only one habitat, a very strict division of habitats that are stable in time and space, or a situation in which all species can be found everywhere (Levins 1968; Clavel *et al.* 2011). The latter would indicate the existence of only one generalist in an otherwise specialised community, such that in each there should be no overlap in species composition in the sites that the generalist inhabits. In real life, however it is more likely that these values diverge from the extreme values of 0 and 1. Both nematode and enchytraeid RNWs were contained between 0.5 and 1 (Chapter 2 Table S2 and Table 3.3), indicating on average an overlap in species composition between sites larger than 50%, and therefore, an overall tendency towards habitat generalism. This would be in agreement with many assumptions about soil fauna, and the flexibility that soil fauna can exhibit in terms of food availability (Luxton 1972; Ponsard and Arditì 2000) and resilience to disturbance (Griffiths and Philippot 2013). But we cannot neglect the fact that in soil studies it is almost impossible to sample the entire community and that this could have affected the RNWs calculated in our study. Firstly, a community, in its most strict definition should include every organism that inhabits the same habitat and that trophically interact. In the soil, this would imply sampling and identifying every organism in a given habitat, from primary producers (such as roots) to top predators (for example moles). Secondly, even when a large proportion of the community is included in a study, taxonomic differences between for example earthworms (with an average site species richness of 4) (Phillips *et al.* 2019) and nematodes (with up to 53 species in cultivated land) (Boag and Yeates 1998) make it difficult to combine these groups into one study. But most important of all is the fact that even if we limit ourselves to the identification of one taxonomic group (such as nematodes or enchytraeids), it is likely that we do not capture every species present in a habitat, and rare species or those harder to extract from the soil are likely to be under-represented. Additionally, in the case of nematodes we could not assess a species specific RNW, since identification of nematodes to the species level are complicated and time consuming using morphological approaches. Here DNA extractions, could be of incredible use in future studies, but the ability to distinguish soil organisms to species level using DNA techniques needs to be supported by adequate genome databases, which are still incomplete for most soil organisms (Singh *et al.* 2009). The last two points, a systematic under-sampling of rare species, and a broad taxonomic scope could lead to an overall increase in the number of overlapping taxa and an overall tendency towards higher

levels of generalism. In fact, our results in Chapter 3 support this claim: on the one hand leaving out rare species decreased the RNW of almost all target species; on the other, a broader taxonomic scope led to a larger RNW, for example with the analysis of *Cognettia sphagnetorum* and *C. chlorofila* as one species complex (the situation before 2019) or as separate species (Martinsson 2019). Moreover, enchytraeid species complexes had the broadest RNW of all target species. Thus the taxonomic scope of the calculations of specialisation is of importance, as generalism even at the level of species, can be the collection of individual specialists (Bolnick *et al.* 2003). Once again, DNA techniques can shed light on the existence of cryptic species that could have slightly different strategies by combining the use of DNA extractions with morphological identifications (Blouin 2002). Future studies might consider including a larger part of the soil community into the calculation of RNW, and tracking the effects of calculating RNW at different taxonomic levels.

Applying this approach on soils comes with another limitation: what defines a habitat? A habitat is the “area that can be used by a particular organism to support survival and/or reproduction” and it can be studied at different spatial scales: from microhabitats, which include the factors necessary for an individual’s survival to macrohabitats, which include all the factors necessary for the survival of a population of a given organism (Stamps 2008). The protocol proposed by (Fridley *et al.* 2007) is actually intended to prevent the scientist from defining the habitat of a species. The first step in the protocol is selecting the sites in which a target taxon is present, and by doing so, the protocol is picking out the habitats of that species. The bias I would like to discuss is introduced earlier, during the sampling of the soil community. The distribution of organisms in the soil can be patchy, and affected by small variations in soil moisture, organic matter content or nutrient fluxes. The rhizosphere and nearby soil can support different communities (Zhang *et al.* 2019). The spatial distribution of soil species can also include a vertical component, as for example enchytraeid species might show stratification due to species specific requirements (Juan-Ovejero *et al.* 2019), but there are also daily and seasonal variations in soil temperature that differ strongly depending on the vegetation cover (Sándor and Fodor 2012). But the sampling at NSMN, for example, was done as part of an effort to assess biological soil quality at the farm level. As such, the sampling campaign included the collection of cores from all fields within a farm. These cores were then mixed to create one bulk sample per farm. Each sample from the NSMN was the collection of several habitats, which could have led to assumptions of co-occurrence that do not represent the actual habitat overlaps of soil taxa.

One last aspect to discuss regarding our approach to calculate RNW is the diversity of habitats that were included in our studies. For Chapter 2, we selected a representative sample of Dutch systems, and for Chapter 3, we used information gathered from 81 European arable lands, grasslands and forests. While both datasets are of good quality, the sampling procedures associated with each were not designed for the purpose of calculating RNW. This means that this site selection might have excluded habitats that are important to specialist species. EcoFinders intended to sample ecosystems that were representative of European landscapes, and consequently focused on cropland, grasslands and forests. The NSMN included a representative sample of Dutch ecosystems (Rutgers *et al.* 2009) and it was complemented to include under-represented habitats such as heathlands or dune meadows, but in the Netherlands there are very few nutrient rich soils that are not being used for agricultural production. If specialism is related to nutrient availability, we would expect to find specialists also in such systems. Supplementing these datasets with data from diverse ecosystems in terms of nutrient availability and disturbance and gathering field level information on management practices could help in the advancement of our understanding of soil ecology and the effects of human impact upon them.

Co-occurrence based methods of niche width estimation offer a great opportunity for soil ecology, not limited to soil nematodes and enchytraeids, but applicable to other organisms as well. This would require further harmonisation of sampling techniques, and complete DNA genome databases, calculations of RNW using co-occurrence methods combined with multivariate statistics could serve to advance our understanding of the evolutionary constraints that lead to the development of specific niche widths in soil fauna.

List of references

- Adhikari, K., and A. E. Hartemink. 2016. Linking soils to ecosystem services - A global review. *Geoderma* 262: 101-111. <http://dx.doi.org/10.1016/j.geoderma.2015.08.009>.
- Adl, S. M., D. C. Coleman, and F. Read. 2006. Slow recovery of soil biodiversity in sandy loam soils of Georgia after 25 years of no-tillage management. *Agriculture, ecosystems & environment* 114 (2-4): 323-334.
- Akaike, H. 1973. Maximum likelihood identification of Gaussian autoregressive moving average models. *Biometrika* 60 (2): 255-265.
- Alkemade, J. R. M., and M. L. P. van Esbroek. 1994. Naar een effecten voorspellings model voor de bodemfauna: BOEF. Netherlands Institute for Health and the Environment (RIVM). Bilthoven, The Netherlands.
- Alons, G. 2017. Environmental policy integration in the EU's common agricultural policy: greening or greenwashing? *Journal of European Public Policy* 24 (11): 1604-1622. <http://dx.doi.org/10.1080/13501763.2017.1334085>.
- Amossé, J., K. Dózsa-Farkas, G. Boros, G. Rochat, G. Sandoz, B. Fournier, E. A. Mitchell, and R.-C. Le Bayon. 2016. Patterns of earthworm, enchytraeid and nematode diversity and community structure in urban soils of different ages. *European Journal of Soil Biology* 73: 46-58.
- Anderson, R. V., D. C. Coleman, C. V. Cole, and E. T. Elliott. 1981. Effect of the Nematodes *Acrobeloides* Sp. and *Mesodiplogaster Lheritieri* on Substrate Utilization and Nitrogen and Phosphorous Mineralization in Soil. *Ecology* 62 (3): 549-555. <http://dx.doi.org/10.2307/1937720>.
- Andrassy, I. 1992. A short census of free-living nematodes. *Fundamental and Applied Nematology* 15 (2): 187-188.
- Bampa, F., L. O'Sullivan, K. Madena, T. Sandén, H. Spiegel, C. B. Henriksen, B. B. Ghaley, et al. 2019. Harvesting European knowledge on soil functions and land management using multi-criteria decision analysis. *Soil Use and Management* 35 (1): 6-20. <http://dx.doi.org/https://doi.org/10.1111/sum.12506>.
- Bardgett, R. D. 2002. Causes and consequences of biological diversity in soil. *Zoology (Jena)* 105 (4): 367-74. <http://dx.doi.org/10.1078/0944-2006-00072>.
- Bardgett, R. D., D. K. Leemans, R. Cook, and P. J. Hobbs. 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology and Biochemistry* 29 (8): 1285-1294. [http://dx.doi.org/https://doi.org/10.1016/S0038-0717\(97\)00019-9](http://dx.doi.org/https://doi.org/10.1016/S0038-0717(97)00019-9).
- Bardgett, R. D., R. D. Lovell, P. J. Hobbs, and S. C. Jarvis. 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology and*

References

- Biochemistry* 31 (7): 1021-1030. [http://dx.doi.org/https://doi.org/10.1016/S0038-0717\(99\)00016-4](http://dx.doi.org/https://doi.org/10.1016/S0038-0717(99)00016-4).
- Bardgett, R. D., T. W. Speir, D. J. Ross, G. W. Yeates, and H. A. Kettles. 1994. Impact of pasture contamination by copper, chromium, and arsenic timber preservative on soil microbial properties and nematodes. *Biology and Fertility of soils* 18: 71-79.
- Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515 (7528): 505-511.
- Barton, K. 2019. MuMIn: Multimodel Inference.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823*.
- Behan-Pelletier, V., and G. Newton. 1999. Computers in biology: Linking soil biodiversity and ecosystem function - The taxonomic dilemma. *BioScience* 49 (2): 149-153.
- Bengtsson, J., J. Ahnström, and A. C. WEIBULL. 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. *Journal of applied ecology* 42 (2): 261-269.
- Beylich, A., and U. Graefe. 2007. Investigations on the enchytraeid fauna in floodplain soils of the Lower Middle Elbe. *Folia Facultatis Scientiarum Naturalium Universitatis Masarykianae Brunensis, Biologia* 110: 107-122.
- Beylich, A., U. Graefe, and D.-C. Elsner. 2015. Response of microannelids to tillage at soil-monitoring sites in Schleswig-Holstein, Germany. *Soil Organisms* 87: 121-135.
- Bhadoria, P., J. Kaselowsky, N. Claassen, and A. Jungk. 1991. Phosphate diffusion coefficients in soil as affected by bulk density and water content. *Zeitschrift für Pflanzenernährung und Bodenkunde* 154 (1): 53-57.
- Bilotta, G. S., R. E. Brazier, P. M. Haygarth, and D. L. Sparks. 2007. "The Impacts of Grazing Animals on the Quality of Soils, Vegetation, and Surface Waters in Intensively Managed Grasslands." In *Advances in Agronomy*, 237-280. Academic Press.
- Birkhofer, K., T. M. Bezemer, J. Bloem, M. Bonkowski, S. Christensen, D. Dubois, F. Ekelund, et al. 2008. Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. *Soil Biology and Biochemistry* 40 (9): 2297-2308.
- Bivand, R. 2017. classInt: Choose Univariate Class Intervals.
- Blakely, J. K., D. A. Neher, and A. L. Spongberg. 2002. Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. *Applied Soil Ecology* 21 (1): 71-88.

- Bloem, J., and A. M. Breure. 2003. "Chapter 8 Microbial indicators." In *Trace Metals and other Contaminants in the Environment*, edited by B. A. Markert, A. M. Breure and H. G. Zechmeister, 259-282. Elsevier.
- Blouin, M. S. 2002. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *International journal for parasitology* 32 (5): 527-531.
- Boag, B., and G. W. Yeates. 1998. Soil nematode biodiversity in terrestrial ecosystems. *Biodiversity & Conservation* 7 (5): 617-630.
- Boesten, J., and L. Van der Pas. 2000. Movement of water, bromide and the pesticides ethoprophos and bentazone in a sandy soil: the Vredepeel data set. *Agricultural Water Management* 44 (1-3): 21-42.
- Bohanec, M. 2008. DEXi: Program for Multi-Attribute Decision Making User's Manual. *Ljubljana, Slovenia: Institut Jozef Stefan*.
- Bohanec, M., and V. Rajkovič. 1990. DEX: An expert system shell for decision support. *Sistemica* 1 (1): 145-157.
- Bohanec, M., M. Žnidaršič, V. Rajkovič, I. Bratko, and B. Zupan. 2013. DEX methodology: three decades of qualitative multi-attribute modeling. *Informatica* 37 (1).
- Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist* 161 (1): 1-28.
- Bommarco, R., D. Kleijn, and S. G. Potts. 2013. Ecological intensification: harnessing ecosystem services for food security. *Trends in Ecology & Evolution* 28 (4): 230-238. <http://dx.doi.org/https://doi.org/10.1016/j.tree.2012.10.012>.
- Bommarco, R., G. Vico, and S. Hallin. 2018. Exploiting ecosystem services in agriculture: for increased food security. *Global Food Security* 17: 57-63. <http://dx.doi.org/https://doi.org/10.1016/j.gfs.2018.04.001>.
- Bongers, M., and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. *Tree* 14 (6): 224-228.
- Bongers, T. 1988. *De nematoden van Nederland: een identificatietabel voor de in Nederland aangetroffen zoetwater-en bodembewonende nematoden*: Koninklijke Nederlandse Natuurhistorische Vereniging Zeist.
- Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83 (1): 14-19. <http://dx.doi.org/dx.doi.org/10.1007/BF00324627>.

References

- Bongers, T. 1999. The maturity index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. *Plant and Soil* 212 (1): 13-22. <http://dx.doi.org/10.1023/A:1004571900425>.
- Bongers, T., and M. Bongers. 1998. Functional diversity of nematodes. *Applied Soil Ecology* 10 (3): 239-251.
- Bongers, T., H. van der Meulen, and G. Korthals. 1997. Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions. *Applied Soil Ecology* 6 (2): 195-199.
- Bouwman, L., and W. Arts. 2000. Effects of soil compaction on the relationships between nematodes, grass production and soil physical properties. *Applied Soil Ecology* 14 (3): 213-222.
- Bradford, M. A., and J. E. Newington. 2002. With the worms: Soil biodiversity and ecosystem functioning. *Biologist* 49 (3): 127-130.
- Briar, S. S., P. S. Grewal, N. Somasekhar, D. Stinner, and S. A. Miller. 2007. Soil nematode community, organic matter, microbial biomass and nitrogen dynamics in field plots transitioning from conventional to organic management. *Applied Soil Ecology* 37 (3): 256-266.
- Briones, M. a. J. I., and P. Ineson. 2002. Use of ¹⁴C carbon dating to determine feeding behaviour of enchytraeids. *Soil Biology and Biochemistry* 34 (6): 881-884.
- Briones, M. J. I. 2014. Soil fauna and soil functions: a jigsaw puzzle. *Frontiers in Environmental Science* 2 (7). <http://dx.doi.org/10.3389/fenvs.2014.00007>.
- Brondizio, E., J. Settele, and S. Díaz. 2019. IPBES. 2019 Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. *Bonn, Germany*.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Machler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal* 9 (2): 378-400.
- Brussaard, L. 2012. Ecosystem services provided by the soil biota. *Soil ecology and ecosystem services*: 45-58.
- Brussaard, L., P. C. De Ruiter, and G. G. Brown. 2007. Soil biodiversity for agricultural sustainability. *Agriculture, ecosystems & environment* 121 (3): 233-244.
- Buchan, D., M. T. Gebremikael, N. Ameloot, S. Sleutel, and S. De Neve. 2013. The effect of free-living nematodes on nitrogen mineralisation in undisturbed and disturbed soil cores. *Soil Biology & Biochemistry* 60: 142-155. <http://dx.doi.org/10.1016/j.soilbio.2013.01.022>.

- Buchanan, M., and L. D. King. 1992. Seasonal fluctuations in soil microbial biomass carbon, phosphorus, and activity in no-till and reduced-chemical-input maize agroecosystems. *Biology and Fertility of Soils* 13 (4): 211-217. <http://dx.doi.org/10.1007/bf00340578>.
- Büchi, L., and S. Vuilleumier. 2016. Ecological strategies in stable and disturbed environments depend on species specialisation. *Oikos* 125 (10): 1408-1420.
- Bünemann, E. K., G. Bongiorno, Z. Bai, R. E. Creamer, G. De Deyn, R. de Goede, L. Fleskens, et al. 2018. Soil quality – A critical review. *Soil Biology and Biochemistry* 120: 105-125. <http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2018.01.030>.
- Bünemann, E. K., G. D. Schwenke, and L. Van Zwieten. 2006. Impact of agricultural inputs on soil organisms - A review. *Australian Journal of Soil Research* 44 (4): 379-406. <http://dx.doi.org/10.1071/SR05125>.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference: A Practical Information-Theoretic approach*. New York: Springer.
- Cameron, E. K., I. S. Martins, P. Lavelle, J. Mathieu, L. Tedersoo, F. Gottschall, C. A. Guerra, et al. 2018. Global gaps in soil biodiversity data. *Nature ecology & evolution* 2 (7): 1042-1043.
- Carboni, M., D. Zelený, and A. T. Acosta. 2016. Measuring ecological specialization along a natural stress gradient using a set of complementary niche breadth indices. *Journal of Vegetation Science* 27 (5): 892-903.
- Carrascosa, M., S. Sanchez-Moreno, and J. L. Alonso-Prados. 2015. Effects of organic and conventional pesticides on plant biomass, nematode diversity and the structure of the soil food web. *Nematology* 17 (1): 11-26.
- Carrascosa, M., S. Sánchez-Moreno, and J. L. Prados-Alonso. 2014. Relationships between nematode diversity, plant biomass, nutrient cycling and soil suppressiveness in fumigated soils. *European Journal of Soil Biology* 62: 49-59.
- CBS. 2016. StatLine: Bodemgebruik; naar gebruiksvorm en gemeente. CBS Statistics Netherlands. edited by CBS. Den Haag / Heerlen.
- CBS. 2019. Centraal Bureau voor de Statistiek. Agrimatie - informatie over de agrosector. Retrieved from: <https://www.agrimatie.nl/ThemaResultaat.aspx?subpubID=2232&themaID=2286&indicatorID=2097>.
- Chalupský, J. 1992. Terrestrial Enchytraeidae (Oligochaeta) and Parergodrilidae (Polychaeta) from Sweden, with description of a new enchytraeid species. *Zoologica Scripta* 21 (2): 133-150.
- Chambers, J. M., and T. J. Hastie. 1992. *Statistical models in S*. Vol. 251: Wadsworth & Brooks/Cole Advanced Books & Software Pacific Grove, CA.

References

- Chan, K. 2001. An overview of some tillage impacts on earthworm population abundance and diversity—implications for functioning in soils. *Soil and Tillage Research* 57 (4): 179-191.
- Chelinho, S., K. D. Sautter, A. Cachada, I. Abrantes, G. Brown, A. C. Duarte, and J. P. Sousa. 2011. Carbofuran effects in soil nematode communities: Using trait and taxonomic based approaches. *Ecotoxicology and environmental safety* 74 (7): 2002-2012.
- Chen, D. M., Z. C. Lan, S. J. Hu, and Y. F. Bai. 2015. Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. *Soil Biology & Biochemistry* 89: 99-108.
<http://dx.doi.org/10.1016/j.soilbio.2015.06.028>.
- Chen, J., and H. Ferris. 1999. The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. *Soil Biology and Biochemistry* 31 (9): 1265-1279.
- Clavel, J., R. Julliard, and V. Devictor. 2011. Worldwide decline of specialist species: toward a global functional homogenization? *Frontiers in Ecology and the Environment* 9 (4): 222-228.
- Clay, N., T. Garnett, and J. Lorimer. 2019. Dairy intensification: Drivers, impacts and alternatives. *Ambio*. <http://dx.doi.org/10.1007/s13280-019-01177-y>.
- Coleman, D. C. 2008. From peds to paradoxes: linkages between soil biota and their influences on ecological processes. *Soil Biology and Biochemistry* 40 (2): 271-289.
- Coleman, D. C., and W. B. Whitman. 2005. Linking species richness, biodiversity and ecosystem function in soil systems. *Pedobiologia* 49 (6): 479-497.
- Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, et al. 1997. The value of the world's ecosystem services and natural capital. *Nature* 387 (6630): 253-260. <http://dx.doi.org/10.1038/387253a0>.
- Creamer, R. E., M. Zwetsloot, J. van Leeuwen, H. Martens, D. Brus, I. Simo Yosa, L. Hemerik, et al. 2019. Monitoring Schema for Regional and European Application, Testing and Assessment of Indicators for Five Soil Functions. LANDMARK.
- Cribari-Neto, F., and A. Zeileis. 2010. Beta regression in R. *Journal of Statistical Software* 34 (2).
- Crittenden, S., T. Eswaramurthy, R. De Goede, L. Brussaard, and M. Pulleman. 2014. Effect of tillage on earthworms over short-and medium-term in conventional and organic farming. *Applied Soil Ecology* 83: 140-148.
- Cycoń, M., and Z. Piotrowska-Seget. 2009. Changes in bacterial diversity and community structure following pesticides addition to soil estimated by cultivation technique. *Ecotoxicology* 18 (5): 632-642.

- de Goede, R. G., and T. Bongers. 1994. Nematode community structure in relation to soil and vegetation characteristics. *Applied Soil Ecology* 1 (1): 29-44.
- De Goede, R. G., and B. Verschoor. 2000. The nematode extraction efficiency of the Oostenbrink elutriator-cottonwool filter method with special reference to Nematode Body Size and Life Strategy. *Nematology* 2 (3): 325-342.
- de Graaff, M.-A., N. Hornslein, H. L. Throop, P. Kardol, and L. T. A. van Diepen. 2019. "Chapter One - Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis." In *Advances in Agronomy*, edited by Donald L. Sparks, 1-44. Academic Press.
- De Vos, A. 2011. Sustainable exploitation of saline resources: ecology, ecophysiology and cultivation of potential halophyte crops.
- De Vries, J. J. 2007. "Groundwater." In *Geology of the Netherlands*, edited by T. E. Wong, D.A.J Batjes and J de Jager, 295–315. Amsterdam, The Netherlands: Royal Netherlands Academy of Arts and Sciences.
- Debeljak, M., A. Trajanov, V. Kuzmanovski, J. Schröder, T. Sandén, H. Spiegel, D. P. Wall, et al. 2019. A Field-Scale Decision Support System for Assessment and Management of Soil Functions. *Frontiers in Environmental Science* 7 (115).
<http://dx.doi.org/10.3389/fenvs.2019.00115>.
- Decaëns, T. 2010. Macroecological patterns in soil communities. *Global Ecology and Biogeography* 19 (3): 287-302. <http://dx.doi.org/10.1111/j.1466-8238.2009.00517.x>.
- Decaëns, T., P. Margerie, M. Aubert, M. Hedde, and F. Bureau. 2008. Assembly rules within earthworm communities in North-Western France—A regional analysis. *Applied Soil Ecology* 39 (3): 321-335. <http://dx.doi.org/https://doi.org/10.1016/j.apsoil.2008.01.007>.
- Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S. Villeger, and N. Mouquet. 2010. Defining and measuring ecological specialization. *Journal of Applied Ecology* 47 (1): 15-25.
- Devictor, V., R. Julliard, J. Clavel, F. Jiguet, A. Lee, and D. Couvet. 2008. Functional biotic homogenization of bird communities in disturbed landscapes. *Global Ecology and Biogeography* 17 (2): 252-261. <http://dx.doi.org/10.1111/j.1466-8238.2007.00364.x>.
- Devictor, V., and A. Robert. 2009. Measuring community responses to large-scale disturbance in conservation biogeography. *Diversity and Distributions* 15 (1): 122-130.
- Didden, W. M. 1993. Ecology of terrestrial Enchytraeidae. *Pedobiologia (Jena)* 37 (1): 2-29.
- Dominati, E., M. Patterson, and A. Mackay. 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecological Economics* 69 (9): 1858-1868.

References

- Dormann, C. F., O. Schweiger, I. Augenstein, D. Bailey, R. Billeter, G. De Blust, R. DeFilippi, et al. 2007. Effects of landscape structure and land-use intensity on similarity of plant and animal communities. *Global Ecology and Biogeography* 16 (6): 774-787.
- Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014. Assemblage Time Series Reveal Biodiversity Change but Not Systematic Loss. *Science* 344 (6181): 296-299. <http://dx.doi.org/10.1126/science.1248484>.
- Ducatez, S., R. Tingley, and R. Shine. 2014. Using species co-occurrence patterns to quantify relative habitat breadth in terrestrial vertebrates. *Ecosphere* 5 (12): 1-12.
- Dufour, F. C. 2000. Groundwater in the Netherlands: Facts and figures. Delft: Netherlands Institute of Applied Geoscience TNO.
- Dunn, O. J. 1964. Multiple comparisons using rank sums. *Technometrics* 6 (3): 241-252.
- DuPont, S. T., H. Ferris, and M. Van Horn. 2009. Effects of cover crop quality and quantity on nematode-based soil food webs and nutrient cycling. *Applied Soil Ecology* 41 (2): 157-167. <http://dx.doi.org/10.1016/j.apsoil.2008.10.004>.
- Edaphobase. Edaphobase data warehouse for soil organisms. Accessed via the Edaphobase Data-Query Portal, <https://portal.edaphobase.org>, on [2020-09-25].
- Eisenhauer, N., S. Cesarz, R. Koller, K. Worm, and P. B. Reich. 2012. Global change belowground: impacts of elevated CO₂, nitrogen, and summer drought on soil food webs and biodiversity. *Global Change Biology* 18 (2): 435-447. <http://dx.doi.org/10.1111/j.1365-2486.2011.02555.x>.
- Emmerson, M., M. B. Morales, J. J. Onate, P. Batry, F. Berendse, J. Liira, T. Aavik, et al. 2016. "How Agricultural Intensification Affects Biodiversity and Ecosystem Services." In *Advances in Ecological Research, Vol 55: Large-Scale Ecology: Model Systems to Global Perspectives*, edited by A. J. Dumbrell, R. L. Kordas and G. Woodward, 43-97.
- Ettema, C. H., and T. Bongers. 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. *Biology and Fertility of Soils* 16 (2): 79-85. <http://dx.doi.org/10.1007/BF00369407>.
- Ettema, C. H., and D. A. Wardle. 2002. Spatial soil ecology. *Trends in Ecology & Evolution* 17 (4): 177-183. [http://dx.doi.org/https://doi.org/10.1016/S0169-5347\(02\)02496-5](http://dx.doi.org/https://doi.org/10.1016/S0169-5347(02)02496-5).
- Ferrari, S., and F. Cribari-Neto. 2004. Beta regression for modelling rates and proportions. *Journal of Applied Statistics* 31 (7): 799-815.
- Ferris, H. 1999. Nemaplex: The "Nematode-Plant expert information system". <http://nemaplex.ucdavis.edu/index.htm>. University of California.
- Ferris, H. 2010a. Contribution of Nematodes to the Structure and Function of the Soil Food Web. *Journal of Nematology* 42 (1): 63-67.

- Ferris, H. 2010b. Form and function: Metabolic footprints of nematodes in the soil food web. *European Journal of Soil Biology* 46 (2): 97-104.
<http://dx.doi.org/10.1016/j.ejsobi.2010.01.003>.
- Ferris, H., T. Bongers, and R. G. M. d. Goede. 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied soil ecology* 18: 13-29.
- Ferris, H., and H. Tuomisto. 2015. Unearthing the role of biological diversity in soil health. *Soil Biology and Biochemistry* 85: 101-109.
<http://dx.doi.org/10.1016/j.soilbio.2015.02.037>.
- Ferris, H., R. Venette, and S. Lau. 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. *Applied Soil Ecology* 3 (2): 161-175.
- Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, et al. 2005. Global Consequences of Land Use. *Science* 309 (5734): 570-574.
<http://dx.doi.org/10.1126/science.1111772>.
- Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, et al. 2011. Solutions for a cultivated planet. *Nature* 478 (7369): 337-342.
<http://dx.doi.org/10.1038/nature10452>.
- Forge, T. A., S. Bittman, and C. G. Kowalenko. 2005. Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer. *Soil Biology and Biochemistry* 37 (10): 1751-1762.
<http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2004.11.013>.
- Fox, J. 1997. *Applied regression analysis, linear models, and related methods*. United States of America: Sage Publications, Inc.
- Fox, J., S. Weisberg, D. Adler, D. Bates, G. Baud-Bovy, S. Ellison, D. Firth, et al. 2012. Package 'car'. *Vienna: R Foundation for Statistical Computing*.
- Frazaõ, J., R. G. M. de Goede, T. E. Salánki, L. Brussaard, J. H. Faber, M. Hedde, and M. M. Pulleman. 2019. Responses of earthworm communities to crop residue management after inoculation of the earthworm *Lumbricus terrestris* (Linnaeus, 1758). *Applied Soil Ecology* 142: 177-188. <http://dx.doi.org/https://doi.org/10.1016/j.apsoil.2019.04.022>.
- Freckman, D. W., and C. H. Ettema. 1993. Assessing nematode communities in agroecosystems of varying human intervention. *Agriculture, Ecosystems and Environment* 45 (239-261).
- Fridley, J. D., D. B. Vandermaast, D. M. Kuppinger, M. Manthey, and R. K. Peet. 2007. Co-occurrence based assessment of habitat generalists and specialists: a new approach for the measurement of niche width. *Journal of Ecology* 95 (4): 707-722.
<http://dx.doi.org/10.1111/j.1365-2745.2007.01236.x>.

References

- Fried, G., S. Petit, and X. Reboud. 2010. A specialist-generalist classification of the arable flora and its response to changes in agricultural practices. *BMC ecology* 10 (1): 20.
- Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. *Annual review of Ecology and Systematics* 19 (1): 207-233.
- Gaston, K. J., T. M. Blackburn, and J. H. Lawton. 1997. Interspecific abundance-range size relationships: an appraisal of mechanisms. *Journal of Animal Ecology*: 579-601.
- Gebremikael, M. T., H. Steel, D. Buchan, W. Bert, and S. De Neve. 2016. Nematodes enhance plant growth and nutrient uptake under C and N-rich conditions. *Scientific reports* 6: 32862.
- Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, P. Ceryngier, et al. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology* 11 (2): 97-105. <http://dx.doi.org/10.1016/j.baaec.2009.12.001>.
- Geisen, S., D. H. Wall, and W. H. van der Putten. 2019. Challenges and opportunities for soil biodiversity in the anthropocene. *Current Biology* 29 (19): R1036-R1044.
- Gems, D. 2000. Longevity and ageing in parasitic and free-living nematodes. *Biogerontology* 1 (4): 289-307.
- Giller, P. 2012. *Community structure and the niche*. Springer Science & Business Media.
- Giller, P. S. 1996. The diversity of soil communities, the 'poor man's tropical rainforest'. *Biodiversity & Conservation* 5 (2): 135-168.
- Graefe, U., and A. Beylich. 2003. Critical values of soil acidification for annelid species and the decomposer community. *Newsletter on Enchytraeidae* 8: 51-55.
- Graefe, U., and R. M. Schmelz. 1999. Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. *Newsletter on Enchytraeidae* 6: 59-67.
- Green, F., R. Harding, and H. Oliver. 1984. The relationship of soil temperature to vegetation height. *Journal of climatology* 4 (3): 229-240.
- Greenacre, M. J. 1984. *Theory and applications of correspondence analysis*. London: Academic Press.
- Griffiths, B. S., J. Faber, and J. Bloem. 2018. Applying Soil Health Indicators to Encourage Sustainable Soil Use: The Transition from Scientific Study to Practical Application. *Sustainability* 10 (9). <http://dx.doi.org/10.3390/su10093021>.
- Griffiths, B. S., and L. Philippot. 2013. Insights into the resistance and resilience of the soil microbial community. *FEMS microbiology reviews* 37 (2): 112-129.
- Gross, J. 2003. Variance inflation factors. *R News* 3 (1): 13-15.

- Guerra, C. A., A. Heintz-Buschart, J. Sikorski, A. Chatzinotas, N. Guerrero-Ramírez, S. Cesarz, L. Beaumelle, et al. 2020. Blind spots in global soil biodiversity and ecosystem function research. *Nature Communications* 11 (1): 1-13.
- Gutierrez, C., C. Fernandez, M. Escuer, R. Campos-Herrera, M. E. B. Rodriguez, G. Carbonell, and J. A. R. Martin. 2016. Effect of soil properties, heavy metals and emerging contaminants in the soil nematodes diversity. *Environmental Pollution* 213: 184-194. <http://dx.doi.org/10.1016/j.envpol.2016.02.012>.
- Haygarth, P. M., and K. Ritz. 2009. The future of soils and land use in the UK: Soil systems for the provision of land-based ecosystem services. *Land Use Policy* 26: S187-S197.
- Helling, C. S., G. Chesters, and R. B. Corey. 1964. Contribution of Organic Matter and Clay to Soil Cation-Exchange Capacity as Affected by the pH of the Saturating Solution. *Soil Science Society of America Journal* 28 (4): 517-520. <http://dx.doi.org/10.2136/sssaj1964.03615995002800040020x>.
- Henneron, L., L. Bernard, M. Hedde, C. Pelosi, C. Villenave, C. Chenu, M. Bertrand, C. Girardin, and E. Blanchart. 2015. Fourteen years of evidence for positive effects of conservation agriculture and organic farming on soil life. *Agronomy for Sustainable Development* 35 (1): 169-181. <http://dx.doi.org/10.1007/s13593-014-0215-8>.
- Herren, G. L., J. Habraken, L. Waeyenberge, A. Haegeman, N. Viaene, M. Cougnon, D. Reheul, H. Steel, and W. Bert. 2020. Effects of synthetic fertilizer and farm compost on soil nematode community in long-term crop rotation plots: A morphological and metabarcoding approach. *PLOS ONE* 15 (3): e0230153. <http://dx.doi.org/10.1371/journal.pone.0230153>.
- Hill, M. O. 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54 (2): 427-432.
- Hoek, J., D. van Balen, W. Haagsma, W. van den Berg, P. van Asperen, W. Sukkel, J. J. de Haan, and J. Bloem. 2019. Bodemindicatoren in BASIS: Identificatie van de belangrijkste biologische en chemische bodemparameters (“bodemindicatoren”) in het project BASIS over de periode 2009-2016. . Wageningen University & Research, Business unit Open Teelten.
- Hollander, M., and D. A. Wolfe. 1999. *Nonparametric Statistical Methods, Solutions Manual*. New York: Wiley.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian journal of statistics*: 65-70.
- Houba, V., I. Novozamsky, and D. Van Dijk. 1998. Certification of an air-dry soil for pH and extractable nutrients using one hundredth molar calcium chloride. *Communications in soil science and plant analysis* 29 (9-10): 1083-1090.

References

- Huang, A. 2017. Mean-parametrized Conway–Maxwell–Poisson regression models for dispersed counts. *Statistical Modelling* 17 (6): 359-380.
- Hurvich, C. M., and C.-L. Tsai. 1989. Regression and time series model selection in small samples. *Biometrika* 76 (2): 297-307.
- Hutchinson, G. E. 1957. Concluding remarks. population studies: animal ecology and demography. . *Cold Spring Harbor Symposia on Quantitative Biology* 22 (415–427).
- Ibarra, J. T., and K. Martin. 2015. Biotic homogenization: loss of avian functional richness and habitat specialists in disturbed Andean temperate forests. *Biological Conservation* 192: 418-427.
- ICZN. 2018. (International Commission on Zoological Nomenclature), 2018. Opinion 2423 (Case 3689)—Cognettia Nielsen & Christensen, 1959 (Annelida, Oligochaeta, Enchytraeidae): conditional precedence given over Euenchytraeus Bretscher, 1906 and Chamaedrillus Friend, 1913. *The Bulletin of Zoological Nomenclature* 75 (1): 279-281.
- IPCC. 2013. Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A. Xia, Y., Bex, V. Midgley, P. M. In *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*.
- ISO. 2007. ISO (International Organization for Standardization). 2007. Soil quality - sampling of soil invertebrates - Part 3: Sampling and soil Extraction of enchytraeids, ISO 23611-3: 2007, Geneva, Switzerland.
- Ito, T., M. Araki, T. Higashi, M. Komatsuzaki, N. Kaneko, and H. Ohta. 2015. Responses of soil nematode community structure to soil carbon changes due to different tillage and cover crop management practices over a nine-year period in Kanto, Japan. *Applied Soil Ecology* 89: 50-58. <http://dx.doi.org/10.1016/j.apsoil.2014.12.010>.
- Jaccard, P. 1912. The distribution of the flora in the alpine zone. 1. *New phytologist* 11 (2): 37-50.
- Jänsch, S., and J. Römbke. 2003. Ökologische Charakterisierung ausgewählter Enchytraeenarten hinsichtlich relevanter Bodeneigenschaften. *Umweltwissenschaften und Schadstoff-Forschung* 15 (2): 95-105.
- Jänsch, S., J. Römbke, and W. Didden. 2005. The use of enchytraeids in ecological soil classification and assessment concepts. *Ecotoxicology and environmental safety* 62 (2): 266-277.
- Jenks, G. F. 1967. The data model concept in statistical mapping. *International yearbook of cartography* 7 (1): 186-190.

- Jiao, J. G., B. B. Liu, M. Mao, C. L. Ye, L. Yu, F. Hu, and H. X. Li. 2015. Characteristics of soil nematode community of different agricultural areas in Jiangsu Province, China. *Chinese Journal of Applied Ecology* 26 (11): 3489-3496.
- Jihua, W., S. Ciyu, and C. Jiakuan. 2010. Effect of microbivorous nematodes on plant growth and soil nutrient cycling: a review.
- Jouquet, P., J. Dauber, J. Lagerlöf, P. Lavelle, and M. Lepage. 2006. Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. *Applied Soil Ecology* 32 (2): 153-164. <http://dx.doi.org/10.1016/j.apsoil.2005.07.004>.
- Juan-Ovejero, R., E. Benito, M. E. Barreal, J. Rodeiro, and M. J. I. Briones. 2019. Tolerance to fluctuating water regimes drives changes in mesofauna community structure and vertical stratification in peatlands. *Pedobiologia* 76: 150571.
- Kapusta, P., and Ł. Sobczyk. 2015. Effects of heavy metal pollution from mining and smelting on enchytraeid communities under different land management and soil conditions. *Science of The Total Environment* 536: 517-526. <http://dx.doi.org/https://doi.org/10.1016/j.scitotenv.2015.07.086>.
- Kerfahi, D., J. Park, B. M. Tripathi, D. Singh, D. L. Porazinska, I. Moroenyane, and J. M. Adams. 2017. Molecular methods reveal controls on nematode community structure and unexpectedly high nematode diversity, in Svalbard high Arctic tundra. 40 (4): 765-776. <http://dx.doi.org/10.1007/s00300-016-1999-6>.
- Kibblewhite, M. G., K. Ritz, and M. J. Swift. 2008. Soil health in agricultural systems. *Philos Trans R Soc Lond B Biol Sci* 363 (1492): 685-701. <http://dx.doi.org/10.1098/rstb.2007.2178>.
- Kladivko, E. J. 2001. Tillage systems and soil ecology. *Soil & Tillage Research* 61 (1-2): 61-76. [http://dx.doi.org/10.1016/s0167-1987\(01\)00179-9](http://dx.doi.org/10.1016/s0167-1987(01)00179-9).
- Kleiber, C., and A. Zeileis. 2008. *Applied econometrics with R*: Springer Science & Business Media.
- Kleijn, D., R. Bommarco, T. P. Fijen, L. A. Garibaldi, S. G. Potts, and W. H. van der Putten. 2019. Ecological intensification: bridging the gap between science and practice. *Trends in ecology & evolution* 34 (2): 154-166.
- Knapp, S., and M. G. A. van der Heijden. 2018. A global meta-analysis of yield stability in organic and conservation agriculture. *Nature Communications* 9 (1): 3632. <http://dx.doi.org/10.1038/s41467-018-05956-1>.
- Korner-Nievergelt, F., T. Roth, S. Von Felten, J. Guélat, B. Almasi, and P. Korner-Nievergelt. 2015. *Bayesian data analysis in ecology using linear models with R, BUGS, and Stan*. Academic Press.

References

- Korthals, G. W., A. D. Alexiev, T. M. Lexmond, J. E. Kammenga, and T. Bongers. 1996. Long-term effects of copper and pH on the nematode community in an agroecosystem. *Environmental Toxicology and Chemistry* 15 (6): 979-985. [http://dx.doi.org/10.1897/1551-5028\(1996\)015<0979:lteo>2.3.co;2](http://dx.doi.org/10.1897/1551-5028(1996)015<0979:lteo>2.3.co;2).
- Korthals, G. W., T. C. Thoden, W. van den Berg, and J. H. M. Visser. 2014. Long-term effects of eight soil health treatments to control plant-parasitic nematodes and *Verticillium dahliae* in agro-ecosystems. *Applied Soil Ecology* 76: 112-123. <http://dx.doi.org/https://doi.org/10.1016/j.apsoil.2013.12.016>.
- Korthals, G. W., A. van de Ende, H. van Megen, T. M. Lexmond, J. E. Kammenga, and T. Bongers. 1996. Short-term effects of cadmium, copper, nickel and zinc on soil nematodes from different feeding and life-history strategy groups. *Applied soil ecology* 4: 107-117.
- Kotze, D. J., and R. B. O'Hara. 2003. Species decline—but why? Explanations of carabid beetle (Coleoptera, Carabidae) declines in Europe. *Oecologia* 135 (1): 138-148. <http://dx.doi.org/10.1007/s00442-002-1174-3>.
- Kuchenbuch, R., N. Claassen, and A. Jungk. 1986. Potassium availability in relation to soil moisture. *Plant and Soil* 95 (2): 233-243.
- Kukul, S. S., R. Rehana, and D. K. Benbi. 2009. Soil organic carbon sequestration in relation to organic and inorganic fertilization in rice–wheat and maize–wheat systems. *Soil and Tillage Research* 102 (1): 87-92. <http://dx.doi.org/https://doi.org/10.1016/j.still.2008.07.017>.
- Lavelle, P., T. Decaëns, M. Aubert, S. b. Barot, M. Blouin, F. Bureau, P. Margerie, P. Mora, and J.-P. Rossi. 2006. Soil invertebrates and ecosystem services. *European journal of soil biology* 42: S3-S15.
- Lefebvre, M., E. Midler, and P. Bontems. 2020. Adoption of Environment-Friendly Agricultural Practices with Background Risk: Experimental Evidence. *Environmental & Resource Economics*: 24. <http://dx.doi.org/10.1007/s10640-020-00431-2>.
- Legendre, P., and L. F. Legendre. 2012. *Numerical ecology*. Vol. 24: Elsevier.
- Legendre, P., J. Oksanen, and C. J. ter Braak. 2011. Testing the significance of canonical axes in redundancy analysis. *Methods in Ecology and Evolution* 2 (3): 269-277.
- Levins, R. 1968. *Evolution in changing environments: some theoretical explorations*. Princeton University Press.
- Liu, T., X. Chen, F. Hu, W. Ran, Q. Shen, H. Li, and J. K. Whalen. 2016. Carbon-rich organic fertilizers to increase soil biodiversity: Evidence from a meta-analysis of nematode communities. *Agriculture, Ecosystems and Environment* 232: 199-207. <http://dx.doi.org/10.1016/j.agee.2016.07.015>.
- Luxton, M. 1972. Studies on the oribatid mites of a Danish beech wood soil. I. Nutritional biology.

- Ma, Q., H. Yu, X. Liu, Z. Xu, G. Zhou, and Y. Shi. 2018. Climatic warming shifts the soil nematode community in a desert steppe. *Climatic Change* 150 (3-4): 243-258.
- Manthey, M., and J. D. Fridley. 2009. Beta diversity metrics and the estimation of niche width via species co-occurrence data: reply to Zeleny. *Journal of Ecology* 97 (1): 18-22.
- Martinsson, S. 2019. A morphology-based identification key to the *Cognettia* species of the world (Clitellata: Enchytraeidae). *SOIL ORGANISMS* 91 (2): 37-43-37-43.
- Martinsson, S., and C. Erséus. 2014. Cryptic diversity in the well-studied terrestrial worm *Cognettia sphagnetorum* (Clitellata: Enchytraeidae). *Pedobiologia* 57 (1): 27-35.
- Martinsson, S., E. Rota, and C. Erséus. 2015. Revision of *Cognettia* (Clitellata, Enchytraeidae): re-establishment of *Chamaedrillus* and description of cryptic species in the sphagnetorum complex. *Systematics and Biodiversity* 13 (3): 257-277.
- Matches, A. G. 1992. Plant response to grazing: A review. *Journal of Production Agriculture* 5 (1): 1-7.
- Matson, P. A., W. J. Parton, A. G. Power, and M. J. Swift. 1997. Agricultural intensification and ecosystem properties. *Science* 277 (5325): 504-509.
- McDaniel, M. D., L. K. Tiemann, and A. S. Grandy. 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecological Applications* 24 (3): 560-570. <http://dx.doi.org/10.1890/13-0616.1>.
- Montanarella, L., D. J. Pennock, N. McKenzie, M. Badraoui, V. Chude, I. Baptista, T. Mamo, et al. 2016. World's soils are under threat. *SOIL* 2 (1): 79-82. <http://dx.doi.org/10.5194/soil-2-79-2016>.
- Morris, S. J., and C. B. Blackwood. 2007. "The ecology of soil organisms." In *Soil Microbiology, Ecology and Biochemistry*, 195-229. Elsevier.
- Mueller, K. E., D. M. Blumenthal, Y. Carrillo, S. Cesarz, M. Ciobanu, J. Hines, S. Pabst, et al. 2016. Elevated CO₂ and warming shift the functional composition of soil nematode communities in a semiarid grassland. *Soil Biology and Biochemistry* 103: 46-51. <http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2016.08.005>.
- Mulder, C., D. De Zwart, H. J. Van Wijnen, A. J. Schouten, and A. M. Breure. 2003. Observational and simulated evidence of ecological shifts within the soil nematode community of agroecosystems under conventional and organic farming. *Functional Ecology* 17 (4): 516-525. <http://dx.doi.org/10.1046/j.1365-2435.2003.00755.x>.
- Mulder, C., and J. A. Vonk. 2011. Nematode traits and environmental constraints in 200 soil systems: scaling within the 60–6000 μ m body size range. *Ecology* 92 (10): 2004-2004. <http://dx.doi.org/doi:10.1890/11-0546.1>.

References

- Mulholland, B., and M. A. Fullen. 1991. Cattle trampling and soil compaction on loamy sands. *Soil Use and Management* 7 (4): 189-193. <http://dx.doi.org/10.1111/j.1475-2743.1991.tb00873.x>.
- Neher, D. A. 2001. Role of nematodes in soil health and their use as indicators. *Journal of Nematology* 33 (4): 161-168.
- NEN-ISO 5793. 2010. Soil - Determination of phosphate in soil extractable with ammoniumlactate-acetic acid buffer (P-AL).
- NEN-ISO 12099. 2010. Fodder, Cereals and Milled Cereal Products – Guidelines for the Application of Near Infrared Spectrometry.
- Nielsen, U. N., E. Ayres, D. H. Wall, G. Li, R. D. Bardgett, T. Wu, and J. R. Garey. 2014. Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties. *Global Ecology and Biogeography* 23 (9): 968-978.
- Nkem, J. N., D. H. Wall, R. A. Virginia, J. E. Barrett, E. J. Broos, D. L. Porazinska, and B. J. Adams. 2006. Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. *Polar Biology* 29 (4): 346-352.
- Norris, K. 2008. Agriculture and biodiversity conservation: opportunity knocks. *Conservation Letters* 1 (1): 2-11.
- Nowak, E. 2004. Enchytraeids (Oligochaeta) in the agricultural landscape. *Polish Journal of Ecology* 52 (2): 115-122.
- Nowak, E. 2007. Enchytraeids [Enchytraeidae, Oligochaeta] in midfield shelterbelts of different age and in adjoining croplands. *Polish Journal of Ecology* 4 (55).
- O'Sullivan, L., R. Creamer, R. Fealy, G. Lanigan, I. Simo, O. Fenton, J. Carfrae, and R. Schulte. 2015. Functional Land Management for managing soil functions: A case-study of the trade-off between primary productivity and carbon storage in response to the intervention of drainage systems in Ireland. *Land use policy* 47: 42-54.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlenn, P. R. Minchin, et al. 2018. vegan: Community Ecology Package.
- Oostenbrink, M. 1960. "Estimating nematode populations by some selected methods." In *Nematology*, edited by J N Sasser and W R Jenkins, 85-102. Chapel Hill, NC, USA: The University of North Carolina Press.
- Orgiazzi, A., R. D. Bardgett, and E. Barrios. 2016b. *Global soil biodiversity atlas*: European Commission.
- Orgiazzi, A., P. Panagos, Y. Yigini, M. B. Dunbar, C. Gardi, L. Montanarella, and C. Ballabio. 2016a. A knowledge-based approach to estimating the magnitude and spatial patterns of potential threats to soil biodiversity. *Science of The Total Environment* 545-546: 11-20. <http://dx.doi.org/https://doi.org/10.1016/j.scitotenv.2015.12.092>.

- Pankhurst, C., B. Hawke, H. McDonald, C. Kirkby, J. Buckerfield, P. Michelsen, K. O'Brien, V. Gupta, and B. Doube. 1995. Evaluation of soil biological properties as potential bioindicators of soil health. *Animal Production Science* 35 (7): 1015-1028.
- Pannek, A., M. Manthey, and M. Diekmann. 2016. Comparing resource-based and co-occurrence-based methods for estimating species niche breadth. *Journal of Vegetation Science*.
- Paoletti, M. G., and M. Hassall. 1999. Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. *Agriculture, ecosystems & environment* 74 (1): 157-165.
- Pe'er, G., A. Bonn, H. Bruelheide, P. Dieker, N. Eisenhauer, P. H. Feindt, G. Hagedorn, et al. 2019. Action needed for the EU Common Agricultural Policy to address sustainability challenges. *People and Nature*.
- Pelosi, C., and J. Römbke. 2016. Are Enchytraeidae (Oligochaeta, Annelida) good indicators of agricultural management practices? *Soil Biology and Biochemistry* 100: 255-263.
- Peres-Neto, P. R., P. Legendre, S. Dray, and D. Borcard. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* 87 (10): 2614-2625.
- Phillips, H., C. Guerra, M. Bartz, M. Briones, G. Brown, T. Crowther, O. Ferlian, et al. 2019. Global distribution of earthworm diversity. *Science* 366: 480-485.
<http://dx.doi.org/10.1126/science.aax4851>.
- Pianka, E. R. 1970. On r-and K-selection. *The american naturalist* 104 (940): 592-597.
- Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. *Journal of theoretical biology* 13: 131-144.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models.
- Pires, L. F., J. A. Borges, J. A. Rosa, M. Cooper, R. J. Heck, S. Passoni, and W. L. Roque. 2017. Soil structure changes induced by tillage systems. *Soil and Tillage Research* 165: 66-79.
- Pohlert, T. 2014. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR).
- Ponsard, S., and R. Arditì. 2000. What can stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) tell about the food web of soil macro-invertebrates? *Ecology* 81 (3): 852-864.
[http://dx.doi.org/10.1890/0012-9658\(2000\)081\[0852:Wcsina\]2.0.Co;2](http://dx.doi.org/10.1890/0012-9658(2000)081[0852:Wcsina]2.0.Co;2).
- Porre, R. J., J. W. van Groenigen, G. B. De Deyn, R. G. de Goede, and I. M. Lubbers. 2016. Exploring the relationship between soil mesofauna, soil structure and N₂O emissions. *Soil Biology and Biochemistry* 96: 55-64.

References

- Postma-Blaauw, M. B., R. G. de Goede, J. Bloem, J. H. Faber, and L. Brussaard. 2010. Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology* 91 (2): 460-473.
- Postma-Blaauw, M. B., R. G. de Goede, J. Bloem, J. H. Faber, and L. Brussaard. 2012. Agricultural intensification and de-intensification differentially affect taxonomic diversity of predatory mites, earthworms, enchytraeids, nematodes and bacteria. *Applied soil ecology* 57: 39-49.
- Pothula, S. K., P. S. Grewal, R. M. Auge, A. M. Saxton, and E. C. Bernard. 2019. Agricultural intensification and urbanization negatively impact soil nematode richness and abundance: a meta-analysis. *Journal of nematology* 51: 1-17. <http://dx.doi.org/10.21307/jofnem-2019-011>.
- Prins, H., J. Jager, R. Stokkers, and M. van Asseldonk. 2018. Damage to Dutch agricultural and horticultural crops as a result of the drought in 2018. Extent of crop yield losses and mitigating and adaptive measures taken by farmers and growers. In *Factsheet* Wageningen Economic Research.
- Ptatscheck, C., B. Gansfort, and W. Traunspurger. 2018. The extent of wind-mediated dispersal of small metazoans, focusing nematodes. *Scientific reports* 8 (1): 6814.
- Pulleman, M., R. Creamer, U. Hamer, J. Helder, C. Pelosi, G. Peres, and M. Rutgers. 2012. Soil biodiversity, biological indicators and soil ecosystem services—an overview of European approaches. *Current Opinion in Environmental Sustainability* 4 (5): 529-538.
- Quist, C. W. 2017. *Impact of trophic ecologies on the whereabouts of nematodes in soil*, Wageningen University & Research, The Netherlands.
- Quist, C. W., G. Gort, P. Mooijman, D. J. Brus, S. van den Elsen, O. Kostenko, M. Vervoort, et al. 2019. Spatial distribution of soil nematodes relates to soil organic matter and life strategy. *Soil Biology and Biochemistry* 136: 107542. <http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2019.107542>.
- Quist, C. W., G. Gort, C. Mulder, R. H. P. Wilbers, A. J. Termorshuizen, J. Bakker, and J. Helder. 2017. Feeding preference as a main determinant of microscale patchiness among terrestrial nematodes. *Molecular Ecology Resources* 17 (6): 1257-1270. <http://dx.doi.org/10.1111/1755-0998.12672>.
- Quist, C. W., M. Schrama, J. J. de Haan, G. Smant, J. Bakker, W. H. van der Putten, and J. Helder. 2016. Organic farming practices result in compositional shifts in nematode communities that exceed crop-related changes. *Applied Soil Ecology* 98: 254-260. <http://dx.doi.org/10.1016/j.apsoil.2015.10.022>.
- R Core Team. 2019. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

- Rantalainen, M.-L., J. Haimi, H. Fritze, and H. Setälä. 2006. Effects of small-scale habitat fragmentation, habitat corridors and mainland dispersal on soil decomposer organisms. *Applied Soil Ecology* 34 (2): 152-159. <http://dx.doi.org/https://doi.org/10.1016/j.apsoil.2006.03.004>.
- Rawls, W. J. 1983. Estimating soil bulk density from particle size analysis and organic matter content. *Soil Science* 135 (2): 123-125.
- Raynaud, X., and N. Nunan. 2014. Spatial ecology of bacteria at the microscale in soil. *PLoS One* 9 (1): e87217.
- RIVM 2019-0026. 2019. Agricultural practices and water quality on farms registered for derogation in 2017 edited by S. Lukács, P.W. Blokland, H. Prins, A. Vrijhoef, D. Fraters and C.H.G. Daatselaar. Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM).
- Römbke, J., S. Jänsch, H. Höfer, F. Horak, M. Roß-Nickoll, D. Russell, and A. Toschki. 2013. State of knowledge of enchytraeid communities in German soils as a basis for biological soil quality assessment. *Soil Org* 85 (2): 123-146.
- Römbke, J., R. M. Schmelz, and C. Pelosi. 2017. Effects of organic pesticides on enchytraeids (Oligochaeta) in agroecosystems: laboratory and higher-tier tests. *Frontiers in Environmental Science* 5: 20.
- Rotches-Ribalta, R., S. Ruas, K. D. Ahmed, M. Gormally, J. Moran, J. Stout, B. White, and D. O. hUallachain. 2020. Assessment of semi-natural habitats and landscape features on Irish farmland: New insights to inform EU Common Agricultural Policy implementation. *Ambio*: 14. <http://dx.doi.org/10.1007/s13280-020-01344-6>.
- Royall, R. 1997. *Statistical evidence: a likelihood paradigm*. Vol. 71: CRC press.
- Ruess, L., and H. Ferris. 2004. Decomposition pathways and successional changes. *Nematology Monographs and Perspectives* 2: 547-556.
- Rutgers, M., C. Mulder, A. J. Schouten, J. Bloem, J. J. Bogte, A. M. Breure, L. Brussaard, et al. 2008. Soil ecosystem profiling in the Netherlands with ten references for biological soil quality Bilthoven: RIVM.
- Rutgers, M., A. J. Schouten, J. Bloem, N. van Eekeren, R. G. M. de Goede, G. Akkerhuis, A. van der Wal, et al. 2009. Biological measurements in a nationwide soil monitoring network. *European Journal of Soil Science* 60 (5): 820-832. <http://dx.doi.org/10.1111/j.1365-2389.2009.01163.x>.
- Rutgers, M., H. Van Wijnen, A. Schouten, C. Mulder, A. Kuiten, L. Brussaard, and A. Breure. 2012. A method to assess ecosystem services developed from soil attributes with stakeholders and data of four arable farms. *Science of the Total Environment* 415: 39-48. <http://dx.doi.org/10.1016/j.scitotenv.2011.04.041>.

References

- Sánchez-Moreno, S., and H. Ferris. 2007. Suppressive service of the soil food web: effects of environmental management. *Agriculture, ecosystems & environment* 119 (1-2): 75-87.
- Sanchez-Moreno, S., N. L. Nicola, H. Ferris, and F. G. Zalom. 2009. Effects of agricultural management on nematode-mite assemblages: Soil food web indices as predictors of mite community composition. *Applied Soil Ecology* 41 (1): 107-117.
<http://dx.doi.org/10.1016/j.apsoil.2008.09.004>.
- Sandén, T., A. Trajanov, H. Spiegel, V. Kuzmanovski, N. P. A. Saby, C. Picaud, C. B. Henriksen, and M. Debeljak. 2019. Development of an Agricultural Primary Productivity Decision Support Model: A Case Study in France. *Frontiers in Environmental Science* 7 (58). <http://dx.doi.org/10.3389/fenvs.2019.00058>.
- Sándor, R., and N. Fodor. 2012. Simulation of Soil Temperature Dynamics with Models Using Different Concepts. *The Scientific World Journal* 2012: 590287.
<http://dx.doi.org/10.1100/2012/590287>.
- Sapkota, T. B. 2012. "Conservation tillage impact on soil aggregation, organic matter turnover and biodiversity." In *Organic fertilisation, soil quality and human health*, 141-160. Springer.
- Sarukhan, J., A. Whyte, R. Hassan, R. Scholes, N. Ash, S. T. Carpenter, P. L. Pingali, et al. 2005. Millenium ecosystem assessment: Ecosystems and human well-being.
- Schmelz, R. M., and R. Collado. 2010. A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta).
- Schmelz, R. M., R. Collado, and J. Römbke. 2015. Case 3689 Cognettia Nielsen & Christensen, 1959 (Annelida, Oligochaeta, enchytraeidae): proposed precedence over Euenchytraeus Bretscher, 1906 and Chamaedrillus Friend, 1913. *The Bulletin of Zoological Nomenclature* 72 (3): 186-192.
- Schmelz RM, R. M., A. Beylich, G. Boros, K. Dózsa-Farkas, and U. Graefe. 2017. How to deal with cryptic species in Enchytraeidae, with recommendations on taxonomical descriptions. *OPUSCULA ZOOLOGICA (BUDAPEST)* 48 (S2): 45-51.
- Scholefield, D., and D. M. Hall. 1986. A recording penetrometer to measure the strength of soil in relation to the stresses exerted by a walking cow. *Journal of Soil Science* 37 (1): 165-176. <http://dx.doi.org/10.1111/j.1365-2389.1986.tb00016.x>.
- Schon, N. L., A. D. Mackay, R. A. Gray, C. van Koten, and M. B. Dodd. 2017. Influence of earthworm abundance and diversity on soil structure and the implications for soil services throughout the season. *Pedobiologia* 62: 41-47.
<http://dx.doi.org/https://doi.org/10.1016/j.pedobi.2017.05.001>.
- Schröder, J. J., R. P. O. Schulte, R. E. Creamer, A. Delgado, J. van Leeuwen, T. Lehtinen, M. Rutgers, et al. 2016. The elusive role of soil quality in nutrient cycling: a review. *Soil Use and Management* 32 (4): 476-486. <http://dx.doi.org/10.1111/sum.12288>.

- Schulte, R. P. O., F. Bampa, M. Bardy, C. Coyle, R. E. Creamer, R. Fealy, C. Gardi, et al. 2015. Making the Most of Our Land: Managing Soil Functions from Local to Continental Scale. *Frontiers in Environmental Science* 3 (81). <http://dx.doi.org/10.3389/fenvs.2015.00081>.
- Schulte, R. P. O., R. E. Creamer, T. Donnellan, N. Farrelly, R. Fealy, C. O'Donoghue, and D. O'hUallachain. 2014. Functional land management: A framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. *Environmental Science & Policy* 38: 45-58. <http://dx.doi.org/https://doi.org/10.1016/j.envsci.2013.10.002>.
- Schulte, R. P. O., L. O'Sullivan, D. Vrebos, F. Bampa, A. Jones, and J. Staes. 2019. Demands on land: Mapping competing societal expectations for the functionality of agricultural soils in Europe. *Environmental Science & Policy* 100: 113-125. <http://dx.doi.org/https://doi.org/10.1016/j.envsci.2019.06.011>.
- Schuppenhauer, M. M., R. Lehmitz, and W. E. Xylander. 2019. Slow-moving soil organisms on a water highway: aquatic dispersal and survival potential of Oribatida and Collembola in running water. *Movement ecology* 7 (1): 20.
- Seppelt, R., C. F. Dormann, F. V. Eppink, S. Lautenbach, and S. Schmidt. 2011. A quantitative review of ecosystem service studies: approaches, shortcomings and the road ahead. *Journal of Applied Ecology* 48 (3): 630-636. <http://dx.doi.org/10.1111/j.1365-2664.2010.01952.x>.
- Shannon, C. E. 1948. A mathematical theory of communication. *Bell system technical journal* 27 (3): 379-423.
- Siderius, W., and H. De Bakker. 2003. Toponymy and soil nomenclature in the Netherlands. *Geoderma* 111 (3-4): 521-536.
- Siebert, J., M. Ciobanu, M. Schädler, and N. Eisenhauer. 2020. Climate change and land use induce functional shifts in soil nematode communities. *Oecologia* 192 (1): 281-294.
- Sieriebriennikov, B., H. Ferris, and R. G. de Goede. 2014. NINJA: An automated calculation system for nematode-based biological monitoring. *European Journal of Soil Biology* 61: 90-93.
- Silva, J. V., T. R. Tenreiro, L. Spätjens, N. P. R. Anten, M. K. van Ittersum, and P. Reidsma. 2020. Can big data explain yield variability and water productivity in intensive cropping systems? *Field Crops Research* 255: 107828. <http://dx.doi.org/https://doi.org/10.1016/j.fcr.2020.107828>.
- Singh, B., C. Campbell, S. Sørensen, and J. Zhou. 2009. Soil genomics. *Nature reviews. Microbiology* 7: 756; author reply 756-7. <http://dx.doi.org/10.1038/nrmicro2119-c1>.
- Sissingh, H. 1971. Analytical technique of the Pw method, used for the assessment of the phosphate status of arable soils in the Netherlands. *Plant and Soil* 34 (1): 483-486.
- Snyder, C. S., T. W. Bruulsema, T. L. Jensen, and P. E. Fixen. 2009. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture*,

References

- Ecosystems & Environment* 133 (3): 247-266.
<http://dx.doi.org/https://doi.org/10.1016/j.agee.2009.04.021>.
- Stamps, J. 2008. "Habitat." In *Encyclopedia of Ecology*, edited by Sven Erik Jørgensen and Brian D. Fath, 1807-1810. Oxford: Academic Press.
- Steel, H., and H. Ferris. 2016. Soil nematode assemblages indicate the potential for biological regulation of pest species. *Acta Oecologica* 73: 87-96.
<http://dx.doi.org/https://doi.org/10.1016/j.actao.2016.03.004>.
- Staniczenko, P. P. A., J. C. Kopp, and S. Allesina. 2013. The ghost of nestedness in ecological networks. *Nature Communications* 4 (1): 1391. <http://dx.doi.org/10.1038/ncomms2422>.
- Stone, D., P. Blomkvist, N. B. Hendriksen, M. Bonkowski, H. B. Jørgensen, F. Carvalho, M. B. Dunbar, et al. 2016. A method of establishing a transect for biodiversity and ecosystem function monitoring across Europe. *Applied Soil Ecology* 97: 3-11.
<http://dx.doi.org/https://doi.org/10.1016/j.apsoil.2015.06.017>.
- Strona, G., P. Galli, D. Seveso, S. Montano, and S. Fattorini. 2014. Nestedness for Dummies (NeD): a user-friendly web interface for exploratory nestedness analysis. *Journal of Statistical Software* 59 (1): 1-9.
- Stubbs, T. L., A. C. Kennedy, and W. F. Schillinger. 2004. Soil ecosystem changes during the transition to no-till cropping. *Journal of crop improvement* 11 (1-2): 105-135.
- Sun, X., X. Zhang, S. Zhang, G. Dai, S. Han, and W. Liang. 2013. Soil nematode responses to increases in nitrogen deposition and precipitation in a temperate forest. *PloS one* 8 (12): e82468-e82468. <http://dx.doi.org/10.1371/journal.pone.0082468>.
- Ter Braak, C. J. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67 (5): 1167-1179.
- Tilman, D., C. Balzer, J. Hill, and B. L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences* 108 (50): 20260-20264. <http://dx.doi.org/10.1073/pnas.1116437108>.
- Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, et al. 2001. Forecasting Agriculturally Driven Global Environmental Change. *Science* 292 (5515): 281-284. <http://dx.doi.org/10.1126/science.1057544>.
- Timper, P., R. Davis, G. Jagdale, and J. Herbert. 2012. Resiliency of a nematode community and suppressive service to tillage and nematicide application. *Applied Soil Ecology* 59: 48-59.
- Trajanov, A., H. Spiegel, M. Debeljak, and T. Sandén. 2019. Using data mining techniques to model primary productivity from international long-term ecological research (ILTER) agricultural experiments in Austria. *Regional Environmental Change* 19 (2): 325-337.
<http://dx.doi.org/10.1007/s10113-018-1361-3>.

- Treseder, K. K. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology letters* 11 (10): 1111-1120.
- Tsiafouli, M. A., E. Thébault, S. P. Sgardelis, P. C. De Ruiter, W. H. Van Der Putten, K. Birkhofer, L. Hemerik, et al. 2015. Intensive agriculture reduces soil biodiversity across Europe. *Global change biology* 21 (2): 973-985.
- Tuck, S. L., C. Winqvist, F. Mota, J. Ahnström, L. A. Turnbull, and J. Bengtsson. 2014. Land-use intensity and the effects of organic farming on biodiversity: A hierarchical meta-analysis. *Journal of Applied Ecology* 51 (3): 746-755. <http://dx.doi.org/10.1111/1365-2664.12219>.
- Turbé, A., A. De Toni, P. Benito, P. Lavelle, P. Lavelle, N. R. Camacho, W. H. Van Der Putten, E. Labouze, and S. Mudgal. 2010. Soil biodiversity: functions, threats and tools for policy makers.
- Ugarte, C. M., E. R. Zaborski, and M. M. Wander. 2013. Nematode indicators as integrative measures of soil condition in organic cropping systems. *Soil Biology and Biochemistry* 64: 103-113. <http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2013.03.035>.
- van Capelle, C., S. Schrader, and J. Brunotte. 2012. Tillage-induced changes in the functional diversity of soil biota – A review with a focus on German data. *European Journal of Soil Biology* 50: 165-181. <http://dx.doi.org/10.1016/j.ejsobi.2012.02.005>.
- Van de Broek, M., C. B. Henriksen, G. B. Bhim, E. Lugato, V. Kuzmanovski, A. Trajanov, M. Debeljak, et al. 2019. Assessing the climate regulation potential of agricultural soils using a decision support tool adapted to stakeholders' needs and possibilities. *Frontiers in Environmental Science* 7: 131.
- van der Sluis, T., B. Pedroli, S. B. P. Kristensen, G. Lavinia Cosor, and E. Pavlis. 2016. Changing land use intensity in Europe – Recent processes in selected case studies. *Land Use Policy* 57: 777-785. <http://dx.doi.org/https://doi.org/10.1016/j.landusepol.2014.12.005>.
- Van Der Wurff, A. W. G., S. A. E. Kools, M. E. Y. Boivin, P. J. Van Den Brink, H. H. M. Van Megen, J. A. G. Riksen, A. Doroszuk, and J. E. Kammenga. 2007. Type of disturbance and ecological history determine structural stability. *Ecological Applications* 17 (1): 190-202. [http://dx.doi.org/10.1890/1051-0761\(2007\)017\[0190:todaych\]2.0.co;2](http://dx.doi.org/10.1890/1051-0761(2007)017[0190:todaych]2.0.co;2).
- van Dijk, J., W. A. M. Didden, F. Kuenen, P. M. van Bodegom, H. A. Verhoef, and R. Aerts. 2009. Can differences in soil community composition after peat meadow restoration lead to different decomposition and mineralization rates? *Soil Biology and Biochemistry* 41 (8): 1717-1725. <http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2009.05.016>.
- van Eekeren, N., L. Bommelé, J. Bloem, T. Schouten, M. Rutgers, R. de Goede, D. Reheul, and L. Brussaard. 2008. Soil biological quality after 36 years of ley-arable cropping, permanent grassland and permanent arable cropping. *applied soil ecology* 40 (3): 432-446.

References

- van Leeuwen, J. P., R. E. Creamer, D. Cluzeau, M. Debeljak, F. Gatti, C. B. Henriksen, V. Kuzmanovski, et al. 2019. Modeling of Soil Functions for Assessing Soil Quality: Soil Biodiversity and Habitat Provisioning. *Frontiers in Environmental Science* 7 (113). <http://dx.doi.org/10.3389/fenvs.2019.00113>.
- Van Vliet, P., L. West, P. Hendrix, and D. Coleman. 1993. "The influence of Enchytraeidae (Oligochaeta) on the soil porosity of small microcosms." In *Soil Structure/Soil Biota Interrelationships*, 287-299. Elsevier.
- Vazquez, C., R. G. de Goede, G. W. Korthals, M. Rutgers, A. J. Schouten, and R. Creamer. 2019. The effects of increasing land use intensity on soil nematodes: A turn towards specialism. *Functional Ecology* 33 (10): 2003-2016.
- Venables, W. N., and B. D. Ripley. 2002. *Modern Applied Statistics with S*. Fourth ed. New York: Springer.
- Venail, P. A., R. C. MacLean, T. Bouvier, M. A. Brockhurst, M. E. Hochberg, and N. Mouquet. 2008. Diversity and productivity peak at intermediate dispersal rate in evolving metacommunities. *Nature* 452 (7184): 210.
- Wagg, C., S. F. Bender, F. Widmer, and M. G. van der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences* 111 (14): 5266-5270.
- Wardle, D. A., and K. E. Giller. 1996. The quest for a contemporary ecological dimension to soil biology. *Soil Biology & Biochemistry* 28 (12): 1549-1554.
- Yeates, G. W., T. d. Bongers, R. De Goede, D. Freckman, and S. Georgieva. 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of nematology* 25 (3): 315.
- Yeates, G. W., V. A. Orchard, T. W. Speir, J. L. Hunt, and M. C. C. Hermans. 1994. Impact of pasture contamination by copper, chromium, arsenic timber preservative on soil biological activity. *Biology and Fertility of soils* 18: 200-208.
- Zelený, D. 2009. Co-occurrence based assessment of species habitat specialization is affected by the size of species pool: reply to Fridley et al. (2007). *Journal of Ecology* 97 (1): 10-17.
- Zhang, P., B. Li, J. Wu, and S. Hu. 2019. Invasive plants differentially affect soil biota through litter and rhizosphere pathways: a meta-analysis. *Ecology Letters* 22 (1): 200-210. <http://dx.doi.org/10.1111/ele.13181>.
- Zhao, J., J. Xiao, W. Zhang, Z. Fu, M. Zhang, T. Liu, Q. Tan, and K. Wang. 2019. A method for estimating nematode body lengths for use in the calculation of biomass via a simplified formula. *Soil Biology and Biochemistry*.

- Zhong, S., H.-c. Zeng, and Z.-q. Jin. 2017. Influences of different tillage and residue management systems on soil nematode community composition and diversity in the tropics. *Soil Biology and Biochemistry* 107: 234-243.
- Zsolnay, A., and H. Görlitz. 1994. Water extractable organic matter in arable soils: Effects of drought and long-term fertilization. *Soil Biology and Biochemistry* 26 (9): 1257-1261. [http://dx.doi.org/https://doi.org/10.1016/0038-0717\(94\)90151-1](http://dx.doi.org/https://doi.org/10.1016/0038-0717(94)90151-1).
- Zuur, A., E. Ieno, N. Walker, A. Saveliev, and G. Smith. 2009. *Mixed effects models and extensions in ecology with R*. Edited by M. Gail, K. Krickeberg, JM Samet, A. Tsiatis and W Wong. Statistics for Biology and Health. New York, NY: Springer Science and Business Media.
- Zuur, A. F., and E. N. Ieno. 2016. A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution* 7 (6): 636-645.
- Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution* 1 (1): 3-14.
- Zwetsloot, M. J., J. van Leeuwen, L. Hemerik, H. Martens, I. Simó Josa, M. Van de Broek, M. Debeljak, et al. 2020. Soil multifunctionality: Synergies and trade-offs across European climatic zones and land uses. *European Journal of Soil Science* n/a (n/a). <http://dx.doi.org/10.1111/ejss.13051>.

Summary

Soils are key to the delivery of ecosystem services such as primary productivity, water regulation and purification, nutrient provision and recycling, climate regulation and carbon sequestration or biodiversity and habitat provision. However, until recently, the concept and measurement of soil quality was focused mostly on the delivery of primary productivity, and most assessments of soil quality neglected the delivery of other soil-based ecosystem services (or soil functions). Soils can deliver several functions simultaneously, but which functions are delivered or have the potential to be delivered depends on soil properties, environmental conditions and associated soil management practices.

The processes that determine the delivery of one soil function can play a role in the delivery of other functions leading to the existence of synergies and trade-offs between soil functions that we do not yet fully understand, due in part to the difficulty of measuring several soil functions simultaneously. For example, high primary productivity might be a result of external agricultural inputs (e.g. fertilizers and pesticides), intensive crop rotations and/or management practices that are disruptive to the soil system (e.g. tillage, heavy machinery), but these management techniques have direct negative impacts on climate regulation, due to associated emissions of greenhouse gasses, and biodiversity and habitat provision due to acidification of the soil that renders the soil uninhabitable for some organisms. In order to sustainably meet agronomic objectives, it is important that we understand the trade-offs and synergies that occur between soil functions, as well as what management decisions and environmental conditions promote multifunctionality.

Soil biota play an important role in the delivery of soil functions. Despite their role in the delivery of ecosystem services, the intensification of agricultural land is one of the largest threats to soil biodiversity in Europe. The impacts on soil biota can take many forms. Sometimes (for example in the addition of manure) agricultural impacts lead to a flush of growth of specific organisms (bacteria and bacterial feeding organisms) other times the impact will lead to the exclusion of an organism from the ecosystem (either by causing its death (pesticides) or by changing the environmental conditions (reduced porosity or changes in pH). In fact, by tracking the soil biota, scientists can derive a lot of information about the type of disturbances and limitations that are being imposed on the soil ecosystem. The loss of specialist species as a consequence of land use intensity has been documented for many above ground organisms. The loss of specialists is a problematic issue, since it leads to the loss of functional diversity, which could (in the soils) translate to a change in soil functions. And yet, we do not know

whether the impacts associated with agricultural practices have a larger effect on soil specialists than generalists, amongst others because the quantification of niche width in soil organisms is very complex.

The main objective of this thesis was to study the effects of land use on different aspects of the soil system, particularly the effects on soil biodiversity. More specifically, I calculated the habitat niche width of nematodes (often used as indicators of different aspects of the soil status) and enchytraeids and classified them from specialists to generalists (i), studied the effect of land use intensity on the community weighted specialisation of soil organisms (ii), studied the effects of long-term agricultural practices on biological soil quality (iii), and studied the multifunctionality of agricultural soils, and the synergies and trade-offs that occur between biodiversity, primary productivity and nutrient cycling (iv). The thesis contains a general introduction (Chapter 1), four research chapters (Chapters 2-5) and a general discussion (Chapter 6).

In **Chapter 2** I calculated the realised niche width for soil nematodes using co-occurrence data mostly collected by the Netherlands Institute for Health and the Environment (RIVM) and I compared the resulting niche widths with different ecological traits, as an effort to better understand the drivers of realised niche width in soil nematodes. I then calculated the nematode taxon richness, diversity and abundance as well as an index of community specialisation (I_{CS}) and explored the differences in these indicators due to land use intensity. The resulting realised niche widths did not correlate with ecological traits such as feeding group, body mass or c-p class. Contrary to our expectations, the lowest community specialisation levels were found in soils with the lowest human intervention (shrubland-woodland ecosystems), while grasslands, dairy farms, and arable farms had an overall higher level of specialisation. I found highest richness and diversity at intermediate levels of disturbance (grasslands and dairy farms). The lowest abundances were found on shrubland-woodland systems. Assessing niche widths via co-occurrence matrices opens the door to estimating the soil community's niche breadth, for which resource-based methods are difficult to implement.

In **Chapter 3** I classified enchytraeid species according to their degree of habitat specialisation using co-occurrence matrices, and tested the robustness of the calculated niche widths to scenarios designed to tackle issues of under-sampling, biased sampling campaigns and the legacy-effect. To do so I used data gathered from 81 sites across Europe in three land use types of increasing land use intensity (forestry, grassland and arable land). I then calculated the enchytraeid I_{CS} for each site and tested whether increasing land use intensity led to changes in the I_{CS} . *Cognettia sphagnetorum* was the most specialised enchytraeid species and *Enchytronia parva* the least. Tolerance to pH

might explain at least the position of these two extreme species in the generalist to specialist scale. The resulting niche width ranks were robust to scenarios simulating under-sampling, but the tests underlined the importance of using the same sampling effort in all sites when calculating niche width from co-occurrence methods. I found no evidence to support the hypothesis that land use intensity had an effect on the level of community specialisation of enchytraeids. This chapter revealed how much there is still to understand regarding the niche width and community level specialisation of soil organisms.

In **Chapter 4** I investigated how and whether biological soil quality (as measured by the nematode community) has developed over time in cropping systems situated on two contrasting soil textures and under different management regimes in The Netherlands, using time-series analyses. I gathered data from two experiments, the *Soil Health Experiment* in Vredepeel (SHE) and the *Broekemahoeve Applied Soil Innovation Systems* experiment in Lelystad (BASIS) that have been sampled on several occasions, first in 2007 and 2009 respectively. Additionally, I explored the changes in biological soil quality in 20 commercial farms sampled first in 2001 and at least two times thereafter (the last sampling event was in 2018). I found changes in the nematode community through time in all three systems, but these were not consistent across the systems or treatments. In fact, while there were significant trends in time, very few were affected by land management (organic or conventional) or by treatment (conventional tillage or minimum tillage). In both experimental sites I observed an increase in nematode richness, and in SHE I also observed an increase in the structure and enrichment indices over time. This trend was not matched by commercial farms, where we found an overall decrease in the nematode structure and an increase in the I_{CS} , indicating a widespread decrease in the soil's food-web structure possibly associated with an increase in land use intensity. We observed that the nematode community continues to change over time, but whether the observed trend reflects an increase of the intensity of land management in time or that the effects of sustained agricultural land management continue to alter the nematode community over time remains uncertain.

In **Chapter 5** I evaluated the supply of primary productivity (PP), nutrient cycling (NC) and biodiversity and habitat provision (B-HP) of 31 grasslands and 21 croplands in the Netherlands using multi-criteria decision models developed by the EU Horizon 2020 Landmark project. To fulfil the data requirements of these models, I used data gathered by the RIVM and Wageningen Economic Research on soil parameters and economic performance respectively. The multi-criteria decision models showed that 38% of the farms had a medium to high supply of all three soil functions, whereas only one cropland had a high supply for all three. Forty-eight per cent of the farms were

characterized by a high supply of PP and NC. We observed a clear trade-off between these two functions and B-HP. Multivariate statistical analyses indicated that higher organic inputs combined with a lower mineral fertilization concur with higher biodiversity scores while maintaining a medium delivery of PP and NC. This chapter highlights the need for systematic collection of management-related data for the assessment of soil functions. Multifunctionality can be achieved in agricultural soils; however, without specifically managing for it, biodiversity might come at a loss.

Chapter 6 addresses the main findings of my thesis and provides a discussion regarding several aspects of my work, including the issue of multifunctionality, the effects of agricultural impacts on soil biodiversity, the role of the I_{CS} as a biological indicator as well as the niche width of soil organisms as quantified using co-occurrence matrices. I also provide suggestions for future study regarding these issues that include (but are not limited to) the refinement of the models used for the calculation of soil functions to be better suited to national measurements, a systematic review of the effects of land use on indicators of soil quality to aid in the interpretation of surveys of multifunctionality, the calculation of the I_{CS} using data from controlled experiments where diverse impacts are explored and lastly the role of dataset selection in the calculation of the realised niche widths of soil biota.

Samenvatting

De bodem is essentieel voor de levering van ecosysteemdiensten, zoals primaire productie, regulering en zuivering van water, nutriëntenvoorziening en -recycling, klimaatregulatie en het vastleggen van koolstof, of biodiversiteit- en habitatsvoorziening. Echter, wanneer het aankomt op het definiëren en meten van bodemkwaliteit, lag de focus tot voor kort voornamelijk op het leveren van primaire productie en werd de levering van andere ecosysteemdiensten (of bodemfuncties) genegeerd. Bodems zijn in staat om tegelijkertijd verschillende functies te leveren, maar welke (potentiële) functies een bodem kan leveren hangt af van de bodemeigenschappen, de omstandigheden van het milieu en bijbehorend bodembeheer.

De processen die de levering van één bodemfunctie bepalen, kunnen ook een rol spelen in de levering van andere functies. Dit kan leiden tot de vorming van synergiën en compromissen tussen verschillende bodemfuncties, welke we tot op heden nog niet volledig doorgronden. Dit komt deels doordat het tegelijkertijd meten van verschillende bodemfuncties veel uitdagingen met zich meebrengt.

Hoge primaire productie kan bijvoorbeeld een resultaat zijn van externe invoer vanuit de landbouw (bv. bemesting en pesticiden), intensieve gewasrotatie of beheerbeleid dat het bodemsysteem schaadt (bv. grondbewerking of zware machines). Deze beheertechnieken hebben echter een direct negatief effect op de klimaatregulatie, omdat deze technieken resulteren in de uitstoot van broeikasgassen. Ook hebben deze praktijken een negatief effect op de biodiversiteit en habitatsvoorziening van de bodem, omdat de grond hierdoor verzuurt, wat weer resulteert in een onbewoonbare bodem voor sommige organismen.

Wanneer we onze agronomische doelen op een duurzame manier willen bereiken, moeten we de compromissen en synergiën, die spelen tussen bodemfuncties, begrijpen, alsmede welk managementbeleid en welke milieuomstandigheden multifunctionaliteit bevorderen.

Het bodemleven speelt een belangrijke rol in het leveren van ecosysteemdiensten. Desondanks is de intensivering van de landbouw één van de grootste bedreigingen voor de diversiteit van het Europese bodemleven. Deze bedreigingen hebben op verschillende wijze impact op de bodemleven.

De bemesting van landbouwgrond kan soms leiden tot een groeispuurt van een specifiek organisme, denk bijvoorbeeld aan bacteriën of organismen die zich voeden met bacteriën. Op andere momenten kan een impact resulteren in het verdwijnen van een organisme uit het ecosysteem. Dit kan zijn door sterfte (wanneer pesticiden worden

toegevoegd) of doordat de omstandigheden in het milieu veranderen, denk bijvoorbeeld aan verminderde porositeit of veranderde pH-waardes.

Door het volgen van het bodemleven, kunnen wetenschappers in werkelijkheid veel informatie inwinnen over het type verstoring en de beperkingen die worden opgelegd aan een ecosysteem.

Het intensiveren van de landbouw heeft als consequentie dat bovengronds veel specialistische soorten verdwijnen. Dit is problematisch, omdat het in veel gevallen leidt tot het verlies van functionele diversiteit. In de bodem kan dit zich vertalen naar een verschuiving van de geleverde bodemfunctie. We weten echter niet of de impacts die landbouwpraktijken met zich meebrengen, in de bodem groter zijn voor specialistische of generalistische soorten. Dit komt onder andere doordat het erg moeilijk is om een correcte kwantificatie te maken voor de breedte van de niche van bodemorganismen.

Dit proefschrift focust voornamelijk op het bestuderen van de effecten van landgebruik op verschillende aspecten van het bodemsysteem, in het bijzonder het effect op de biodiversiteit in de bodem. Hiervoor berekende ik de habitat-nichebreedte van nematoden, die vaak gebruikt worden als indicator voor verschillende aspecten van de bodemgesteldheid, en potwormen. Deze heb ik geclassificeerd, van specialist tot generalist(i), om vervolgens het effect van intensief landgebruik op het gewogen gemiddelde specialisme van een gemeenschap van bodemorganismen te bestuderen(ii), het langetermijneffect van agricultuur op de biologische bodemkwaliteit (iii), de multifunctionaliteit van landbouwbodems en de synergiën en compromissen die voorkomen tussen biodiversiteit, primaire productie en de nutriëntenkringloop (iv) te bestuderen. Deze thesis omvat een algemene introductie (Hoofdstuk 1), vier wetenschappelijke hoofdstukken (Hoofdstuk 2-5) en een algemene discussie (Hoofdstuk 6).

In **Hoofdstuk 2** berekende ik de werkelijke niche-wijdte voor in de bodem levende nematoden. Hiervoor gebruikte ik gegevens over samenlevende soorten, voornamelijk verzameld door het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) en vergeleek de nichebreedte met verschillende ecologische kenmerken, in een poging om de verschillende drijfveren van de werkelijke nichebreedte voor in de bodem levende nematoden te begrijpen. Hierna berekende ik de soortenrijkdom, diversiteit en abundantie, en ook een index voor het gemeenschappelijke specialisme (I_{cs}) van nematoden en verkende de verschillen in deze indicatoren, als gevolg van de intensiteit van landgebruik. De hieruit volgende werkelijke nichebreedte bleek niet te correleren met ecologische kenmerken zoals voedingsklasse, lichaamsgewicht of c-p klasse. Tegen onze verwachtingen in, bleken we de laagste waarden van gemeenschappelijk specialisme te vinden in bodems die weinig menselijke invloed kennen (struweel- en

bossige ecosystemen), terwijl in graslanden, melkveehouderijen en agrarische bodems een gemiddeld hoger gemeenschappelijk specialisme gevonden werd. Ik vond de hoogste soortenrijkdom en -diversiteit in de gemiddelde klasse van verstoring (graslanden en melkveehouderijen). De laagste abundantie werd gevonden in struweel- en bossige systemen. Het wegen/beoordelen van de nichebreedte via samenlevingsmatrices geeft kansen om de werkelijke niche-wijdte van een complexe bodemgemeenschap te schatten, waar de implementatie van resultaten uit experimenteel onderzoek naar verschillende parameters heel veel uitdaging geeft.

In **Hoofdstuk 3** heb ik potwormen geclassificeerd middels hun niveau van habitat-specialisme, waarvoor ik samenlevings-matrices gebruikte. Ook testte ik hoe robuust de berekende nichebreedte is in verschillende scenario's, speciaal ontworpen om problemen bij onderbemonstering, eenzijdige bemonsteringprotocollen en het erfenis-effect aan het licht te brengen. Hiervoor heb ik data gebruikt van 81 verschillende plekken, verdeeld over Europa, in drie categorieën van landgebruik, oplopend in intensiteit (Bosgebied, grasland en bouwland). Hierna berekende ik voor elke plek de I_{cs} van potwormen en testte ik of de I_{cs} verandert bij oplopende intensiteit van landgebruik. Hieruit bleek, dat *Cognettia sphagnetorum* de meest specialistische soort was, en *Enchytronia parva* de minst specialistische. De positie van deze soorten op de schaal van generalist tot specialist kan wellicht verklaard worden door hun pH-tolerantie. In het scenario met onderbemonstering was de geobserveerde nichebreedte robuust, maar de tests benadrukten wel het belang van het gebruik van gelijkwaardige bemonstering-efforts, wanneer de nichebreedte wordt berekend met samenlevings-gegevens. Ik heb geen bewijs gevonden die ondersteunend is aan de hypothese dat de intensiteit van landgebruik een effect heeft op het niveau van het gemeenschappelijke specialisme van potwormen. Dit hoofdstuk laat zien dat er nog veel inzicht te winnen is op het gebied van niche-breedte en specialisme op het gemeenschapsniveau van bodemorganismen.

In **Hoofdstuk 4** onderzocht ik hoe, en of, biologische bodemkwaliteit (gemeten door analyse van de nematodengemeenschap) zich gaandeweg heeft ontwikkeld in gewassystemen, gelegen op twee contrasterende bodemtexturen en onder verschillende managementregimes in Nederland. Hiervoor analyseerde ik het effect van het tijdsverloop. Ik verzamelde data van twee experimenten, het *Soil Health Experiment* in Vredepeel (SHE) en het *Broekemahoeve Applied Soil Innovation Systems* experiment (BASIS), waar op verschillende tijdstippen bemonsterd is, beginnend in 2007 en 2009. Ook onderzocht ik de veranderingen in de biologische bodemkwaliteit in 20 commerciële boerderijen, die voor het eerst bemonsterd werden in 2001 en vervolgens ten minste nog twee maal. De laatste bemonsteringen vonden plaats in 2018. Ik ontdekte, door de tijd heen, wijzigingen in de nematodengemeenschap in alle drie

systemen, maar deze veranderingen waren niet consistent in de systemen of de verschillende behandelingen. Het landbeheer (biologisch of conventioneel) en de grondbehandeling (conventionele of minimale grondbewerking) bleek vrijwel nooit de oorzaak van de geobserveerde trends over tijd. Op beide experimentele locaties observeerde ik een toename van de soortenrijkdom van nematoden, en op de locatie SHE zag ik ook een toename in de structuur- en verrijkingsindicatoren over tijd. Deze trend werd niet waargenomen in commerciële boerderijen, waar ik een algemene afname zag in de nematodenstructuur en een toename in de I_{cs} , wat duidt op een wijdverspreide afname van de structuur in het voedselweb, wat mogelijk kan worden geassocieerd met een toename van de intensiteit van grondbewerking. We observeerden dat een nematodengemeenschap door de tijd verandert, maar of de waargenomen trend de reflectie is van een toename in landbeheerintensiteit door de tijd heen, of het effect van een stabiel agrarisch landbeheer, blijft onzeker.

In **Hoofdstuk 5** evalueerde ik het aanbod van primaire productie (PP), nutriëntenvoorziening en -recycling (NC) en biodiversiteit en habitatsvoorziening (B-HP) van 31 graslanden en 21 akkerbouwlanden in Nederland. Hiervoor gebruikte ik multi-criteria beslissingsmodellen, ontwikkeld door de EU Horizon 2020 Landmark project. Deze modellen eisen veel data, en om aan deze vereisten te voldoen heb ik gegevens gebruikt over bodemparameters en economisch resultaten van boerderijen, verzameld door het RIVM en Wageningen Economic Research. Deze multi-criteria beslissingsmodellen lieten zien dat 38% van alle boerderijen een midden tot hoog aanbod van alle drie bodemfuncties had, waar enkel één akkerbouwland hoog scoorde in alle drie de bodemfuncties. Achtenveertig procent van de boerderijen werd gekenmerkt door een hoog aanbod in PP en NC. We zagen een duidelijke compromis tussen deze twee bodemfuncties en B-HP. Multi-variate statistische analyses duiden erop dat een hogere organische input, gecombineerd met een lagere kunstbemesting samengaan met een hogere B-HP score en tegelijkertijd een medium-hoge score behielden voor PP en NC. Dit hoofdstuk belicht de noodzaak van het systematisch verzamelen van management gerelateerde gegevens ter beoordeling van bodemfuncties. Multifunctionaliteit kan bereikt worden in landbouwgrond, echter, wanneer er hiervoor geen specifiek beheer wordt ingevoerd, kan biodiversiteit verloren gaan.

Hoofdstuk 6 behandelt de belangrijkste bevindingen van mijn proefschrift en stelt verschillende aspecten van mijn werk ter discussie, zoals het onderwerp multifunctionaliteit, de impact die agricultuur heeft op de biodiversiteit van de bodem, de rol die de I_{cs} speelt als biologische indicator alsmede de nichebreedte van bodemorganismen wanneer deze wordt gekwantificeerd door het gebruik van

samenlevingsmatrices. Ook geef ik suggesties omtrent toekomstige studies rondom deze kwesties. Deze bevatten, maar zijn niet beperkt tot, het verfijnen van modellen die we gebruiken voor het berekenen van bodemfuncties, zodat deze beter geschikt zijn voor metingen op nationaal niveau, een systematische beoordeling van de effecten van landgebruik op indicatoren voor bodemkwaliteit, om bij te dragen aan de interpretatie van onderzoeken naar multifunctionaliteit, het berekenen van de Ics, gebruikmakend van gegevens uit gecontroleerde experimenten waarin verscheidene effecten worden verkend en afsluitend de rol van de geselecteerde dataset in het berekenen van de reële niche-breedte van bodembiota.

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About the author

Carmen Vázquez Martín was born on the 26th of August of 1986 in Madrid, Spain. She grew up in a small town close to the mountains. Here she started enjoying being surrounded by nature, a feeling that has grown since and that led Carmen to study Environmental Sciences at the Universidad Autónoma de Madrid. Here she discovered a new found love for ecology, which led her to join the ERASMUS program at the University of South Eastern Norway specialising on alpine ecology.



The time in Norway only strengthened her interest in ecology, conservation, and sustainability, which lead her to pursue a master's degree in Forest and Nature conservation at Wageningen University, with a specialisation in Ecology. Her thesis work on the prevalence of the bimodal activity pattern on wild animals awoke a new interest: the development of statistical tools to aid in the analysis and understanding of ecological data. In 2015 Carmen started a PhD with the department of Soil Quality (now the department of Soil Biology) in Wageningen University, evaluating the effects of different management strategies on biological soil quality. She defended her PhD in June of 2021. Currently she is working as a postdoctoral researcher in the department of Soil Biology in Wageningen. In this new position she combines her passion for education and science by assisting with education tasks on several courses and exploring the issue of scale in assessing multifunctionality in Dutch soils.

Peer reviewed publications

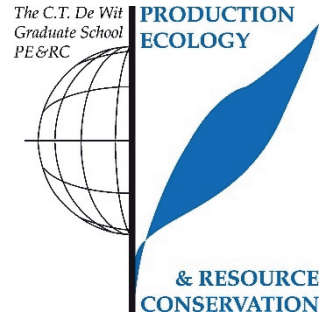
Vazquez, C, de Goede, RGM, Rutgers, M, de Koeijer, TJ, Creamer, RE. Assessing multifunctionality of agricultural soils: Reducing the biodiversity trade-off. *Eur J Soil Sci.* 2020; 1– 16. <https://doi.org/10.1111/ejss.13019>

Vazquez, C, de Goede, RGM, Korthals, GW, Rutgers, M, Schouten, AJ, Creamer, R. The effects of increasing land use intensity on soil nematodes: A turn towards specialism. *Funct Ecol.* 2019; 33: 2003– 2016. <https://doi.org/10.1111/1365-2435.13417>

Vazquez, C, Rowcliffe, JM, Spoelstra, K, Jansen, PA. Comparing diel activity patterns of wildlife across latitudes and seasons: Time transformations using day length. *Methods Ecol Evol.* 2019; 10: 2057– 2066. <https://doi.org/10.1111/2041-210X.13290>

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)

- Potential of Evenness and Richness as Bioindicators of soil quality

Post-graduate courses (5.7 ECTS)

- Soil ecology and the planetary boundaries; PE&RC (2016)
- The role of soil fauna on soil organic matter dynamics; Centre for Functional Ecology (2016)
- Multivariate analysis; PE&RC (2016)
- Zero inflated models; PE&RC (2018)

Invited review of (unpublished) journal manuscripts (2 ECTS)

- Nematology: nematode communities in oasis (2018)
- Soil biology and biochemistry: nematode and crop rotations (2018)

Deficiency, refresh, brush-up courses (4.6 ECTS)

Data science Capstone project; John Hopkins University (2016)

Machine learning; John Hopkins University (2017)

Competence strengthening / skills courses (1.9 ECTS)

- Information literacy PhD including Endnote introduction; Wageningen UR Library (2016)
- Lecturing; Wageningen UR (2019)

Scientific integrity / ethics in science activity (0.3 ECTS)

- Data management and planning; Wageningen UR Library (2016)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC First years weekend (2015)
- PE&RC Day (2015)
- PE&RC Midterm weekend (2017)

Discussion groups / local seminars / other scientific meetings (5.25 ECTS)

- PE&RC Ecological theory and application (2016)
- Plant-soil-microbe interactions for crop and pest management (2016)
- Tipping points in pest management: what is the evidence? (2016)
- Symposium on nature conservation (2016)
- Current themes in ecology (2016)
- PE&RC Plant soil interactions (2016-2020)
- Frontiers in ecology: the soil-plant interphase (2017)
- PhD Symposium: convener (2017)
- Netherlands annual ecology meeting (2017)
- Landmark meeting (2018)
- Renew: restoration network Wageningen (2019)

International symposia, workshops and conferences (8 ECTS)

- Netherlands annual ecology meeting; the Netherlands (2016)
- 3rd International symposium on nematodes as environmental indicators; Ireland (2017)
- Netherlands annual ecology meeting; the Netherlands (2018)
- Wageningen soil conference; the Netherlands (2019)

Societally relevant exposure (0.15 ECTS)

- Werken met bodemleven: a workshop for the sustainable management of agricultural soils (2020)

Lecturing / supervision of practicals / tutorials (5.4 ECTS)

- Soil and landscape variability (2016-2018)
- Identification of terrestrial and freshwater nematodes for ecologists/environmentalists (2016-2019)
- Trending topics in soil water and chemistry (2017)

MSc thesis supervision (4 ECTS)

- The effects of drought and flooding on the nematode community
- Assessing multifunctionality in Dutch agricultural systems

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