Biodiversity of Ciliated Protozoa in Soil Ecosystems and Assessment of Their Potential as Bio-indicators of Soil Quality

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ABSTRACT

Biodiversity of Ciliated Protozoa in Soil Ecosystems and Assessment of Their Potential as Bio-indicators of Soil Quality

The wide range of goods and services provided by terrestrial ecosystems are dependent on soil function. There is an increasing requirement for policy to monitor soils to improve agricultural productivity and protect soil quality. Soil biota, including protists, have fundamental roles in the services provided by soils, and have been considered as potential effective indicators to assess soil quality. Ciliated protozoa are abundant phagotrophic microorganisms in soil and play important roles in food webs by controlling smaller microorganisms and recycling organic matter. They can also be affected by soil properties, presenting a potential for their use as bio-indicators of soil quality. In the present study, a comparison of the abundance and species richness of soil ciliates and soil properties in the natural and agricultural soils was carried out in order to establish the relationships between soil ciliates and soil properties, and to investigate the potential of soil ciliates as bio-indicators of soil quality. Both field and pot based experiments were used and a wide range of methods was employed to determine soil properties. The abundance and species richness of soil ciliates were determined after 4 days and 10 days of incubation using the non-flooded petri dish method. The results showed that there were significant differences in soil properties amongst the soils investigated and there was a significantly higher abundance of soil ciliates in the natural soils. The seasonal variation in soil ciliate abundance was also clearly seen in the natural habitat. However, there were no significant differences in the total species richness of soil ciliates amongst the three ecosystems investigated. Three potential new species of ciliates were recorded, and one of them is fully described here at morphological and molecular levels. Soil ciliate abundance and total species richness of soil ciliates in the three ecosystems together had positive correlations with soil moisture, soil organic matter, available concentrations of phosphorus, ammonium, the available concentrations of Cu, Ni and Zn, total microbial activity and activity of β-glycosidase, but a negative correlation between ciliate abundance and soil pH was found. No correlations between the abundance of soil ciliates and the available concentrations of K and S and activity of acid-phosphomonoesterase in the three ecosystems together were found. Interestingly, strong negative correlations between ciliate species richness and available concentrations of measured trace metals were shown in the natural soil only. Copper at the highest

treatment concentration (960 mg kg^{-1}) decreased significantly the total microbial activity, but stimulated the abundance of soil ciliates after 4 days of incubation due to increased numbers of two species, *Homalogastra setosa* and *Chilodonella uncinata*. Similarly, insecticide cypermethrin increased the abundance of soil ciliates, but *Homalogastra setosa* started to be limited at concentration of 320 mg kg^{-1}. This species was also affected by glyphosate concentrations of 16.82 and 33.6 mg kg^{-1}. The structure of the soil ciliate community changed at the highest concentrations of all pollutants i.e., Cu, cypermethrin and glyphosate at 15 days after application. Overall, this research shows that the abundance and species richness of soil ciliates had strong correlations with soil properties. Hence, they have been shown to be potential bio-indicators for the presence of nutrients, soil pH, soil texture, and contamination from trace metals and pesticides.

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List of abbreviations and Common terms

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
AZM	Adoral Zone of Membranelles
BC	Buccal cirrus
BI	Bayesian inference
BOD	Biochemical Oxygen Demand
$CaCl_2$	Calcium Chloride
CC	Caudal cirri
DK	Dorsal kinety
DMK	Dorso-marginal kinety
e	Endoral membrane
FC	Frontal cirri
FDA	Fluorescein diacetate
FVC	Fronto-ventral cirri
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
H_2O_2	Hydrogen Peroxide
ICP-OES	Inductively Coupled Plasma-Optimal Emission Spectrometry
KCl	Potassium Chloride
K_2HPO_4	Potassium Phosphate Dibasic
KNO_3	Potassium Nitrate
LD	Lethal Dose
LMC	Left marginal row of cirri
M	Median
Max	Maximum
Min	Minimum

$MgSO_4.7H_2O$	Magnesium Sulphate Heptahydrate
ML	Maximum likelihood
Modified universal buffer	is described by Tabatabai (1994)
MPN	Most Probable Number
NH_4^+	Ammonium
NH_3-N	Ammoniacal-Nitrogen
NO_3^--N	Nitrate-Nitrogen
NO_2^-	Nitrite
OD	Oven-Dry Weight
p	Paroral membrane
PCR	Polymerase Chain Reaction
*p*NP	*p*-nitrophenol
*p*NPG	*p*- nitrophenyl-β-glucoside
*p*NPP	*p*-nitrophenyl phosphate
PO_4^{2-}	Phosphate
PTVC	Pre-transverse ventral cirrus
PVC	Post-oral ventral cirrus
RMC	Right marginal row of cirri
rDNA	Ribosomal Deoxyribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SD	Standard deviation
SE	Standard Error
SO_4^{2-}	Sulphate
SOM	Soil Organic Matter
SES	Soil Extract added Salts
TAN	Total ammoniacal nitrogen
TC	Transverse cirri
TOC	Total Organic Carbon

Trace metal	Metallic and metalloid elements that typically occur in soil at concentrations less than 1000 mg kg^{-1}
VC	Ventral cirri
WHC	Water holding capacity
$\overline{x}$:	Arithmetic mean

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Declaration

I declare that the work in this thesis was made in accordance with the requirements of the University's Code of Practice for Research Degrees and that it has not been submitted for any other academic award. The work is the candidate's own. Work is done with the assistance of others as indicated.

Chapter 1. INTRODUCTION

1.1 Overview

The wide range of goods and services provided by terrestrial ecosystems are dependent on soil functions, thus soil quality plays an important role in agricultural production (Chee 2004). Degradation of soil quality due to the existence of contaminants in soil will affect the quantity and quality of agricultural products as well as the soils capacity to produce them (Baishya 2015). Consequently, there is an increasing requirement for monitoring and policies aimed towards improving agricultural productivity (Lal 2012). For example, zero net land degradation by 2030 is one of the main goals of the United Nations Conference of Sustainable Development, Rio+20 (Lal 2012). Soil biota, such as bacteria, protozoa and nematodes, have fundamental roles in the services provided by soils, and have been considered as potentially effective indicators of soil quality and may therefore be useful in monitoring systems (Karlen et al. 1997). Bio-indicators are very sensitive to natural and human disturbances, such as using pesticides and removal of topsoil (Foissner 1999b; Asif et al. 2018), making then useful in the monitoring of soil quality in agricultural ecosystems. In general, ciliated protozoa are single-celled and with high sensitivity to environmental conditions (Foissner 1994, 1999b). They respond quickly to both environmental changes and soil contamination and have rapid population growth (Foissner 1994, 1999b; Esteban and Finlay 2010). Hence, they are potential soil biotic indicators.

Ciliated protozoa are dikaryotic unicellular organisms. Micro-organisms of this group are easier to identify than many other eukaryotic microbes, with identification based on their special characteristics such as locomotion, infraciliature, and nuclei. The species richness and absolute abundance of protozoa in some soil habitats have been recorded, but many species remain undescribed (Foissner 1997a, 1997b; Foissner et al. 2002; Foissner 2016).

The role of ciliates as bio-indicators has been demonstrated in other environments, especially in activated-sludge sewage treatment works. Many ciliate species can be indicators of the status of the sludge, such as BOD (Biochemical Oxygen Demand), the lack of dissolved oxygen in aeration tank and sludge retention time (Madoni 1994; Lee et al. 2004; Madoni 2011). Ciliates have also been widely used as bio-indicators of water quality (Esteban et al. 1991; Madoni 1994; Lee et al. 2004; Madoni 2011).

Only a small amount of research on the community structure of soil ciliates and the relationship between them and some soil characteristics has been carried out (Ekelund and Rønn 1994; Li et al. 2010a; Li et al. 2010b). This indicated that the abundance of soil ciliates had a strong correlation with soil physical and chemical parameters. Furthermore, numerous studies on assessing the effects of contaminants, such as trace metals and pesticides, on protozoa in general and ciliates in particular have shown that they are useful bio-indicators in natural and agricultural ecosystems (Foissner 1994, 1997c; Díaz et al. 2006; Geisen et al. 2018). Despite this, soil protozoa are less studied than other soil organisms such as nematodes, bacteria or fungi, and many ciliate species remain undescribed (Geisen et al. 2018). Research on soil protistology also needs to connect to research on other soil organisms (Geisen et al. 2018).

Soil protists, i.e., amoebae, ciliates, and flagellates are also useful for plants, because they can interact with native bacteria to produce negative effects on plant diseases. They may stimulate antibiotic production through stimulating growth of plant-growth promoting bacterial species. Thus, protists will improve the ability of bacteria to supress plant diseases. This contributes to the main goals of sustainable agricultural development (Geisen et al. 2018).

Natural and agricultural soils are different in soil properties; for example pH, concentration of contaminants, and content of organic matter (Trivedi et al. 2016). In addition, natural ecosystems usually suffer from fewer disturbances than agricultural ecosystems, which are subject to pesticide use, ploughing, which leads to the burial of topsoil, and application of fertilisers, which increases soil trace metals. Consequently, how soil conditions impact on ciliate diversity in natural and agricultural soils and how soil ciliates might potentially be used as biological indicators of soil quality is an area in need of further research. This PhD research will investigate and compare the biodiversity of soil ciliates in natural and agricultural soils and determine if soil ciliates can be used as bio-indicators of soil quality to aid in its monitoring.

1.2 Literature review

1.2.1 Soil quality

1.2.1.1 Definition of soil quality

Soil quality has been defined as the capacity of a soil to function within ecosystem boundaries to sustain plant-animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen et al. 1997). Soil quality is

related to the sustainability of soil use and management, so the notion of soil quality must include soil productivity, soil fertility, soil degradation, and environmental quality (De La Rosa and Sobral 2008). While the definition of soil quality embraces physical, chemical and biological characteristics, soil health places more emphasis on biodiversity and ecological functions (Bruggen and Semenov 2000; Van Bruggen and Semenov 2000; Allen et al. 2011). In general, the terms “soil quality” and “soil health” are considered synonyms (Wolfe 2006), but De La Rosa and Sobral (2008) defined the integrated concept of soil quality, which includes inherent soil quality (or named soil suitability which may include soil texture, depth to bedrock, type of clay, cation-exchange capacity) and dynamic soil quality (or soil health) (Figure 1.1). Thus, in the framework of this project, the term soil quality will be used.

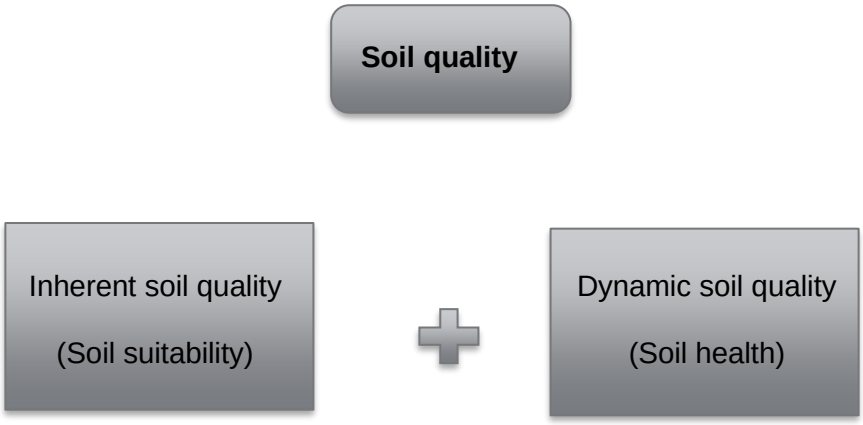


Figure 1.1 Graphic of soil quality concept integrating soil suitability and soil health (adapted from De La Rosa and Sobral 2008).

1.2.1.2 Physical, chemical, and biological indicators for soil quality

Soil systems can fulfil many different functions, in which the soil must provide basic functions, including: (i) physical, chemical and biological environments for living organisms; (ii) regulate and distribute water flow, storage and re-cycle nutrients and other elements; (iii) support biological activity and diversity for plant growth and animal productivity; (iv) filter, buffer, degrade, immobilize and detoxify organic and inorganic substances; (vi) provide mechanical support for living organisms and their structures (Nortcliff 2002). The valuation of soil quality in relation to these functions may be a combination of different forms of soil information which depends on different purposes of soil use, e.g. agricultural production, forest, nature conservation.

The most significant and widely accepted concept of soil quality at the global level is with respect to agricultural production (Nortcliff 2002). It is well-documented that sustainable agricultural production must be supported by conservative measures to ensure long-term maintenance of the activity (Nortcliff 2002). Thus, there is an increasing demand to assess soil quality in the development of sustainable agricultural production. However, standard methods of soil analysis need to be developed to meet the requirement (Nortcliff 2002). Bio-indicators may be used as an effective method in such assessment. An indicator is chosen to assess soil quality when analytic methods are developed fully (Nortcliff 2002) in order to describe or determine what that indicator signifies. Suitable soil quality indicators are simple and measurable attributes of soil used to assess quality with respect to given functions. Indicators can be physical, chemical and biological soil characteristics. It is necessary to choose appropriate attributes for the assessment. There is a large number of determined soil parameters which are divided into three broad groups and presented in Table 1 (De La Rosa and Sobral 2008).

According to Nortcliff (2002), the selection of soil attributes should be based on seven features: (i) purpose of land use; (ii) relationship between soil function and indicator; (iii) the ease and reliability of their measurement; (iv) spatial and temporal patterns of variation and the importance of this variation; (v) the sensitivity of the measurement to changes in soil management and use; (vi) comparability with routine sampling and monitoring programs; (vii) and the necessary skills for the use and interpretation of the indicator. The selection of any type of soil indicators depends on the nature of soil function under consideration, for examples Gómez et al. (1999) selected only six soil indicators to assess sustainability of agricultural production of an olive farm.

Table 1.1 Soil attributes used as indicators of soil quality (from De La Rosa and Sobral 2008).

Grouping type	Soil indicators
Physical attributes	Soil texture
	Stoniness
	Soil structure
	Bulk density
	Porosity
	Soil compaction
	Aggregate strength and stability
	Soil crusting
	Drainage
	Water retention
	Infiltration
	Hydraulic conductivity
	Topsoil depth
Chemical attributes	Color
	pH
	Salinity
	Carbonate content
	Sodium saturation
	Cation exchange capacity
	Plant nutrients
	Toxic elements
Biological attributes	Organic material content
	Fractions of organic materials
	Population of organisms
	Nematode communities
	Microbial biomass
	Respiration rate
	Mycorrhizal associations
	Enzyme activities
	Fatty acid profiles
	Bioavailability of pollutants

1. 2.2 Protozoa and the diversity of free living ciliated protozoa

1.2.2.1 Protozoa

Protozoa are phagotrophic, unicellular and eukaryotic organisms. Free-living protozoa can be divided into three main morphological-functional groups: Ciliates, Flagellates and Amoeboid (Finlay and Esteban 2013). Their small size, high abundance and ability to form cysts makes them easy to disperse; consequently, most free-living protozoa are cosmopolitan (Finlay 2002; Fenchel and Finlay 2004). They may occur in any aquatic or damp habitat, such as the oceans, snow and ice, fresh and brackish water, soils and even endolithic habitats in which free water is not present at all times (Agatha et al. 1990; Finlay et al. 2000; Pfister et al. 2002; Finlay and Esteban 2013). As with other eukaryotic organisms, most protozoan species have preferences for specific habitat types. Some major groups are only found in the sea, for example the foraminifera. Many species of ciliated protozoa only prevalently exist in marine (Esteban and Finlay 2004), or freshwater environments (Finlay and Esteban 1998; Finlay and Clarke 1999) or in soil habitats (Esteban et al. 2006). Generally, freshwater and marine habitats are the most common environments to find free-living protozoan species.

It is estimated that there are about 36,400 free-living protozoan species, but Mora et al. (2011) claim that the same number of species might be present in the world's oceans alone. If their biodiversity is compared to that of animals or plants, the number of species is relatively small because of their global ubiquity. Protozoa are also the most abundant phagotrophic microorganisms in the biosphere, capturing and ingesting food particles. Many species in both marine and fresh waters are capable of phagotrophy and phototrophy - the so-called mixotrophy, because they have endosymbiotic alga, chloroplasts or sequestered chloroplasts (Stoecker et al. 1987; Finlay et al. 1988; Stoecker et al. 1988; Esteban et al. 2010) that allow photosynthesis.

Protozoa play important roles in ecosystems as part of the microbial loop (Figure 1.2). Their functional role arises from their microscopic size which ranges from 20 µm to 200 µm, but some can reach several millimetres (Finlay and Esteban 2013). Their prey is usually smaller microorganisms and protozoa control bacterial abundances in freshwater, marine and soil environments by grazing actively on them (Fenchel 1982; Berninger et al. 1991; Finlay et al. 2000). Protozoa also stimulate microbial activity in oxic and anoxic environments (Finlay and Esteban 2013) by stimulating decomposition of organic matter, and by increasing the rate of

turnover of essential nutrients and carbon; hence, they help the increase of microbial biomass (Finlay et al. 1988; Finlay 1997; Esteban et al. 2006; Geisen et al. 2018). Protozoa are therefore crucial in the transfer of biomass within microbial food webs and beyond; the presence of protozoa in the rhizosphere have been shown to increase plant biomass by 30-80% (Bonkowski et al. 2000). In addition, protozoa are used as indicators of quality of water and soil (Esteban et al. 1991; Foissner 1994, 1997c; Lee et al. 2004; Madoni 2011).

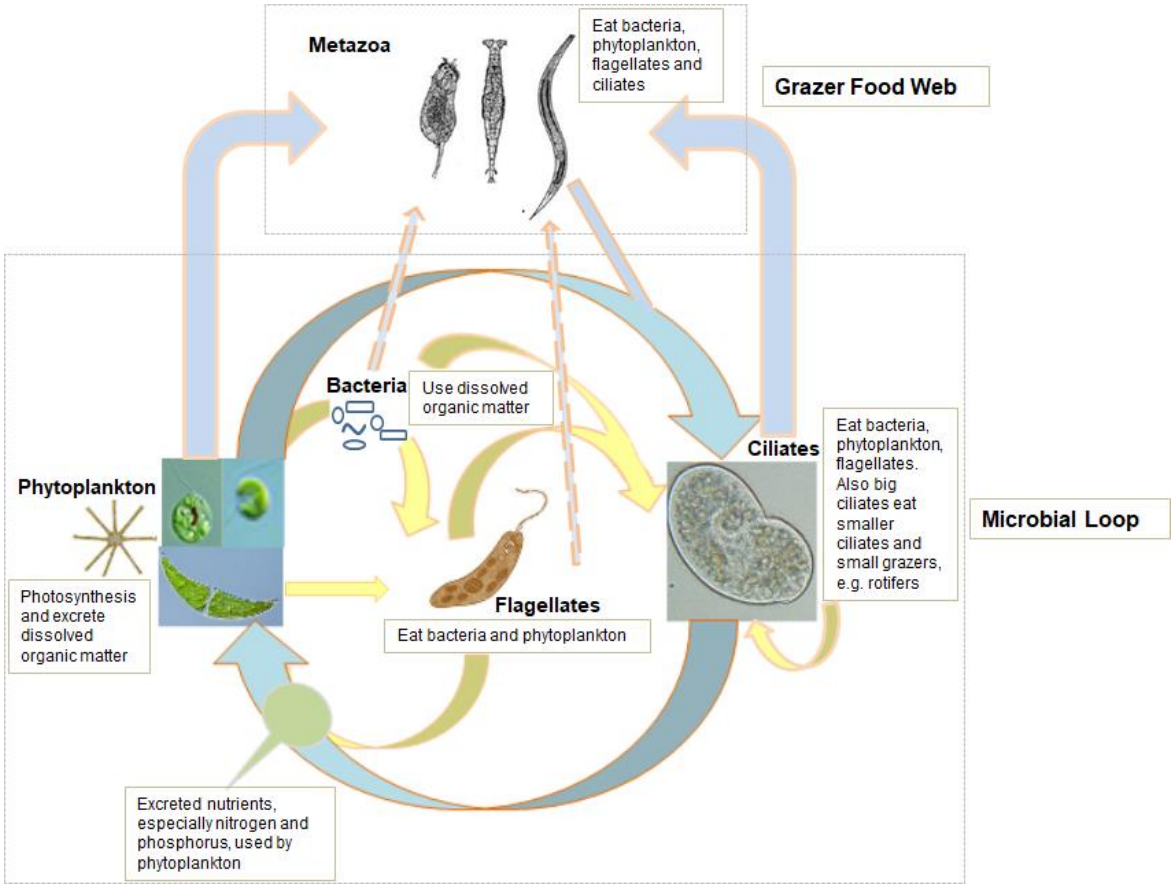


Figure 1.2 The microbial loop linked to the grazer food web (adapted from Finlay and Esteban 2013). Note: in soil, fungi (not shown here) might also be food for flagellates, ciliates and metazoans

1.2.2.2 Ciliated protozoa

Ciliated protozoa are a distinct group of protists which are characterised by some specific traits. Firstly, they are dikaryotic organisms. One nucleus is the micronucleus responsible for reproduction; the other nucleus is the macronucleus, which carries out the vegetative function. Secondly, they use cilia for locomotion and feeding which derived from kinetosomes with three fibrillar associates (microtubules, microfibrils and external ciliature). In addition, ciliates have the ability to reproduce asexually, and sexually by conjugation; the latter allows genetic

material to be exchanged between two cells (Lee et al. 1985; Lynn and Corliss 1991; Finlay and Esteban 2013).

There are about 3,000 species of free-living ciliates (Finlay and Esteban 1998), although Foissner (1999a) stated that it is more likely that free-living ciliates number up to 30,000 species. Nevertheless, the number of global ciliate species is relatively low, probably because free-living ciliates are ubiquitous (Lynn and Corliss 1991; Finlay and Esteban 1998; Finlay and Esteban 2013). Many species can also be successfully cultured in the laboratory, which facilitates research in ciliates.

There have been many new soil ciliate species described or re-described (Foissner 1995, 1997b, 1998; Foissner et al. 2002; Foissner 2016) with species remaining undiscovered (Foissner 1997a, 2016). This demonstrates that more intensive research on the diversity of soil ciliated protozoa is needed.

1.2.3 The influence of soil characteristics on ciliated protozoa

1.2.3.1 The influence of soil physical parameters on ciliated protozoa

There is increasing evidence of the impact of soil physical characteristics on soil protozoa and ciliates, for example, soil texture, soil moisture, soil temperature, and soil compaction (Cowling 1994; Ekelund and Rønn 1994; Foissner 1999b; Li et al. 2010a).

Ekelund and Rønn (1994) showed that soil texture and structure, and soil moisture are two specially important factors for soil protozoa. Occurrence of distinct protozoan groups, including ciliates in different aggregates in the same soil may differ (Vargas and Hattori 1990), because soil aggregates will strongly restrict movement of protozoan cells between soil particles at low moisture. If soil moisture is sufficiently high, movement is possible, which increases the dispersal of protozoan communities. It is recognised that the effect of soil moisture is the most significant factor governing the abundance of soil ciliates (Li et al. 2010a).

Soil temperature is also one of the major physical factors which affects soil protozoa (Cowling 1994). In an experiment to consider the effect of altered temperature on desert protozoa, Darby et al. (2006) showed that optimal range for growth was from 4 ^{o}C to 26 ^{o}C and no existence of active cells was found when cultured at 37 ^{o}C. Ciliates were most abundant at 15 ^{o}C and 26 ^{o}C in the experiment. Simultaneously, field observation suggested that desert protozoa may be adapted to a specific desert temperature and precipitation regime (Darby et al. 2006). Thus, it seems that soil ciliates have temperature adaptations to their environment. This is further

supported by Buitkamp (1979) who demonstrated that local species were adapted to the temperature conditions of their local habitats (Table 1.2). The research of Buitkamp (1979) also pointed out that most ciliate species had the best growth near the highest mean annual temperature. Similarly, this suggestion is reported in field data and laboratory experiments by Petz et al. (1985) when testing temperature adaptation of the autochthonous soil ciliate species *Grossglockneria acuta* from spruce forest near Upper Austria. The results indicated that *G. acuta* was adapted to low soil temperature, with the highest individual numbers at 4.5 °C. It was also shown in field observation during the study that the abundance and occurrence of this species were higher and more regular in alpine soils than in lowland soils, i.e., under lower temperature.

Furthermore, soil compaction also has inhibitory effect on soil protozoa. Ciliates decreased significantly when soil compaction increased, probably due to decrease of soil pore space and lower moisture, thus affecting protozoa (Foissner 1999b).

Table 1.2 Temperature range (5-40 °C) for some soil ciliate species from distinct world regions (adapted from Buitkamp (1979).

Species	Temperate zone (Bonn, Germany)		Tropical zone (Africa)		
	Pasture	Mixed forest	Burned savannah	Unburned savannah	Gallery forest
Colpoda inflata	5-35	10-30	15-35	15-30	20-40
Colpoda steinii	10-40	5-30	15-35	20-30	15-35
Cyrtolophosis elongata	10-35	10-25	20-35	20-35	20-30
Cyrtolophosis mucicola	20-25	10-25	25-35	20-35	20-40
Gonostomum affine	10-35	5-30	15-40	25-30	20-35
Hemisincirra kahli	20	10-30	20-35	20-35	20-35

1.2.3.2 The influence of soil chemical properties on soil ciliated protozoa

The effect of pH on soil ciliates

Soil pH is generally found to be the most important factor determining the availability of metals. As soil pH decreases, metal availability tends to increase and *vice versa* (Berbecea et al. 2011). This may have important consequences for living organisms, e.g. poor crop growth on acidic soils may be the result of available aluminium and manganese reaching levels toxic to plants. Soil pH also critically affects organisms such as bacteria and fungi through influence on soil enzyme activity, as each enzyme requires a specific pH level to function optimally (Eivazi and Tabatabai 1988; Margesin and Schinner 1994). Consequently, an acidic soil pH (pH < 5.5) has severe effects on bacterial communities (Li et al. 2017).

Soil pH also has influence on soil ciliated protozoa. Foissner et al. (2005) illustrated that there was a positive correlation between ciliate species number and soil pH, i.e., the number of species decreases with decreasing pH. Li et al. (2010b) studied the community structure and biodiversity of soil ciliates in a mangrove forest in China. The results showed that abundance of ciliates was strongly and negatively correlated with soil pH.

The effect of nutrients/fertilisers on soil ciliates

Generally, soil protozoa, and in particular ciliates, are influenced by many environmental factors. In addition to the factors mentioned above, organic and mineral fertilisers, nutrient levels, soil type, litter and humus type are also influencing factors (Cowling 1994; Foissner 1994). Nutrients and fertilisers have marked impacts on the abundance and species richness of ciliates (Cowling 1994; Foissner 1994; Zhang et al. 2012). Li et al. (2010b) found that the abundance of soil ciliates was positively correlated with total concentrations of nitrogen, phosphate and total sulphate in soil, but negatively correlated with total potassium. It was also inferred that land use type in association with various soil properties influenced the abundance of soil protozoa (Shi et al. 2013).

Foissner (1987) illustrated from the data available that fertilisation increases the abundance of most groups of soil organisms, with no exception for protozoa. Positive impacts on the abundance of ciliates were observed after fertilisation treatments. The abundance and species composition of ciliates increased significantly after both mineral and organic fertiliser application, but organic fertilisers caused more significant changes to the micro-fauna than mineral ones (Aescht and Foissner 1992). Zhang (2012) also found that organic fertiliser significantly increased the abundance of soil ciliates under different treatments, including field and greenhouse experiments.

The effect of heavy metals on soil ciliates

Heavy metals are toxic and persistent inorganic pollutants that exist in both terrestrial and aquatic environments. They can also be bio-accumulated and transferred into food chains (Nica et al. 2012). They cannot be degraded and cause serious environmental health problems. Heavy metals have cytotoxic effects on ciliates, and the level of sensitivity of ciliates species depends on the concentration and type of metals, and the specific ciliate strains (Díaz et al. 2006; Martín-González et al. 2006). Díaz et al. (2006) carried out laboratory toxicity tests of the metals Cd, Zn and Cu on some common soil ciliate species, such as *Colpoda steinii*, *Colpoda inflata*, and *Cyrtolophosis elongata*. The results of the tests showed that the toxicity sequence was Cd > Cu > Zn. Also, when assessing impacts of Cd + Zn mixtures, results indicated that Cd cytotoxicity decreased in the presence of certain Zn concentrations. This toxicity sequence is supported by other research which directly evaluated the cytotoxicity of metallic solution or tested cytotoxicity of elutriates from metal contaminated soil on soil ciliates (Forge et al. 1993; Bowers et al. 1997; Campbell et al. 1997; Pratt et al. 1997).

Soil ciliated protozoa are more sensitive to environmental hazards than more commonly used test organisms (e.g., earthworms) (Foissner 1999b). Furthermore, the rapidity of toxicity testing based on the growth of soil ciliates (24 h) makes it possible to assess changes in biological availability of heavy metals much more rapidly than other organisms (Forge et al. 1993). Hence, a soil protozoan-based bioassay can be suitable for monitoring not only bioavailability of heavy metals in soil, but also toxicity (Forge et al. 1993). The toxicity of heavy metals to *Colpoda* spp. in the experiments of Bowers et al. (1997) and Forge (1993) generally fell in the lower range of tested concentrations for all metals, demonstrating the sensitivity of ciliate-based tests (Foissner 1999b).

In general, it seems likely that soil ciliates have a higher resistance to some metals than ciliates from fresh waters or wastewater treatment plants (Díaz et al. 2006), but all cells have mechanisms to detoxify metals. Heavy metal sequestration is the main mechanism of resistance in cells. This sequestration process principally involves the biosynthesis of a specific group of cysteine-rich proteins called metallothioneins (MTs) (Díaz et al. 2006; Martín-González et al. 2006). Indeed, ciliate MTs have been identified and sequenced from some species of *Tetrahymena* (Gutiérrez et al. 2009). It is reported that MT gene expression is enhanced under the influence of Metal Transcription Factor 1 (MTF1) in the presence of elevated levels of at least Cd, Cu and Zn inside the cell (Gutiérrez et al. 2009). However, sequestration is not

the only resistance mechanism to the cytotoxicity of metals. It is possible that there are other mechanisms, such as ATPase efflux pumps (Martín-González et al. 2006). Studies on interactions between soil ciliates and heavy metals are scant however. Only some Colpodid strains isolated from different soil habitats are used in those studies that tested the toxicity of heavy metals to soil ciliates (Díaz et al. 2006). Thus, further research on the potential impact of heavy metals on natural soil ciliate communities is needed to evaluate the possible role of ciliates as bio-indicators and understand the impacts of metal pollution on this important group. This present study will address the relationship between soil ciliates and trace metals.

The effect of pesticides on soil ciliates

The term “pesticide” embraces all herbicides, insecticides, fungicides, arachnicides, rodenticides, wood preservatives, garden chemicals and household disinfectants (Zacharia 2011). Pesticides are the most effective means of pest and weed control and are thus essential to improve the quantity and quality of agricultural products. More than 500 different pesticide formulations are being used worldwide, and agricultural production occupies the biggest share of pesticide use (Azevedo 1998). Many pesticides can persist in ecosystems for a long time. Organochlorine insecticides are typical examples. In this case, they can still be detected in ecosystems 20 years after their use was banned (Szeto and Price 1991; Arias-Estevez et al. 2008).

A pesticide usage survey report showed that there were many types of pesticides used in arable crops in the UK (Garthwaite et al. 2014a; Garthwaite et al. 2014b). The pesticides used agriculturally in the UK are dependent on specific crops or fruits, with different farmers using different types according to the crop being grown. For example, some common herbicides or insecticides used in cereal farming include Iodosulfuron-methyl-sodium/mesosulfuron-methyl, Glyphosate, Lambda-cyhalothrin, Cypermethrin, whilst dessert apple farming typically uses Glyphosate, Dicamba/MCPA/mecoprop-P, Chlorpyrifos, Chlorantraniliprole (Garthwaite et al. 2014a; Garthwaite et al. 2014b).

The effects of pesticides on soil protozoa seem to be significant. Todorov and Golemaski (1992) tested the effects of some pesticides on the growth of laboratory cultures of protozoan species. The results showed that the ciliate *Blepharisma japonicum* can show inhibited reproduction and possible death when exposed to pesticides such as Fundasol, Fuzamicin and Lavendotricin at distinct

concentrations. Likewise, Ekelund et al. (1994) showed that three pesticides, dimethoate, pirimicarb and fenpropimorph, had distinct influences on three different protozoan species, including amoeba, ciliated and flagellated protozoa in a liquid culture experiment. They demonstrated that the ciliate species *Colpoda* sp. was the most sensitive to dimethoate, but at very low concentrations of fenpropimorph or pirimicarb, the growth of *Colpoda* sp. seem to be stimulated. This was supported by the results from Schreiber and Brink (1989) in an *in vitro* toxicity test of pesticides on soil and freshwater ciliate and a flagellate species. The observed results demonstrated that protozoan species responded differently, and normal concentrations applied in the field (i.e., used as per manufacturer's instruction) for chlorex, MCPA (4-chloro-2-methylphenoxy acetic acid) and benlate were toxic for the dominant ciliate species in the test, such as *Colpoda* spp., *Pseudoplatyophrya nana*, *Hausnanniella discoidae*, and *Platyophrya spumacola*. Additionally, Petz and Foissner (1989) conducted a field study to investigate the effects of mancozeb (a fungicide) and lindane (an insecticide) in normal dose and high dose (a tenfold dose) on the soil micro-fauna of a spruce forest. Lindane at a normal dose caused acute toxicity on the number of ciliate individuals, although their populations soon recovered. A high dose of lindane caused serious and long-term effects on the number and community structure of ciliate species. In contrast, mancozeb at normal dose and tenfold dose had insignificant toxicity effects on ciliate species. Consequently, it is likely that insecticides are usually more toxic than fungicides; however, the data is very limited at present.

Ciliate communities demonstrated very pronounced changes caused by pesticides in some studies (Petz and Foissner 1989; Schreiber and Brink 1989; Ekelund et al. 1994), whereas testaceans showed more resistance (Petz and Foissner 1989). This indicated that ciliates are a sensitive group and, therefore, a potentially useful tool for testing the safety of pesticides under field conditions. In addition, the abundance of all groups of free-living protozoa in agricultural soils, including naked amoebae, flagellates, and ciliates, had a marked negative correlation with the concentration of the total residues of chlorinated organic pesticides (hexachlorocyclohexane and dichlorodiphenyltrichloroethane) in the soils (Shi et al. 2013). It is therefore assumed that long-term persistence of the residues of chlorinated organic pesticides may cause effects on the abundance of protozoa in real agricultural ecosystems.

Noticeably, Foissner (1987, 1994) reached four important conclusions about the interaction between protozoa and pesticides: (i) in general, the mode of reaction of

soil protozoa to pesticide stress is the same as that of other organisms; (ii) many protozoan species seem to be just as sensitive to pesticides as other more commonly used test organisms; (iii) insecticides are more toxic than herbicides to protozoa; (iv) insecticides disturb soil protozoa critically; the population is often only fully recovered after 60 days. However, investigations on the side-effects of pesticides to soil protozoa under field conditions are rare when compared to research conducted under laboratory conditions (Foissner 1987, 1994). Consequently, further research to assess impacts of pesticides on soil protozoa is needed to understand the effects that occur in more complex conditions.

1.2.3.3. The influence of soil biological properties on soil ciliated protozoa

The relationship between microflora and soil ciliates

In soil, protozoa including ciliates are important predators of microflora (bacteria and fungi). Under nutrient-stressed condition in soils, protozoa help to regulate the size and diversity of the bacterial communities (Pussard et al. 1994). Moreover, in the presence of protozoa, bacterial production is greater due to the use of nutrient compounds by bacterial released by protozoa as waste (Pussard et al. 1994). Micro-organisms also affect protozoa by producing extracellular metabolites, which play a role in the stimulation of protozoan activity (Pussard et al. 1994). These active substances can induce excystment or stimulate the growth rate of protozoa by increasing their division rate (Pussard et al. 1994).

Pussard et al. (1994) explained the stimulation of bacterial activity by protozoa as follow:

(i) Protozoan predation maintains a higher proportion of young and active bacteria, either by consuming preferentially the bacteria or by delaying the decline of the metabolism that appears when the population density reaches the carrying capacity of the medium. Thereby, the bacterial biomass consumed by protozoa is always recycled, which is the explanation mostly favoured at present. Conversely, protozoa produce substances to stimulate the growth and activity of bacterial populations.

(ii) Protozoa make the medium more favourable for bacterial activity, either by consuming oxygen to prevent a concentration of oxygen that can inhibit nitrogen fixation and so prevent the acidification of the environment.

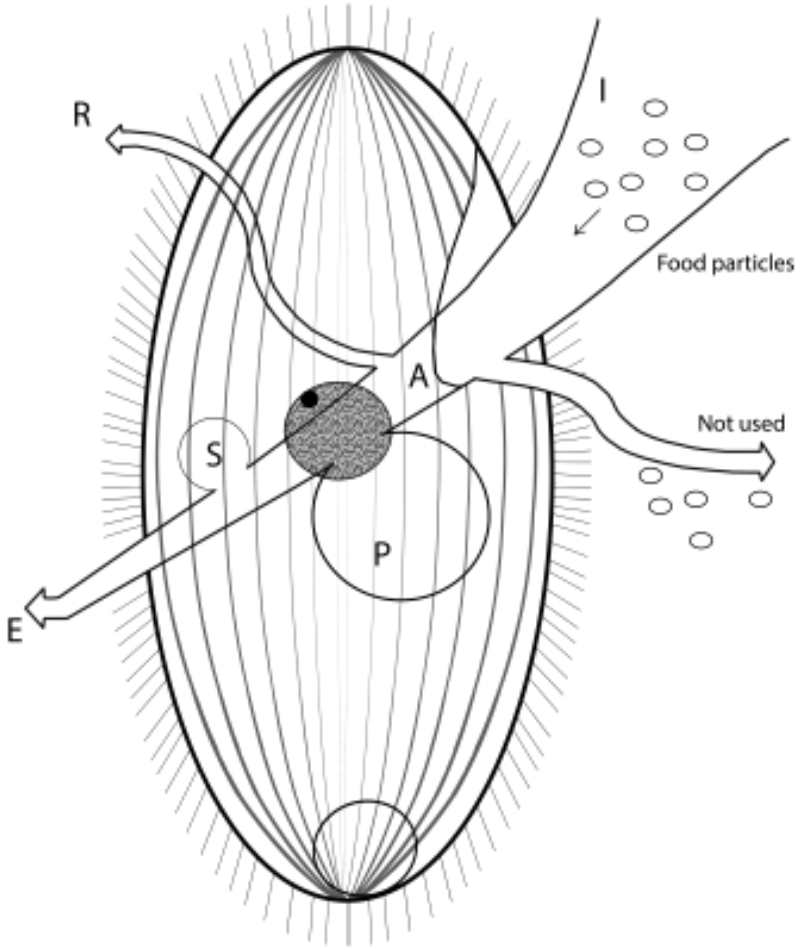


Figure 1.3 The flow of food energy through protozoa. Most of the ingested food (I) is assimilated (A) and used for production (P), respiration (R) and storage (S). Undigested material and nutrients in excess are excreted (E) (adapted from Griffiths (1994).

With respect to agriculture, protozoa play a crucial role in nutrient cycling through their feeding activities (Griffiths 1994; Esteban et al. 2006). The model of the flow of nutrients through protozoa is shown in Figure 1.3. Not all the food ingested by protozoa is utilized. In general, out of all the carbon ingested, 30 % is released through respiration, 30 % excreted and 40 % used for production (Griffiths 1994). Protozoa and their bacterial prey differ in their C:N:P ratios, but protozoa use only 10-40 % of the prey carbon for biomass production, the excess N and P is assumed to be excreted in inorganic or organic form and may therefore be readily available for other soil organisms including plants (Griffiths 1994; Pussard et al. 1994; Bonkowski et al. 2000). According to Foissner (1987), 69 % of total animal respiration in soil is due to protozoa because of their small size and fast reproduction. Hence, the high production rates of protozoa suggest significant

effects on nutrient mineralization through grazing on bacteria (Bonkowski et al. 2000).

Regarding soil enzymes, it is clear soil enzymes play a crucial role in soil organic decomposition and nutrient cycle because they are direct mediators for biological catabolism of soil organic and mineral components (Nielsen et al. 2002). They are often closely related to soil organic material, soil physical properties and microbial activity or biomass, they also change much sooner than other parameters (Nielsen et al. 2002). Consequently, activities of soil enzymes provide early indications of changes in soil quality, which are very important. Soil enzyme activities can be used as measures of microbial activity, soil productivity, and inhibiting effects of pollutants (Nielsen et al. 2002). The available assays for some typical enzymes are well-documented (Table 1.3).

Table 1.3 Soil enzymes as indicators of soil health (from Nielsen et al . 2002).

Soil enzyme	Enzyme reaction	Indicator of
Beta-glucosidase	Cellobiose hydrolysis	C-cycling
Cellulase	Cellulose hydrolysis	C-cycling
Phenol oxidase	Lignin hydrolysis	C-cycling
Urease	Urea hydrolysis	N- cycling
Amidase	N-mineralisation	N- cycling
Phosphatase	Release of PO_4^{2-}	P- cycling
Arylsulphatase	Release of SO_4^{2-}	S- cycling
Dehydrogenase	Electron transport system	Microbial activity
Soil enzymes	Hydrolysis	General organic material degradative enzyme activities

Soil enzymes catalyse reactions of important biochemical processes in soil, thus they provide a meaningful assessment for activity rates of microbes, in which glycosidase, phosphatase and total microbial activities are crucial soil biological parameters (Eivazi and Tabatabai 1977; Nielsen et al. 2002; Stege et al. 2010a).

The glycosidases are a group of carbon cycling enzymes, and catalyse breakdown reactions of low molecular weight carbohydrates to produce sugars as final products

of the degradation process, which are the main energy source for soil microorganisms. β-glucosidase is the most predominant glycosidase in soil (Eivazi and Tabatabai 1988). β-glucosidase is sensitive to soil management and provides an early indication of changes in organic matters status and its turnover, thus it is used as a bioindicator of soil quality (Stege et al. 2010a). Phosphates are important in mineralization of organically-bound phosphorus. In soil, phosphorous bound to organic matter is unavailable for plant uptake, so mineralization is fundamental to provide phosphate to plant and soil organisms. Phosphatases have been intensively studied in agricultural soils and have been shown to be dominant in soils (Eivazi and Tabatabai 1977) and play an important role in releasing inorganic phosphate from organic matter, which can be used by plants, etc. Total microbial activity is a general measure of organic material turnover in natural habitats (Schnürer and Rosswall 1982). It includes a broad group of hydrolytic enzymes which cleave bonds in organic constituents such as starch, cellulose, protein, lipid, lignin etc. This parameter is also closely correlated with the microbial biomass in the soil (Söderström 1977; Lundgren 1981).

Despite the important role of soil enzymes in the processes of organic matter mineralisation and thereby nutrient provision for plants and the interaction between soil protozoa and microflora mentioned above, there seem to be no published research on the relationship between soil ciliates and soil enzyme activities. Thus, this research aims at understanding the correlation between soil enzymes and soil ciliates.

The relationship between organic matter parameter and soil ciliates

Soil organic matter is defined as the total of all organic carbon-containing substances in the soil that consist largely of complex materials of plant and animal residues in various stages of decomposition, substances synthesized microbiologically and/or chemically from the breakdown products, and the living and dead microorganisms and their decomposing remains (Schnitzer 2000). The organic matter content of a soil depends predominantly on inputs from vegetation and removals that depend on the rate of oxidation of the organic matter. However, the soil itself affects the growth of plants and thereby organic matter input and the rate of organic matter oxidation.

The abundance of total protozoa increased after application of a variety of organic mulches, e.g. composts, paper recycling wastes, and hay (Forge et al. 2003). Verhoeven (2001) found similar results when testing the impact of three organic

fertilisers, i.e., straw, rabbit dung, and wheat bran; the abundance of soil ciliated protozoa significantly increased with respect to the control. A strong positive correlation was found between the abundance of soil ciliates and total soil organic matter in the natural soils of China (Li et al. 2010a; Li et al. 2010b).

1.2.4 Soil ciliated protozoa as bio-indicators

Bick (1982) defined that “bio-indicators are, in a broad ecological sense, organisms that can be used for the detection and quantitative characterisation of a certain environmental factor or a complex of environmental factors”. Foissner (1987) suggested the narrower definition, confining bio-indicators to human influences only.

Protozoa have been considered bio-indicators in soil ecosystems (Foissner 1987, 1994, 1997c, 1999b; Gabilondo and Bécares 2014), but there has been little recent research. As mentioned above, protozoa are very sensitive to changes in environmental conditions, which is due to their lack of a cell wall. Protozoa, with rapid growth rates, can react more quickly to environmental changes than other eukaryotic organisms and thus can serve as an early warning system (Foissner 1987, 1994).

Ciliates have a potential for use in bioassays to monitor heavy metal pollution (Foissner 1999b; Díaz et al. 2006; Gutiérrez et al. 2009). When comparing the diversity of ciliates and amoebae in agro-ecosystems with those in natural habitats, Foissner (1997c) found that the species richness of testate amoebae decreased distinctly in agricultural lands, whilst richness of ciliate species was only slightly reduced. So, testate amoebae were considered the most important protozoan indicator group for agro-ecosystems. However, ciliates with only high density in the topmost litter layer of soils were considered an excellent tool to monitor health recovery of soil after soil pollution or heavy disturbance i.e., topsoil removal, the use of pesticides, etc. (Foissner 1994). Petz and Foissner (1989) proved that ciliates were more sensitive to some pesticides than were amoebae. Also, as reviewed above, soil properties have marked influences on soil ciliates. Thus, it is clear that soil ciliates have potential as bio-indicators of soil quality. Therefore, a crucial investigation in the present research is to understand the ciliate communities found in natural soils and compare them to those from farm land that is subject to many activities that can damage soil health.

1.3 Research aim and objectives

There is no doubt that ciliates have a strong connection to soil properties. However, previous studies only investigated a limited range of soil properties (Li et al. 2010a;

Li et al. 2010b) meaning that research on the influence of soil properties on soil ciliates is sparse overall. In addition, research on the biodiversity of soil ciliates is needed because many species remain undiscovered or several species were described a very long time ago, but still require a full description (Esteban et al. 2001b; Foissner 2016)

The aim of this study is to investigate the abundance and species richness of ciliated protozoa in natural and agricultural soils, and to determine the relationship between physio-chemical soil properties and the occurrence of the species and abundance of ciliates in order to determine the usefulness of soil ciliates as bio-indicators of soil quality. In order to do so, investigating biodiversity of soil ciliates and analysing physical and chemical characteristics of soils in the study sites was conducted. Controlled experimental conditions were used to assess the impact of specific contamination factors i.e., the metal copper, herbicide glyphosate and insecticide cypermethrin. In addition, the taxonomy of soil ciliate species found at study sites was investigated to identify rare and new species and to enhance the knowledge of ciliate biodiversity in soils.

The objectives of the current study were:

1. To determine the soil properties in three study sites, i.e., one natural ecosystem and two agricultural ecosystems (Chapter 3);
2. To investigate the abundance and richness of soil ciliated protozoa in the three study sites and to determine correlations between soil ciliates and soil properties in order to determine if soil ciliates are bio-indicators of soil quality (Chapter 4);
3. To assess the impact of specific contamination factors on soil ciliate abundance and species richness (Chapter 6);
4. To examine the taxonomy of soil ciliate species in the three study sites and to identify rare and new species (Chapter 5).

Chapter 2. MATERIALS AND METHODS

2.1 Study sites

Three study sites (Table 2.1 and Figure 2.2) were chosen for the present study: a natural site (East Stoke Fen Nature Reserve); and two agricultural farms (Vicarage Farm, and Corfe Castle Farm). Two 1 x 2 m plots were designated for each study site, the position of each site being selected by a random walk using distances and bearings created using the RANDBETWEEN function in Excel (Table 2.1).

Figure 2.1. Locations of study sites: East Stoke Fen, Vicarage Farm and Corfe Castle Farm near Wareham (UK). Inset: approximate location of the study area in the UK.

Table 2.1 Experimental Sites in Natural and Agricultural ecosystems.

Study site	Plot	Grid reference	Plot size	Use
East Stoke Fen	Plot 1	SY 86565 86612	1 x 2 m	Nature reserve
	Plot 2	SY 86531 86614	1 x 2 m	
Vicarage Farm	Plot 1	SY 79690 81683	1 x 2 m	Agricultural production
	Plot 2	SY 79680 81660	1 x 2 m	
Corfe Castle Farm	Plot 1	SY 96425 83342	1 x 2 m	Agricultural production
	Plot 2	SY 96437 83353	1 x 2 m	

East Stoke Fen Nature Reserve - Natural site

East Stoke Fen Nature Reserve is owned by the Freshwater Biological Association, but managed by the Dorset Wildlife Trust as a biodiversity area. It is of large size, covering 4.5 ha, and is situated to the east of Wool in the River Frome floodplain in Dorset, United Kingdom (Figure 2.2). The terrain ranges from reed-marsh and wet deciduous woodland to oak copse *(http://www.dorsetwildlifetrust.org.uk)*. The area of the study site is quite shaded by trees (Figure 2.2).

Figure 2.2 Natural study site at East Stoke Fen Nature Reserve (Dorset, UK) (Image: author's own collection)

Agricultural Farms

Sampling was conducted in two different farms, i.e., Vicarage Farm in Winfrith Newbourgh and Corfe Castle Farm in Wareham (UK). Vicarage Farm grew spring oats and winter wheat during the study, whilst Corfe Castle Farm grew spring barley and winter turnip over the period of sample collection (Figures 2.3 and 2.4). The altitude of Vicarage Farm and Corfe Castle Farm is 151 m and 12 m above sea level, respectively.

Figure 2.3 The agricultural study site at Corfe Castle Farm in Wareham (UK). (Image: author's own collection)

Figure 2.4 The agricultural study site at Vicarage Farm in Winfrith Newbourgh (UK). (Image: author's own collection)

2.2 Soil sampling

Soil samples from the natural site were collected every month from January 2016 to April 2017, whilst samples from the agricultural sites were collected every month from May 2016 to April 2017. After April 2017, soil samples were taken every

season i.e., July, October and December 2017 for all study sites. On each sampling occasion, samples were collected in the morning and transferred to the River Laboratory in Wareham, which is near the sampling sites.

At each site (natural and farm sites), two samples were collected from two randomly designated 1 x 2 m plots (Table 2.1). A 4 cm diameter stainless steel soil corer was used to collect topsoil samples to a depth of 5 cm, which included any litter layer. On each sampling occasion, eight core samples were taken randomly from within each plot to ensure samples were representative of the plot. The soil collected was removed from the corer and placed into a labelled plastic bag to form a bulk sample for that plot.

Soil and air temperatures in each sampling point at the time of sampling were recorded by using a Checktemp® LC thermometer (Hanna Instruments, Rhode Island, USA).

2.3 Ciliate species richness and abundance

2.3.1 Determination of soil ciliate abundance

In general, methods to retrieve ciliates from soil samples can be divided into two direct and indirect methods (Foissner 1987). Direct methods are only appropriate for estimation of ciliates abundance if fresh, moist soil is used. Active ciliates in soil are low, as they depend on soil moisture (Adl and Coleman 2005). Alternatively, the indirect methods allow ciliate cysts formed owing to dry conditions in the environment to be made active, thus the potential diversity of ciliates in different types of soil can be retrieved. Most probable number (MPN) are indirect methods in which water, organic substances, mineral elements, and/or bacterial food sources are added to enrichment media to encourage the growth of soil protozoa (Foissner 1987). However, MPN methods have negative aspects, for example such methods are well known to select of some particular protozoan species if only one type of bacterial food is added. Moreover, the results of MPN methods vary depending on the enrichment protocol (Foissner 1987). Finlay et al. (2000) developed a method to estimate the potential abundance of free-living protozoa in soil, which overcomes some limitations and problems of the direct and most probable number methods. The key advantage of this method is that nothing more than adding filtered rain water to the soil is required (Finlay et al. 2000). It is suitable for the comparison of protozoa between different soils. In the present study, the method of Finlay et al. (2000) was followed to investigate abundance and richness of soil ciliated protozoa. The details of the method are described below:

Sample treatment

After immediate return to the River Laboratory, soil samples were removed from the original plastic bag and the soil was then homogenised by thorough mixing in a clean 30 cm-diameter glass bowl. A sub-sample of soil (~50 g) was then taken and spread out as a layer in 15 cm diameter glass Petri dish and dried at room temperature (18-22 ^{o}C) for six days. After the soil for determination of ciliates was dried, it was passed through a 4 mm sieve to remove large objects such stones. This material was now called “air-dried soil”. This soil was used for ciliate-related work. The remaining soil was used to analyse soil characteristics

Incubation

To facilitate the development of ciliates, 5 g of air-dried soil was placed into a 5 cm diameter sterile plastic Petri dish. This was replicated three times for each sample. A measured volume of filtered rain water was then added to samples - sufficient to produce a slurry. Whatman® syringe filters with a 0.2 µm diameter pore were used to filter rain water to exclude ciliates and other microbes. Slurry samples were then incubated in the dark at 15 ^{o}C and ciliates were investigated after 4 days and 10 days of incubation.

Enumeration of ciliates

After 4 days and 10 days of incubation, the wet soil prepared as above was pressed to release water. A 50 µl sample from the water runoff was taken and placed on to a glass Sedgewick-Rafter Chamber. Ciliate numbers in the 50 µl subsample were determined under a compound microscope at a magnification of 40-125 X. This was repeated five times for each Petri dish of soil.

Calculations

The number of ciliate cells g^{-1} oven-dried weight of soil = a x (V1+ V2)/(50 x W)

Where:

a: The number of ciliate cells counted in 50 µl sample from the water runoff

V1: Volume of rain water added into 5 g of air-dried soil

V2: Volume of water determined in 5 g of air-dried soil

W: oven- dried weight of 5 g of air-dried soil

2.3.2 Identification of ciliates

During the counting process, the species richness of ciliates was determined by identifying observed ciliates using taxonomic works such as Kahl (1935); Foissner (1987, 1993); Foissner et al. (1991, 1992, 1994, 1995); Foissner et al. (2002); Foissner (2016), and other specialised taxonomic literature. After counting, samples were used for enrichment cultures in order to obtain later populations of ciliates, which enabled further identification; enrichment cultures also facilitate growth of species that were not observed during the counting process. This enrichment is also useful to provide sufficient cells for detailed observations and for silver impregnations. Enrichment cultures are prepared by putting a soil inoculum (approximate 5 g of rewetted soil) into 50 mL sterile culture flasks that contained medium as described in Table 2.2 below.

Table 2.2 Medium used to prepare enrichment cultures of soil ciliates.

Component	**per/flask**
SES*	5 mL
Mineral water	20 mL
Boiled Wheat Grain	1/2 grain
Soil from the Petri dish	~ 5 g of rewetted soil

*SES (Soil Extract added Salts) components (http://www.ccap.ac.ukmedia/SES.pdf):

Component	**per litre**
Stock solutions (1-3)*	20.0 mL each
Soil extract	100.0 mL
Distilled	840.0 mL
***Stock solutions**	**per litre**
(1) K_2HPO_4	1.0 g
(2) $MgSO_4.7H_2O$	1.0 g
(3) KNO_3	10.0 g

All ciliates that grew in the flask were identified under a compound microscope using a combination of glass micro-pipetting and Silver-staining techniques (see below).

Silver-staining techniques

There are many different impregnation and staining techiques to reveal typical characteristics of ciliates, such as vital staining, protargol, silver-carbonate (Fernández-Galiano 1994), and the dry/wet silver nitrate method (Lee and Soldo 1992; Foissner 2014). The ammoniacal silver carbonate impregnation method (Fernández-Galiano 1994) can reveal the kinetosomes, some cortical and cytoplasmic features of ciliates, especially the kinetodesmal fibre and the nuclear apparatus. The method is also quick to do, which is useful as it makes the identification of ciliate species quicker. Consequently, the ammoniacal silver carbonate impregnation method (Fernández-Galiano 1994) was followed in the present study.

The procedure followed was adapted from Fernández-Galiano (1994). The following components are added to a glass beaker (Volume 50 mL)*:

5 mL of distilled water

2 mL of the sample to be studied

2-3 drops of formalin

18-20 drops of bacto-peptone 5 %

10 drops of pure pyridine

1 mL of ammoniacal silver carbonate solution

20 mL of distilled water

*Order of components added as listed from top to bottom

The beaker was then stirred to mix the components, and placed into a water bath at 55 °C. The beaker was left in the water bath until the liquid changes into a brandy-like colour. At this point, the liquid was poured into a glass evaporation dish that had about 50 mL of distilled water in it. The impregnated suspension was left to settle, and then swirled slightly around to concentrate the cells in the centre of the capsule. Finally, a glass micropipette was used to pick up and place the impregnated cells on a microscope slide, which was then covered with a coverslip and observed under a compound microscope.

This method is not good for some ciliates, for example hypotrichs, as the formalin used for cell fixation makes them burst (Foissner 2014).Therefore, for hypotrichs and other fragile ciliates the protargol method described in Foissner (2014) was followed. The procedure followed was adapted from Foissner (2014):

Ciliate cells in the sample to be studied were fixed in commercial Bouin's fluid in a glass beaker for 20 minutes. The ratio of the fixative and the sample fluid was 1:1. The solution contained the fixed ciliate cells were then poured into a 15 mL polypropylene centrifuge tube. The fixed ciliate cells in the centrifuge tube were concentrated and washed three times with tap water to remove Bouin's fluid by centrifugation at 2000 rpm for two minutes. It should be noted in this washing step that centrifuged cells were added enough tap water to bring to 10 mL and shaken vigorously. After centrifuging, the supernatant was carefully decanted, but 1 mL in the tube was kept with taking great care (not to disturb the loosely pelleted ciliates). At the final washing time, about 0.3 mL of concentrated and fixed cells was remained in the tube. One small drop of albumin-glycerol was placed on the centre of a very clean glass slide, and then a small drop of concentrated and fixed cells in the tube was added to the slide. The drops were mixed well with a mounted needle and spread over the middle-third of slide. Usually, eight very clean glass slides were used for a sample. After that, the slides were dried overnight at room temperature. When the slide dried, they were placed in an eight-slot staining jar filled with ethanol 95 % for 20 minutes. Also, an another eight-slot staining jar contained protargol solution 0.5 % was placed in oven at 60 ^{o}C to warm the solution up before the slides contained fixed ciliate cell were place into. After slide dripping for 20 minutes in ethanol 95 %, the slides were rehydrated though ethanol 70 % and washed two tap water steps for 5 minutes each. The slides were then placed in 0.2 % potassium permanganate for 2 minutes before they were transferred to 2.5 % aqueous oxalic acid for 3 minutes. The slides were collected to a staining jar filled with tap water and washed two times with tap water and one time with distilled water for 3 minutes each. The slides were transferred to the warm protargol solution (60 ^{o}C) and impregnated for 40-60 minutes, depending on specific ciliate species. The staining jar contained protargol solution and the slides were removed from the oven. One slide was removed from the jar and dipped into tap water for 1-2 seconds. Then 1-2 drops of ordinary developer were added and ciliate cells were immediately observed for development under a compound microscope. When oral structures of ciliate cells become visible (but not very dark), remove the slide from the stage of microscope, pour off fluid and dip the slide in and out of a container with 500 mL of tap water. To

interrupt the development of ciliate infraciliature, the slide was dripped in 5 % sodium thiosulphate for 5 minutes. The same steps were carried out for the remaining slides. After that, the slides were washed three times with tap water for 3 minutes each. The slides were alternately transferred though ethanol 70,100 and 100 % to dehydrate before they were cleared by two 10 minutes transfers through xylene. Finally, the slides were mounted by synthetic neutral medium and applied cover glass (24x40 mm). They need to leave overnight to harden before any observations.

2.4 Determination of soil physical characteristics

Soil texture refers to the relative proportions of sand, silt and clay in the soil (White 1997). Soil texture has a profound effect on the physio-chemical characteristics of the soil and therefore on the nature of communities that live in, and on the soil (White 1997). The present study adapted the method described by Rowell (1994) to analyse soil structure. The soil was initially treated by wet oxidation which means the oxidation of dissolved or suspended organic components using hydrogen peroxide as an oxidizer. This is necessary as otherwise the organic substances bind the mineral particles, particularly the clays, together. The wet oxidation was conducted as follows: a 10 g sub-sample of fine earth fraction soil (air-dried soil passed through a 2mm sieve) was treated with 30 mL of 6 % H_2O_2. After initial frothing had subsided, the sample was heated to 40 °C in a water bath. After 1.5 h (or when the frothing had stopped), another 70 mL of 6 % H_2O_2 was added and the temperature of the water bath increased to 60 °C. After a further hour of heating or when the frothing has subsided if longer, the temperature of the water bath was increased to 100 °C and the sample heated until frothing stopped, by which time the organic material should have been fully oxidized.

The following stage of the procedure used the physical action of a blender and chemical anti-flocculent to separate and disperse the mineral fraction of the soil. The contents of the beaker from the wet oxidation step were topped up to a volume of about 100 mL and then 10 mL of dispersant (10 % sodium hexametaphosphate) was added. A blender was then used to mix the contents for 45 seconds. Following a 15-second break, the sample was blended again for a further 45 seconds. Adhered soil from the blender was washed with distilled water into the beaker.

The sample was then wet sieved through a 63 µm sieve contained in a funnel into a 500 mL cylinder using a spray of water. Spraying was stopped when 450 mL of water had collected in the measuring cylinder. The volume in the cylinder was filled

up to 500 mL by water used to wash out the funnel. The cylinder was stoppered and used to determine silt and clay fraction.

The sand fraction remaining on the sieve surface was transferred to a weighed 250 mL beaker. The surplus water in the beaker was carefully decanted and the sand fraction dried at 105 ^{o}C for at least 24 h.

To determine silt and clay fraction, the sealed measuring cylinder containing the silt and clay fraction was inverted from 10 to 20 times to ensure an even distribution of particles throughout the water column. The cylinder was then immediately placed on a bench top and 20 mL of the solution in the cylinder was removed as soon as possible from a depth of 10 cm. This contained both silt and clay fractions. The solution was transferred to a 100 mL beaker and was then placed in the oven at 105 ^{o}C for at least 24 h. After 8 h, a further 20 mL of the solution was again sampled from a depth of 10 cm. This liquid contained only clay as silt had sedimented out of this depth. Clay is much slower to settle and the clay content of the sampled solution is still representative of the clay content throughout the cylinder at this time. The sampled solution was again transferred to a beaker and dried in an oven 105 °C for at least 24 h.

Calculations

To calculate the weight of the sand fraction, the weight of the empty beaker was removed from the weight of the beaker which contained sand fraction after drying in the oven. This weight of dried sand was called **S.** After, the weight of the dried silt and clay fractions (recorded as **M**) was calculated by removing the empty weight of the empty beaker from the beaker plus the fractions and multiplied by 25 (i.e., 500 mL/20 mL). Similarly, the mass of the clay fraction (**C)** was also calculated by removing the empty weight of the beaker from the beaker plus the clay fraction and multiplied by 25 (i.e., 500 mL/20 mL). After, the total weight of mineral material in the soil sample was calculated by adding the weight of the sand fraction to the weight of the silt and clay fraction. This value was called **Wt** (i.e., Wt = S + M + C). Also, the weight of the silt fraction was **M – C**.

Percent sand, silt and clay can be calculated by % = (fraction weight/Wt) x 100

2.5 Determination of chemical characteristic

2.5.1 pH

The pH of a solution is defined as:

$$pH = -\log_{10}[H^{+}]$$

Soil pH measurement can be determined in soil suspension when air-dried soil is mixed with distilled water, 0.1 M $CaCl_2$ or 1 M KCl solution at different ratios (Hendershot et al. 2007; Kabała et al. 2016). In the present study, to calculate soil pH, a 10 g sub-sample of air-dried fine soil was put into a 50 mL beaker and 25 mL distilled water was then added. After stirring thoroughly, the beaker was left to stand for 15 minutes. The pH meter was calibrated by using a pH 7.0 buffer solution before the soil pH value was determined in the soil suspension. The measurement of pH was repeated three times for each soil sample using an H-series minilab pH meter (HACH, USA).

2.5.2 Soil oven-dry (OD) weight

To calculate the soil oven-dry weight, an exact 5 g sub-sample of fresh fine soil was placed in a 50 mL beaker, and then transferred to a laboratory oven at 105 ^{o}C. The dry soil was reweighted after leaving at least 24 h in the oven. Oven-dry weight is calculated by: % OD = dry weight/wet weight x 100.

This was repeated three times for each soil samples (Rowell 1994).

Soil moisture was also recorded here by: Moisture (%) = 100 % - % OD

2.5.3 Determination of total major and trace elements in soils by *aqua regia* acid digestion

Digestion of soils with *aqua regia* acid is used for determination of major and trace elements such as heavy metals, Al, Ca, Mg, K, P and S. The method described below is adapted from the method of McGrath and Cunliffe (1985).

A 0.300 g (+/- 0.02 g) sub-sample of fine earth fraction soil was placed into a boiling tube. The exact weight of the soil was recorded. *Aqua regia* was created by adding 9 mL of 37 % HCl and then 3 mL 70 % HNO_3 to the tube. Acids were Primar Plus trace metal grade (Fisher Scientific, Loughborough, UK). The tubes were swirled again to mix the acid and soil and were then placed in a cold heating block. The tube was left to stand at room temperature for at least two hours. The soil in the Fen contained a high level of organic matter and it was necessary to leave this for a longer period to allow sufficient cold digestion of organic material to prevent frothing on heating. To promote digestion, the tube was heated for 60 ^{o}C overnight. Then, the temperature of the heating block was increased to 105 ^{o}C for 1 h to complete soil digestion. An increase of temperature to 150 ^{o}C then followed to evaporate the contents of the boiling tube. The heating was stopped when the tube contents had dried. The dried tube was collected and cooled at room temperature and 20 mL of 5

% HNO_3 was then added to re-suspend the digest. The suspension was then filtered through a Whatman® No. 42 filter paper in to a 15 mL polypropylene centrifuge tube. This was repeated three times for each soil sample. Blanks and appropriate standard reference materials (NRC TH-2) were included in each digested time. All samples were presented for analysis by ICP-OES (Varian Vista-Pro, Varian Pty, Australia).

Calculations

$$\text{Concentration in soil (mg kg}^{-1)}) = \frac{\text{ICP x V}}{\text{W}}$$

Where:

ICP = value recorded by ICP-OES system

V = sample volume

W = sample weight

2.5.4 Determination of extractable (bioavailable) elements in soils

This method used a salt solution of neutral pH (i.e., $CaCl_2$) to determine the concentration of metals and other substances such as phosphates in soils that were potentially available for uptake by organisms (Houba et al. 1990; Novozamsky et al. 1993). These bioavailable metals occur as free metal ions in soil solution or absorbed to cation exchange sites that can be found on clays and organic matters. The method used in the present study was adapted from the extraction method of Houba et al. (1990). A 2 g sub-sample of fine earth fraction soil was placed in a 30 mL polypropylene tube and 20 mL of 0.01 M $CaCl_2$ added. The tube was then placed horizontally on an orbital shaker and shaken at 125 rpm at room temperature (20-22 ^oC) for 3 h exactly. The soil suspension was then filtered through a Whatman® No. 42 filter paper into a 15 mL centrifuge tube and stored samples at minus 18 ^oC until analysis. Each soil sample was repeated three times for this. All samples were presented for analysis by ICP-OES (Varian Vista-Pro, Varian Pty, Australia).

Calculations

$$\text{Concentration in soil (mg kg}^{-1)}) = \frac{\text{ICP x V}}{\text{W}}$$

Where:

ICP = value recorded by ICP-OES system

V = sample volume

W = sample weight

2.5.5 Determination of organic matter by loss on ignition

There are three common methods to determine soil organic carbon or organic matter: loss on ignition; the Walkley and Black oxidizable carbon method; and dry combustion (Konare et al. 2010). However, loss on ignition is an easier and more convenient method than the others (Konare et al. 2010). Therefore, *ca.* 2 g of fine oven dry soil was put in a crucible and heated to 450 ^{o}C for 10 h in a furnace. Ignited soil was then placed in a dissector to cool before re-weighting to determine soil organic matter by the loss on ignition (Rowell 1994).

2.5.6 Determination of total ammoniacal nitrogen and nitrate nitrogen

Extraction of ammoniacal and nitrate with 2 M potassium chloride

This method involves extraction of soil ammoniacal and nitrate with 2 M KCl, which is the generally accepted method because it is simple and rapid (Rowell 1994). Also, adding KCl does not lead to changes in the amount of exchangeable NH_4^+ and NO_3^- nitrogen (Keeney and Nelson 1982). Procedure for the method was adapted from Keeney and Nelson (1982). 4g of fresh soil was placed in a sterile tube and 20 mL of 2 M KCl was added. The tube was stoppered and shaken on an orbital shaker for 1 hour. The soil suspension was then filtered through a Whatman® No. 42 into a clean tube. This extraction solution was used for determine concentrations of ammoniacal and nitrate nitrogen. Three replications were prepared for each sample.

Determination of extractable nitrate nitrogen via vanadium (III) reduction

There are several methods to determine nitrate-N, for example: nitrate reductase, specific ion electrode, chemiluminiscence and copperized cadmium reduction method (Keeney and Nelson 1982; Bartholomew 1984; Aoki et al. 1997). However, the colorimetric method to determine extractable nitrate-N via vanadium reduction combined with detection by Griess reagents is believed to have comparable validity with other methods (Miranda et al. 2001). This method is a sensitive and widely applied procedure, which does not require specialized equipment, expensive reagents or time consuming steps (Miranda et al. 2001; Garcia-Robledo et al. 2014).

Vanadium (III) prepared in HCl solution affects the efficient reduction of nitrate to nitrite and/or nitric oxide, both of which are captured by Griess reagents (which includes sulphanilamide and N-(1-naphthyl)-ethylenediamine) to produce a red dye. The reagent solution, containing vanadium in acid solution and Griess reagents, was mixed with the nitrate samples as extracted above in cuvettes in the ration of 4 mL of reagent to 80 µl extractant. Absorbance was read at 540 nm using a UV/vis spectrophotometer (Varian Carym 50, Varian Pty, Australia) following full colour development, which took 12 hours at room temperature. Nitrate standards with 0, 5, 10, 25 and 50 mg L^{-1} was made up in 2 M KCl to calibrate the UV/vis and allow conversion of absorbance into concentration.

Total ammoniacal nitrogen (TAN) by the Salicylate-Hypochlorite method

A common method for quantitative determining total ammoniacal-N is indophenol blue method (Ma et al. 2014). However, the method is not recommended, because it requires the use of phenol - a particularly hazardous chemical (Molins-Legua et al. 2006). The salicylate-hypochlorite method is safer than the indophenol method because it uses the reaction of ammonia with salicylate and hypochlorite in the presence of sodium nitroprusside (Bower and Holm-Hansen 1980). This method has proved to produce results that are very close to the conventional indophenol blue method (Bower and Holm-Hansen 1980; Le and Boyd 2012). Indeed, accuracy was slightly better for salicylate-hypochlorite method (Le and Boyd 2012).

The procedure followed the method of Le and Boyd (2012). 1 mL of the filtered 2 M KCl soil extracts was diluted in 4 mL of analytical grade water in a clean tube. Then 0.6 mL of salicylate catalyst solution and 1 mL of alkaline hypochlorite solution were pipetted into the tube and mixed well with the sample. The mixed solution was placed in a low light area for 1 hour for colour development, because the pigment produced in reactions in this method is photosensitive. Absorbance was read at 640 nm UV/vis spectrophotometer.

TAN standard solution made up in 2 M KCl at 0, 1, 5, 10, 15, 20 mg L^{-1} was prepared following the same as steps above to calibrate the UV/vis instrument and allow conversion of absorbance into concentration.

Finally, this method gave the concentrations of NH_3-N. Conversion between NH_4^+ and NH_3 is complicated by the equilibrium reaction between the two (Korner et al. 2001):

$$NH_3 + H^+ \Leftrightarrow NH_4^+$$

At pH under 8.0, *ca* 90 % of N will be in the form of ammonium (NH_4^+) (Groeschke et al. 2016). Thus, to convert mg L^{-1} of NH_3-N to mg L^{-1} NH_4^+, multiplication by 1.285 was applied (Barth et al. 2008).

Calculations

The concentration of nitrate-N and ammoniacal-N can be calculated as below:

$$\frac{C \times 20}{W} \quad (mg\ kg^{-1})$$

Where:

C: value recorded by UV/vis

W: dry weight of soil

2.6 Soil microbial properties

2.6.1 Soil glycosidase activity

Glycosidase or glycoside hydrolase is part of a group of hydrolase enzymes that hydrolyse the glycosidic bonds in carbohydrates. The general equation of the reaction is (Eivazi and Tabatabai 1988):

$$\text{Glycosides} + H_2O \longrightarrow \text{Sugar} + \text{Aglycons}$$

Among the glycosidase group, β-glucosidase is significant in the soil carbon cycle and used as a bio-indicator of biological fertility of soil (Zhang et al. 2011). It participates in the final step of cellulose degradation by catalysing hydrolysis of β-glycosidic linkage in cellobiose to release glucose (Singhania et al. 2013).

To determine activity of β-glucosidase, the assay method described by Tabatabai (1994) was used. This method works by measuring the amount of *p*-nitrophenol (*p*NP) released when β-glucosidase enzymes cleave the bond linking this compound to the carbohydrate moiety of *p*- nitrophenyl-β-glucoside (*p*NPG). A 1 g sub-sample of fresh soil (sieved to 2 mm) was placed in a 50 mL conical flask. After that, 0.25 mL of toluene and 4 mL of Tabatabai's (1994) modified universal buffer (pH 6) was added. After swirling the flask for a few seconds, 1 mL of *p*NPG was added to start hydrolysis reactions and the flask was then placed in a shaking incubator set at 37 ^oC and 100 rpm. After one hour, 1 mL of 0.5 M $CaCl_2$ and 4 mL of Tris(hydroxymethyl)aminomethane (THAM) buffer (pH 12) was used to stop β-glucosidase enzyme activity. The soil suspension was filtered through a Whatman® No. 2 filter paper into a 30 mL polypropylene tube. This process was repeated three times for each soil sample. A blank was prepared as above but which excluded the

addition of *p*NPG. This was used to account for any background absorbance arising from matter from the soil that passed through the filter paper. Absorbance of all samples was measured at a wavelength 405 nm by a UV/vis spectrophotometer (Varian Cary 50). Standards were prepared from *p*NP to convert absorbance into µmol *p*- nitrophenol released g^{-1} soil h^{-1}.

Calculations

$$\text{µmol } p\text{- Nitrophenol released g}^{-1}\text{ soil h}^{-1} = \left(\frac{F_s}{Wt_s * S_d} - \frac{F_b}{Wt_b * S_d} \right) / 139.11$$

Where:

F_s: concentration of *p*-nitrophenol in sample

F_b: concentration of *p*-nitrophenol in the blank

Wt_s: weight of soil sample

Wt_b: weight of blank soil

S_d: dry matter content of soil expressed as a decimal (determined as in 5.2).

139.11: Molecular weight of *p*- Nitrophenol

2.6.2 Soil phosphomonoesterase activity

The term phosphatases have been used to describe a broad group of enzymes that catalyse the hydrolysis of phosphate-ester bonds. Although, there are many phosphatases involved in hydrolysis of phosphorus compounds, phosphomonoesterases play the most important role in organic phosphorus mineralization (Anna et al. 2012). They are divided into two distinct categories based on their pH optima, pH 6.5 for acid phosphatases and pH 11.0 for alkaline phosphatases (Eivazi and Tabatabai 1977; Pang and Kolenko 1986). Acid phosphomonoesterase was selected to be determined in the present study because the pH of agricultural soil is often manipulated to pH 6.5 to ensure the greatest availability of nutrients.

The method of Tabatabai (1994) to determine phosphomonoesterase activity was followed. The principle of this assay is the measurement of the amount of *p*-nitrophenol (*p*NP) released when phosphomonoesterases hydrolyses the bond linking *p*NP to the phosphate part of *p*-nitrophenyl phosphate (*p*NPP). A 1 g sub-sample of fresh soil (sieved to 2 mm) was transferred to a 50 mL conical flask. Next, 0.2 mL of toluene and 4 mL of Tabatabai's (1994) modified universal buffer (pH 6.5)

was added to the flask, followed by 1 mL of *p*NPP to begin hydrolysis reactions. The flask was swirled thoroughly and incubated at 37 ^{o}C and 100 rpm for 1 hour, after which 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH was added to stop the enzyme reactions. The soil suspension was filtered through a Whatman® No. 2 filter paper into a 30 mL sterilized tube. This process was repeated three times for each soil sample. The blank, which excluded adding *p*NPG, was prepared as described above, was used to account for any background absorbance arising from matter from the soil that passed through the filter paper. Absorbance of all samples was measured at a wavelength 405 nm by a Varian Cary 50 UV/vis spectrophotometer. Standards were prepared from *p*NP to convert absorbance into µmol *p*- nitrophenol released g^{-1} soil h^{-1}.

Calculations

$$\text{µmol } p\text{- Nitrophenol released g}^{-1}\text{ soil h}^{-1} = \left(\frac{F_s}{Wt_s * S_d} - \frac{F_b}{Wt_b * S_d} \right) / 139.11$$

Where:

F_s: concentration of *p*-nitrophenol in sample

F_b: concentration of *p*-nitrophenol in the blank

Wt_s: weight of soil sample

Wt_b: weight of blank soil

S_d: dry matter content of soil expressed as a decimal (determined as in 5.2).

139.11: Molecular weight of *p*- Nitrophenol

2.6.3 Soil total microbial activity

Total microbial activity is a measure of organic matter turnover in natural environments (Schnürer and Rosswall 1982) and it is used to assess soil quality (Nielsen et al. 2002). Fluorescein diacetate (FDA) hydrolysis is used widely as an accurate and simple assay to measure total microbial activity (Schnürer and Rosswall 1982; Adam and Duncan 2001). A wide range of bacterial and fungal extracellular enzymes and membrane bound enzymes can hydrolyse the bonds between the fluorescein and the acetate, releasing the bright yellow dye fluorescein. The amount of fluorescein produced in the sample extracts can be measured by the absorbance of light at 490 nm. The more fluorescein produced, the greater the microbial activity in the soil.

This method adapted the assay using FDA developed by Adam and Duncan (2001). 15 mL of 60 mM potassium phosphate buffer (pH 7.6) was added to a 50 mL conical flask, which contained a 2 g sub-sample of fresh soil (sieved to 2 mm). 0.2 mL of the 1000 µg mL^{-1} FDA stock solution was then added to start the reactions. Then, the flask was stoppered and moved to an orbital incubator shaker set 30 ^{o}C and 100 rpm for 20 minutes. After this, 15 mL of 2:1 chloroform/methanol was added to terminate the reactions. The contents of the flask were transferred to a 50 mL centrifuge tube and centrifuged at 2000 rpm for 3 minutes to collect the supernatant, which was then passed through a Whatman® No 2 filter paper in a 30 mL polypropylene tube. This was repeated three times for each soil sample, with a blank prepared without the addition of FDA stock used to correct for background absorbance caused by matter from soil, after passing through the filter paper. Fluorescein concentrations of the soil extracts were measure by absorbance at 490 nm using a Varian Cary 50 UV/vis spectrophotometer.

Calculations

After measuring mg fluorescein released per gram of dry soil by the activity of microorganisms and determining the moisture content of the soil, the activity can be calculated by the below formula:

$$\text{µg fluorescein released g}^{-1}\text{ soil h}^{-1} = \left(\frac{F_s * 20}{Wt_s * S_d} - \frac{F_b * 20}{Wt_b * S_d} \right) \text{ x } 3$$

Where:

F_s: concentration of fluorescein in sample

F_b: concentration of fluorescein in the blank

Wt_s: weight of soil sample

Wt_b: weight of blank soil

S_d: dry matter content of soil expressed as a decimal (determined as in 5.2).

2.7 Data analysis

Statistical analysis was conducted with SPSS and R software.

A two-way ANOVA in R vs. 3.4.2 was conducted to find differences between a dependent variable and two independent variables. Data sets were analysed for homogeneity of variance with Levene's test. When this assumption was not met,

data was subject to adjustment using the Heteroscedasticity-Consistent Covariance Matrix Estimator HC3 described by MacKinnon and White (1985) using the R car package (Fox and Weisberg 2011).

One-way ANOVA test in SPSS vs. 20 was conducted to find differences between a dependent variable and an independent variable. Data sets were analysed for homogeneity of variance with Levene's test. When assumptions were not met, data were log10 transformed or Welch's robust ANOVA used as appropriate.

Correlations between variables were determined by Spearman's rank order method, conducted by using SPSS vs. 20.

Chapter 3. DETERMINATION OF SOIL PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES

Abstract

Ciliated protozoa are abundant in soil habitats. They have strong correlations with soil properties because they are sensitive to changes in environmental factors. Therefore, in this Chapter, soil properties including soil texture, pH, moisture, air and soil temperatures, essential nutrient factors, soil organic matter (SOM), inorganic nitrogen, trace metals, and enzyme activities of β-glucosidase, acid phosphomonoesterase and total microbial activity were investigated in the natural and agricultural study sites in order to find correlations between soil ciliates and soil properties, to interpret the health of the soils in the study sites and to assess the potential of soil ciliates as bio-indicators of soil quality, especially to find correlations between soil ciliates and the activity of soil enzymes which have not been investigated in previous studies. The results showed that there were significant differences in the selected soil properties, including air and soil temperatures, available concentrations of K and S, nitrate-N, ammonium, total concentrations of Al, Cr and Pb, total microbial activity, β-glucosidase and acid phosphomonoesterase among the three ecosystems and sampling months. Content of nutrients including available P, SOM and accumulation of ammonium, the enzyme activities of β-glucosidase and total microbial activity, and soil moisture were significantly higher in the natural soil than in the agricultural soils. In addition, the natural habitat had relative uniformity and consistence in all most soil properties over two years 2016 and 2017 when changes by seasonal pattern in soil properties at this site were taken into consideration. In general, the Fen is typical for a natural ecosystem with high content of nutrients, high moisture and high enzyme activities while Vicarage Farm and Corfe Castle Farm have soil characteristics typical of cultivated production.

3.1 Introduction

Whilst soil habitats are a suitable environment for free-living protozoa, including ciliates, it is clear that soil properties influence the soil ciliate community (Foissner et al. 2005; Li et al. 2010a; Li et al. 2010b). Foissner (1987) showed that there are three important factors that affect their existence and activity, namely the structure of porous space, the water supply, and intraspecific and interspecific interactions. There are also indications that soil ciliates are adapted to the soil environment. For

example, species of the class Stichotrichia have elongated and narrow shapes to exist between soil particles (Geisen et al. 2018).

Soil texture is dependent on the relative percentages of sand, silt and clay fractions. Soil texture influences physical and chemical characteristics of the soil, for example, it affects decomposition and stability of soil organic matter and has an effect on nutrient availability (Jha et al. 1996; Plante et al. 2006). Similarly, soil pH also has a major influence on soil characteristics. Acidic soil conditions can restrict the activity of organisms, reduce availability of essential nutrient elements or increase toxicity of trace metals such as aluminium (Moir and Moot 2010; McCauley et al. 2017). These examples demonstrate the important effects that soil physio-chemical parameters have on soil organisms. Relationships amongst soil parameters and between them and soil organisms are, however, quite complicated.

Although some previous studies have considered the relationship between soil ciliates and some soil properties, the work was limited with a narrow range of parameters, such as soil pH, moisture, total P, total C, total K and organic matter. In addition, testing the effects of trace metals has been carried out but, mainly conducted under laboratory conditions and on a limited number of soil ciliate species.

Therefore, this study addressed the current gap in knowledge by investigating a wider range of soil physical, chemical and biological characteristics to find correlations between soil parameters and soil ciliated protozoa, in which correlation between activity of enzymes and concentration of trace metal on field condition and soil ciliates have not been studied in previous studies. In addition, physical, chemical and biological properties are also used as indicators of soil quality (De La Rosa and Sobral 2008). Thus, to find out the correlation between soil ciliates and soil properties, to interpret the health of the soils in three study sites and to assess the potential of soil ciliates as bio-indicators of soil quality, the determination of soil physio-chemical and biological properties is needed. The aim of this chapter is to determine soil physical, chemical and biological properties in natural and agricultural soils. It is hypothesised that there are differences in soil properties between natural and agricultural soils, and seasonal and annual changes over two years 2016 and 2017 in soil properties in the natural Fen soil.

The objectives of the work presented in this chapter are:

i) to establish physical, chemical and biological properties of the natural soil;

ii) to establish physical, chemical and biological properties of the agricultural soils;

to find the difference in soil physical, chemical and biological properties between natural and agricultural soils;

iii) to interpret the health of the soils.

3.2 Materials and Methods

Soil samples (see Chapter 2, section 2.2) were collected every month from January 2016 to April 2017 in the natural ecosystem (East Stoke Fen), and agricultural ecosystems (Vicarage Farm and Corfe Castle Farm) were monthly sampled from May 2016 to April 2017, followed by seasonal sampling in both natural and agricultural ecosystems in July, October, and December 2017. Therefore, the data sets were divided into two different groups to be analysed:

i) Differences in soil parameters between natural and agricultural ecosystems were determined using the data set from twelve consecutive months of sampling from May 2016 to April 2017;

ii) The natural habitat is more appropriate to assess seasonal and annual changes on soil properties than the agricultural habitats because inter-year comparisons are made difficult by the impacts of agricultural production, such as tillage and changes in practice from year to year caused by crop rotation (i.e., different crops were grown between years and fertiliser/pesticide applications were altered correspondingly). Hence, only data from the natural habitat was analysed to determine differences in soil properties between seasons over two years 2016 and 2017. Data from four representative months for four seasons was used: April represents spring, July represents summer, October represents autumn, and December represents winter.

The detailed descriptions of methods to analyse soil parameters can be found in Chapter 2, sections 2.4, 2.5, and 2.6.

A two-way ANOVA was used for all statistical analyses in this Chapter to find differences in soil parameters between natural and agricultural ecosystems and to determine if these changes over the year were different between the two habitats. Statistical analyses were conducted using R vs. 3.4.2 (Team 2017) and significance was determined by $p < 0.05$ in all instances. Data sets were analysed for homogeneity of variance with Levene's test. When this assumption was not met, data was subject to adjustment using the Heteroscedasticity-Consistent Covariance

Matrix Estimator HC3 described by MacKinnon and White (1985) using the R car package (Fox and Weisberg 2011).

3.3 Results

3.3.1 Soil texture

Based on percentages of soil particle fractions, i.e., sand, silt, and clay (Table 3.1), soil texture in the Fen and Corfe Castle Farm were defined as sandy loam; meanwhile soil texture in Vicarage Farm was classified as silt loam by the USDA textural classification.

Table 3.1 Mean of components in soil texture of study sites.

Study site	Plot	Sand (%)	Silt (%)	Clay (%)
East Stoke Fen	Plot 1	71.25	13.77	14.98
	Plot 2	67.97	15.61	16.42
Vicarage Farm	Plot 1	22.73	50.86	26.41
	Plot 2	21.17	53.97	24.86
Corfe Castle Farm	Plot 1	72.32	18.32	9.36
	Plot 2	74.60	15.67	7.72

3.3.2 Soil moisture, temperature and pH

Changes in soil moisture in the natural soil at East Stoke Fen (Fen) from January 2016 to December 2017, and in the agricultural soils at Vicarage Farm and Corfe Castle Farm from May 2016 to December 2017 are shown in Tables 3.2 and 3.3. The highest soil moisture was always recorded at the Fen in all sampling months, followed by Vicarage Farm and moisture at Corfe Castle Farm soil was the lowest. Some months, such as July, August, and September of 2016, moisture was decreased sharply in all ecosystems due to lack of rain in these months.

A two-way ANOVA was conducted to find significant differences in soil moisture among the three ecosystems over the twelve-month sampling period (Table 3.3). The analysis indicated that there were significant differences in soil moisture among ecosystems and months ($F_{(2, 36)} = 187.49$, $p < 0.001$ and $F_{(11, 36)} = 5.2$, $p < 0.001$, respectively), but no significant interaction term between ecosystem and month was found ($F_{(22, 36)} = 0.89$, $p = 0.605$). This means that soil moisture in the three ecosystems followed the same pattern over the 12 months.

Changes in soil moisture in the Fen for the four months representing the four seasons over the two years (2016 and 2017) are presented in Figure 3.1. No significant difference in soil moisture in the natural Fen soil was found for the main effects of year and season by a two-way ANOVA ($F_{(1, 8)} = 1.31$, $p = 0.28$, and $F_{(3, 8)} = 0.77$, $p = 0.539$, respectively).

Soil pH in the Fen was acidic (pH~4.5; Tables 3.2 and 3.3), whilst in agricultural soils pH was variable around neutral and alkaline (pH~7.0 to 8.0). Statistical analysis for data collected in 12 consecutive months from May 2016 to April 2017 (Table 3.3) demonstrated that soil pH was significantly different between ecosystems and months ($F_{(2, 36)} = 340.05$, $p = 0.001$, and $F_{(11, 36)} = 2.32$, $p = 0.02$, respectively). However, no significant interaction term was found ($F_{(22, 36)} = 0.65$, $p = 0.85$), indicating that there was not difference in the way soil pH changed with month among the ecosystems.

Differences in soil pH in the Fen in the months representing the seasons over two years 2016 and 2017 are illustrated in Figure 3.1. There was no significant difference in pH for either main effects of year or season ($F_{(1, 8)} = 0.38$, $p = 0.55$, and $F_{(3, 8)} = 0.09$, $p = 0.959$, respectively).

Table 3.2 Mean (mean ± 1 SE) of soil moisture (%), temperature (°C), and pH in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Moisture	Soil Temp	Air Temp	pH
Jan, 2016	Fen	50.7±0.8	N/A	10.0±0.0	4.5±0.1
Feb, 2016	Fen	53.6±5.9	5.3±0.1	6.4±0.0	4.5±0.2
Mar, 2016	Fen	56.6±0.1	8.7±0.0	12.4±0.1	4.7±0.5
April, 2016	Fen	54.0±3.9	7.1±0.4	9.7±0.03	4.6±0.4
Jul, 2017	Fen	31.7±2.8	17.4±0.2	18.5±0.05	4.3±0.1
	Vicarage	15.8±0.5	16.3±0.0	17.8±0.9	7.1±0.1
	Corfe Castle	10.0±0.01	18.3±0.3	19.3±0.3	8.7±0.0
Oct, 2017	Fen	37.3±3.7	12.8±0.1	16.0±0.2	4.2±0.2
	Vicarage	17.7±0.2	13.8±0.05	15.3±0.05	7.6±0.1
	Corfe Castle	15.3±0.5	15.0±0.05	16.1±0.0	8.1±0.0
Dec, 2017	Fen	44.1±5.2	6.6±0.2	8.2±0.5	4.4±0.3
	Vicarage	21.0±0.3	5.4±0.1	7.1±1.3	8.3±0.05
	Corfe Castle	15.5±1.1	7.7±0.2	9.7±0.1	8.5±0.2

Table 3.3 Mean (mean ± 1 SE) of soil moisture (%), temperature (°C), and pH in three ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	Moisture	Soil Temp	Air Temp	pH
May, 2016	Fen	50.2±7.4	10.3±0.3	12.55±0.2	4.5±0.1
	Vicarage	19.8±0.1	15.1±0.2	16.75±0.05	7.0±0.4
	Corfe Castle	15.9±0.8	17.3±0.4	19.90±0.0	6.6±0.05
Jun, 2016	Fen	49.1±8.8	13.7±0.1	15.05±0.1	4.5±0.3
	Vicarage	16.7±0.9	15.6±0.05	15.80±0.1	7.3±0.0
	Corfe Castle	11.6±1.6	17.9±0.5	20.40±0.8	7.0±0.3
Jul, 2016	Fen	28.4±7.5	15.1±0.1	17.70±1.0	4.3±0.3
	Vicarage	14.0±0.3	18.0±1.1	23.20±0.2	7.1±0.2
	Corfe Castle	10.3±3.2	22.7±0.1	29.65±0.8	6.9±0.05
Aug, 2016	Fen	31.0±5.1	16.9±0.1	17.30±0.0	4.3±0.4
	Vicarage	12.1±0.2	18.5±0.2	24.15±0.05	7.0±0.1
	Corfe Castle	9.7±1.9	23.2±0.3	27.75±0.4	7.1±0.3
Sep, 2016	Fen	33.4±3.1	14.5±0.3	15.40±0.1	4.3±0.5
	Vicarage	21.2±0.3	17.3±0.1	24.40±0.6	7.1±0.1
	Corfe Castle	15.5±0.5	22.1±0.3	25.70±0.3	6.8±0.3
Oct, 2016	Fen	40.9±3.6	9.8±0.1	11.15±0.1	4.5±0.4
	Vicarage	15.9±0.0	10.0±0.1	10.15±0.2	7.1±0.05
	Corfe Castle	15.0±0.6	11.9±0.1	13.10±0.8	7.0±0.05
Nov, 2016	Fen	47.8±5.75	7.1±0.3	7.60±0.0	4.8±0.5
	Vicarage	22.7±0.0	8.8±0.05	12.15±0.2	7.8±0.0
	Corfe Castle	16.8±0.7	9.2±0.0	12.15±0.6	7.3±0.05
Dec, 2016	Fen	48.4±3.2	10.6±0.1	13.3±0.0	4.7±0.4
	Vicarage	25.2±0.6	8.6±0.05	11.20±0.0	7.9±0.1
	Corfe Castle	19.2±1.0	10.6±2.05	10.65±2.0	7.1±0.1
Jan, 2017	Fen	48.9± 2.7	4.5±0.8	6.0±0.2	4.7±0.3
	Vicarage	24.7±0.6	1.0±0.1	1.55±0.1	8.0±0.0
	Corfe Castle	10.5±0.1	1.3±0.0	1.40±0.0	7.9±0.3
Feb, 2017	Fen	49.2±5.7	9.7±0.3	12.65±0.4	4.4±0.4
	Vicarage	20.7±0.3	8.5±0.1	11.0±0.4	7.5±0.05
	Corfe Castle	18.2±1.2	9.6±0.05	11.55±0.3	7.0±0.0
Mar, 2017	Fen	48.1±4.8	11.2±0.05	13.25±0.2	4.8±0.5
	Vicarage	23.4±0.05	8.2±0.05	9.15±0.1	7.4±0.05
	Corfe Castle	15.8±2.9	10.7±0.1	10.45±0.05	7.1±0.2
April, 2017	Fen	39.0±12.2	9.1±0.1	13.45±0.6	4.5±0.5
	Vicarage	11.6±0.30	10.8±0.2	12.35±0.1	7.2±0.0
	Corfe Castle	8.90±1.2	13.9±0.2	14.70±0.0	8.0±0.0

Moving to temperature, higher soil and air temperatures were recorded in the agricultural sites than that in the Fen site in almost all sampled occasions (Tables 3.2 and 3.3). The highest were usually in Corfe Castle Farm. Interestingly, in cold months (in winter or early spring) temperature in the Fen was slightly warmer than in the farms.

Differences in soil and air temperature in natural and agricultural ecosystems over the 12 months from May 2016 to April 2017 (Table 3.3) were significant in both parameters for both main effects of ecosystems and months ($F_{(2, 36)} = 155.76$, $p < 0.001$ and $F_{(11, 36)} = 364.04$, $p < 0.001$; and $F_{(2, 36)} = 155.02$, $p < 0.001$ and $F_{(11, 36)} = 458.17$, $p < 0.001$, respectively). Interaction effect between ecosystem and month were observed in both soil and air temperatures ($F_{(22, 36)} = 17.33$, $p < 0.001$ and $F_{(11, 36)} = 39.45$, $p < 0.001$, respectively). Hence, there were differences in the way soil and air temperature differed among the ecosystems over the 12 month sampling period.

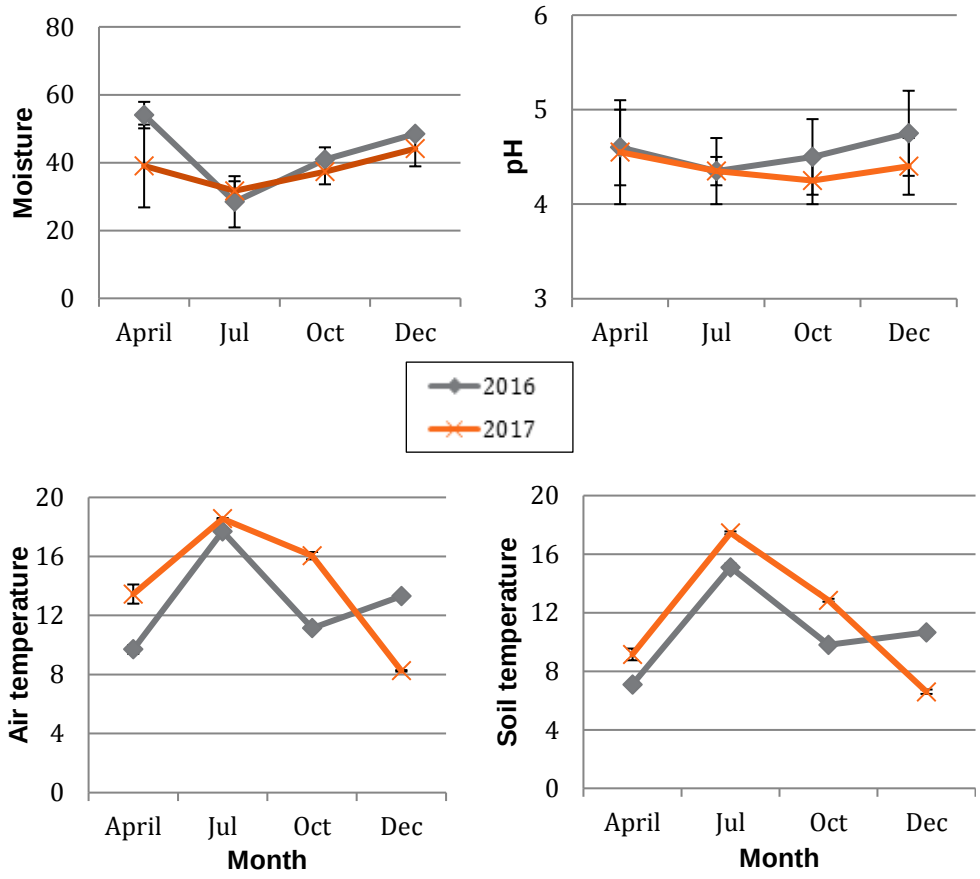


Figure 3.1 Changes in soil pH, moisture (%) and temperature (°C) (mean ± 1SE) in the natural Fen soil of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

In terms of changes in soil and air temperature in the Fen during the seasons over two years, a similar tendency in fluctuation of temperature was shown (Figure 3.1). 2017 had higher soil and air temperatures in spring, summer and autumn in comparison with 2016, but lower than that in winter. The results of statistical analysing showed soil and air temperatures were significantly different in year and season ($F_{(1, 8)} = 33.02$, $p < 0.001$ and $F_{(3, 8)} = 123.89$, $p < 0.001$ and ($F_{(1, 8)} = 32.33$, $p < 0.001$ and $F_{(3, 8)} = 129.16$, $p < 0.001$, for soil and air temperatures respectively). Interaction terms between year and season in soil and air temperatures were significantly different ($F_{(3, 8)} = 128.96$, $p < 0.001$ and $F_{(3, 8)} = 129.19$, $p < 0.001$, respectively), indicating there were differences in effect of year on soil and air temperature by season.

3.3.2 Soil essential chemical elements

Bioavailable concentrations of some soil essential chemical elements in natural and agricultural ecosystems from January 2016 to December 2017, including K, P and S are presented in Tables 3.4 and 3.5. The concentration of bioavailable P was the highest in all sampled months in the Fen compared to the farms, whilst the concentrations of bioavailable K and S fluctuated widely among ecosystems and months. In some sampled months, the concentrations of K and S were higher in the Fen, but not in the others.

The results of the statistical tests analysing the effect of three ecosystems and months on the bioavailable concentrations of K, P and S over the 12 consecutive months from May of 2016 to April of 2017 (Table 3.5) showed that the concentration of K was affected significantly by ecosystem and month $F_{(2, 36)} = 4.59$, $p = 0.016$ and $F_{(11, 36)} = 6.39$, $p < 0.001$, respectively). Similarly, there was significant difference in the concentration of S between habitats and months ($F_{(2, 36)} = 57.65$, $p < 0.001$ and $F_{(11, 36)} = 52.95$, $p < 0.001$, respectively). Interaction terms between ecosystem and month on the available concentrations of K and S were significant ($F_{(22, 36)} = 2.26$, $p = 0.014$ and $F_{(22, 36)} = 34.25$, $p < 0.001$, respectively), indicating there were differences in the effect of ecosystems on the available concentrations of K and S among months.

However, there was only significant difference in the available concentration of P amongst ecosystems, not amongst months ($F_{(2, 36)} = 31.84$, $p < 0.001$ and $F_{(11, 36)} = 0.54$, $p = 0.85$, respectively). No interaction term between ecosystem and month was found ($F_{(22, 36)} = 0.50$, $p = 0.95$). This suggests that there was no difference in the effect of ecosystem on the available concentration of P by month.

Table 3.4 Mean (mean ± 1 SE) of selected soil essential elements (mg kg^{-1}) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Available K	Available P	Available S
Jan, 2016	Fen	280.5±60.8	22.2±18.0	99.7±1.9
Feb, 2016	Fen	275.9±3.1	20.9±10.7	103.1±4.2
Mar, 2016	Fen	226.7±65.4	20.9±18.8	118.3±15.8
April, 2016	Fen	164.8±17.0	9.1±5.6	122.2±1.0
Jul, 2017	Fen	272.9±3.7	10.4±4.5	90.8±25.3
	Vicarage	278.1±18.5	0.5±0.08	59.2±2.9
	Corfe Castle	245.2±2.2	0.7±0.07	45.1±11.1
Oct, 2017	Fen	529.2±111.0	17.4±5.8	153.1±13.5
	Vicarage	729.0±113.1	2.5±0.5	142.4±4.2
	Corfe Castle	284.2±28.9	1.8±0.04	159.9±8.8
Dec, 2017	Fen	374.0±22.5	8.7±5.5	137.5±3.2
	Vicarage	743.7±127.8	3.0±0.02	144.1±5.7
	Corfe Castle	180.4±27.2	1.9±0.1	149.1±11.3

Table 3.5 Mean (mean ± 1 SE) of selected soil essential elements (mg kg^{-1}) in three ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	Available K	Available P	Available S
May, 2016	Fen	167.0±8.2	8.7±5.8	124.7±4.8
	Vicarage	359.3±92.2	1.1±0.1	101.1±5.9
	Corfe Castle	370.1±71.5	0.8±0.1	80.1±4.6
Jun, 2016	Fen	206.6±8.4	15.0±10.4	79.6±10.0
	Vicarage	301.4±65.0	1.7±0.2	84.9±4.9
	Corfe Castle	217.6±37.9	1.2±0.2	88.6±4.6
Jul, 2016	Fen	143.2±41.3	7.9±5.7	78.1±7.3
	Vicarage	173.3±31.4	0.8±0.08	66.0±3.3
	Corfe Castle	213.1±45.1	1.0±0.19	130.7±3.8
Aug, 2016	Fen	156.4±5.3	12.8±4.2	114.9±25.5
	Vicarage	166.4±39.1	1.2±0.4	184.3±11.5
	Corfe Castle	137.0±30.8	0.8±0.1	199.9±6.4
Sep, 2016	Fen	131.9±25.6	9.2±4.9	147.8±0.5
	Vicarage	251.5±46.7	0.8±0.04	202.9±5.5
	Corfe Castle	136.1±18.6	0.9±0.01	208.3±9.6
Oct, 2016	Fen	218.7±14.5	14.7±6.88	360.4±15.1
	Vicarage	239.9±50.1	0.9±0.1	290.6±28.8
	Corfe Castle	154.7±29.9	1.8±0.2	112.4±3.1
Nov, 2016	Fen	350.8±28.5	19.5±12.2	367.1±15.5
	Vicarage	262.6±12.3	1.0±0.1	318.3±7.9
	Corfe Castle	133.0±7.8	1.4±0.17	115.6±7.9
Dec, 2016	Fen	239.7±13.7	13.9±8.77	103.9±23.7
	Vicarage	243.9±3.4	1.2±0.1	103.3±12.2
	Corfe Castle	225.6±49.9	0.8±0.1	158.1±3.8
Jan, 2017	Fen	217.9±25.0	12.3±8.06	95.9±18.8
	Vicarage	220.4±30.2	1.0±0.2	91.5±1.5
	Corfe Castle	157.1±8.6	0.3±0.01	124.0±0.8
Feb, 2017	Fen	192.1±55.7	6.6±1.8	86.8±16.9
	Vicarage	188.3±3.1	1.8±0.08	698.3±74.3
	Corfe Castle	156.6±9.2	0.1±0.005	145.1±9.9
Mar, 2017	Fen	395.2±33.9	25.3±11.19	66.7±17.0
	Vicarage	335.0±20.0	0.9±0.1	35.2±1.3
	Corfe Castle	206.8±9.0	1.2±0.01	31.6±2.7
April, 2017	Fen	283.5±33.97	21.1±7.8	103.5±13.3
	Vicarage	340.7±9.44	1.0±0.08	343.6±2.4
	Corfe Castle	376.4±121.92	0.9±0.16	138.3±65.9

There were large changes in the total concentration of soil essential elements i.e., Ca and P, in the three habitats from January 2016 to December 2017 (Tables 3.6 and 3.7). The concentration of Ca in Vicarage Farm was higher than that in the Fen in all sampling months. In almost all months, Ca concentration in Corfe Castle Farm was the lowest, but in some months of 2017 (from April 2017 to December 2017), the Ca concentration in Corfe Castle Farm increased strongly and was higher in comparison to the Fen, even the concentrations reached to the highest level in the three study ecosystems (Table 3.6). Meanwhile, the lowest total concentration of P was always recorded in Corfe Castle Farm in all sampling months, and the highest concentration was in Vicarage Farm in almost all months.

Table 3.6 Mean (mean ± 1 SE) of selected soil essential elements (mg kg^{-1}) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Total Ca	Total P
Jan, 2016	Fen	1693.0±941.0	611.9±150.3
Feb, 2016	Fen	2477.1± 1088.1	784.8±71.6
Mar, 2016	Fen	2863.0± 2091.5	649.2±111.8
April, 2016	Fen	1965.5± 969.1	585.5±81.3
Jul, 2017	Fen	1772.6± 775.6	577.1±52.8
	Vicarage	3992.8±486.3	699.6±18.7
	Corfe Castle	7524.6±1205.9	381.0±2.4
Oct, 2017	Fen	2121.8± 557.3	787.8±4.9
	Vicarage	4478.4±274.9	943.9±22.9
	Corfe Castle	4539.9±128.4	421.8±0.9
Dec, 2017	Fen	1693.7± 516.1	723.6±76.3
	Vicarage	5733.2±154.6	977.0±26.6
	Corfe Castle	5567.5±1398.3	421.5±13.5

Table 3.7 Mean (mean ± 1 SE) of selected soil essential elements (mg kg^{-1}) in three ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	Total Ca	Total P
May, 2016	Fen	1723.5± 569.8	540.28±60.4
	Vicarage	2939.4±402.7	491.89±52.8
	Corfe Castle	1292.8±84.2	240.12±38.1
Jun, 2016	Fen	1628.1±462.3	538.69±11.8
	Vicarage	4070.2±174.2	396.84±35.8
	Corfe Castle	1388.5±19.5	268.45±21.3
Jul, 2016	Fen	1607.6±607.0	493.43±57.3
	Vicarage	2400.4±513.5	363.48±9.9
	Corfe Castle	1309.4±11.5	227.62±10.6
Aug, 2016	Fen	2016.1±565.6	673.21±21.9
	Vicarage	5169.6±1492.1	797.47±65.9
	Corfe Castle	1548.1±85.9	377.74±1.2
Sep, 2016	Fen	1828.2±700.2	657.62±17.9
	Vicarage	4466.1±893.9	720.38±1.4
	Corfe Castle	1500.5±6.09	373.0±5.8
Oct, 2016	Fen	1800.7±884.5	614.74±91.9
	Vicarage	3503.8±240.3	666.17±8.0
	Corfe Castle	1415.7±71.6	372.34±11.4
Nov, 2016	Fen	2446.4±996.4	823.47±66.0
	Vicarage	5029.7±910.6	800.69±11.0
	Corfe Castle	1715.7±68.1	470.28±1.4
Dec, 2016	Fen	2175.8±542.6	759.29±50.2
	Vicarage	3844.9±245.6	863.93±43.9
	Corfe Castle	1942.5±83.3	505.84±1.9
Jan, 2017	Fen	2418.7±1180.2	727.91±143.5
	Vicarage	4289.4±520.1	778.91±56.6
	Corfe Castle	2034.7±110.9	412.44±6.8
Feb, 2017	Fen	2089.7±686.0	723.65±23.1
	Vicarage	3651.2±330.9	818.51±64.7
	Corfe Castle	1620.7±292.1	345.57±61.1
Mar, 2017	Fen	2310.5±987.1	680.01±38.9
	Vicarage	4987.9±1419.4	706.47±6.4
	Corfe Castle	1879.6±190.4	388.99±8.0
April, 2017	Fen	1977.5±790.5	583.82±74.4
	Vicarage	4436.6±763.0	717.06±31.3
	Corfe Castle	6039.6±1903.2	409.05±16.4

Statistical analysis showed that there were significant main effects of ecosystem and month on the total concentration of Ca in 12 consecutive months (Table 3.7) ($F_{(2, 36)} = 33.71$, $p < 0.001$ and $F_{(11, 36)} = 2.24$, $p = 0.033$, respectively), but there was no significant interaction term between ecosystem and month ($F_{(22, 36)} = 1.32$, $p = 0.22$). Similarly, ecosystem and month significantly affected total concentration of P ($F_{(2, 36)} = 167.48$, $p < 0.001$ and $F_{(11, 36)} = 17.93$, $p < 0.001$, respectively). The interaction was again not significant ($F_{(22, 36)} = 1.69$, $p = 0.07$).

Concerning differences in total and bioavailable concentrations of soil essential elements in the Fen separately, there were large changes in concentrations of all the selected elements during four different seasons over the two years 2016 and 2017 (Figure 3.2). The bioavailable concentration of K in 2017 was higher than that in 2016 over all seasons. The results of a two-way ANOVA analysis indicated that year and season had significant effects on the bioavailable concentration of K ($F_{(1, 8)} = 29.28$, $p < 0.001$ and $F_{(3, 8)} = 5.80$, $p = 0.02$, respectively), but no significant interaction between year and season was found ($F_{(3, 8)} = 2.05$, $p = 0.18$). This means that there was no difference in the way bioavailable K changed over the seasons between the two years.

The bioavailable concentration of S fluctuated widely during the four representative months for the four seasons of 2016 and reached a peak of 360.4 mg kg^{-1} in October of 2016 (Figure 3.2). Significant difference in the concentration of S between year and season was indicated ($F_{(1, 8)} = 17.31$, $p < 0.001$ and $F_{(3, 8)} = 26.18$, $p < 0.001$, respectively) and there was also a significant interaction between season and year ($F_{(3, 8)} = 26.16$, $p < 0.001$).

Trends of the bioavailable and total concentration of P were relatively similar (Figure 3.2). Both measures of P were slightly higher in first three months of 2017 in comparison to 2016, but lower in the month of December. The results of statistical analysis indicated that there were no significant differences in the bioavailable concentration of P over the years and seasons ($F_{(1, 8)} = 0.42$, $p = 0.53$ and $F_{(3, 8)} = 0.49$, $p = 0.69$, respectively). No significant interaction term between year and season was showed ($F_{(3, 8)} = 0.57$, $p = 0.64$). Although there was no significant difference in the total concentration of P over two years ($F_{(3, 8)} = 1.37$, $p = 0.27$), season had significant effect ($F_{(3, 8)} = 4.26$, $p = 0.044$). There was also no interaction effect on the total concentration of P between year and season ($F_{(3, 8)} = 0.99$, $p = 0.44$).

The total concentration of Ca also showed a similar trend as in case of the total concentration of P (Figure 3.2), but the differences in Ca concentrations between two years 2016 and 2017 and seasons were less marked. This was confirmed by a statistical analysis, which showed that there was no significant difference on Ca concentrations between year and season ($F_{(1, 8)}$ = 0.0001, p = 0.99 and $F_{(3, 8)}$ = 0.06, p = 0.97, respectively). Consequently, no interaction effect on Ca concentration between year and season was found ($F_{(3, 8)}$ = 0.99, p = 0. 44).

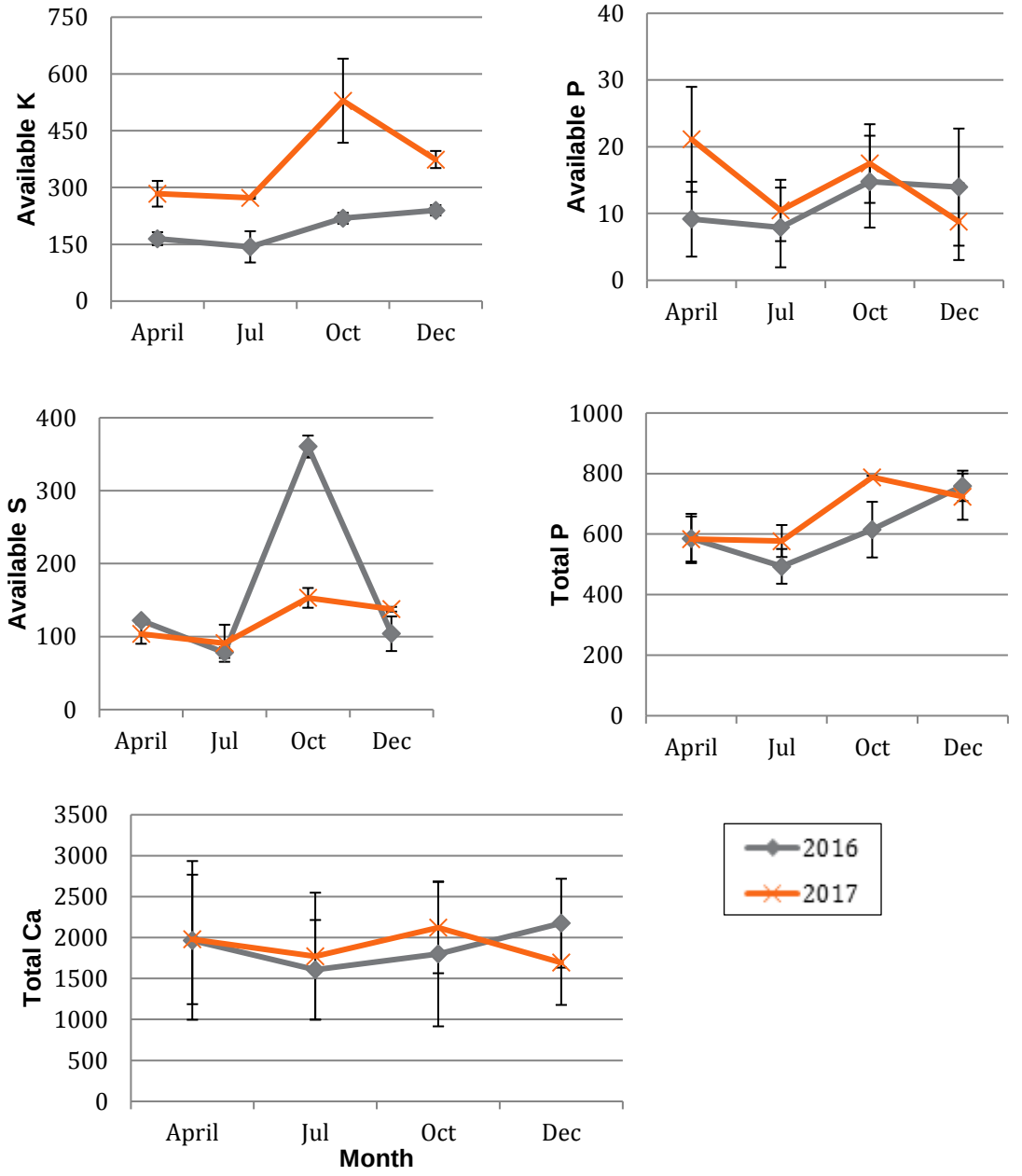


Figure 3.2 Changes in soil essential elements (mg kg^{-1}) in the natural Fen soil of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

3.3.3 Soil organic matter, Nitrate-N and ammonium

Content of soil organic matter (SOM) differed discernibly between the three ecosystems investigated and sampling months (January 2016 to December 2017), the highest percentage of SOM was recorded in the natural Fen soil in all months. Meanwhile, the lowest concentration was found in Corfe Castle Farm (Tables 3.8 and 3.9). The results of two-way ANOVA analysis indicated that type of ecosystem had significant effect on SOM ($F_{(2, 36)} = 162.08$, $p < 0.001$), but there was no significant difference among months ($F_{(11, 36)} = 0.27$, $p = 0.98$). The interaction term between ecosystem and month was also not significant $F_{(22, 36)} = 0.26$, $p = 0.99$).

Table 3.8 Mean (mean ± 1 SE) of SOM (%), nitrate-N (mg kg^{-1}) and ammonium (mg kg^{-1}) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	SOM	Nitrate-N	Ammonium
Jan, 2016	Fen	25.0± 3.9	10.6±7.3	52.7±18.8
Feb, 2016	Fen	27.2±2.2	15.0±3.9	42.2±4.3
Mar, 2016	Fen	35.7±6.3	15.6±7.8	59.3±4.2
April, 2016	Fen	33.3±0.1	10.8±4.9	43.7±4.2
Jul, 2017	Fen	33.2±3.1	2.4±0.7	19.4±3.0
	Vicarage	5.1±0.05	5.8±0.03	10.4±2.7
	Corfe Castle	3.0±0.005	4.7±0.3	9.4±0.2
Oct, 2017	Fen	34.5±5.4	6.0±0.5	105.6±82.8
	Vicarage	7.1±0.4	31.4±4.5	13.9±2.7
	Corfe Castle	3.7±0.1	27.3±5.8	10.4±0.02
Dec, 2017	Fen	30.0±6.4	5.3±0.2	26.1±1.4
	Vicarage	7.4±0.4	9.6±2.4	12.3±1.6
	Corfe Castle	3.3±0.06	2.0±0.5	13.2±1.2

Table 3.9 Mean (mean ± 1 SE) of SOM (%), nitrate-N (mg kg^{-1}) and ammonium (mg kg^{-1}) in three ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	SOM	Nitrate-N	Ammonium
May, 2016	Fen	28.6±3.2	3.2±2.6	58.5±9.1
	Vicarage	4.6±0.4	119.0±49.5	33.0±5.8
	Corfe Castle	3.2±0.1	131.8±11.3	29.8±0.9
Jun, 2016	Fen	32.0±7.8	1.4±1.1	53.7±5.2
	Vicarage	4.9±0.08	63.1±15.5	19.8±0.2
	Corfe Castle	3.1±0.2	10.5±0.7	22.6±2.2
Jul, 2016	Fen	29.0±2.7	2.4±1.4	43.9±1.0
	Vicarage	4.5±0.3	36.5±1.8	15.2±0.01
	Corfe Castle	3.5±0.6	15.0±1.2	21.5±2.6
Aug, 2016	Fen	34.5±10.5	12.3±10.4	42.4±7.2
	Vicarage	5.7±0.5	41.5±3.6	17.0±1.8
	Corfe Castle	2.9±0.1	32.3±2.5	19.2±1.7
Sep, 2016	Fen	24.0± 11.6	3.5±2.7	42.7±7.6
	Vicarage	5.7±0.01	63.3±5.9	17.7±3.8
	Corfe Castle	3.2±0.1	184.6±67.9	55.2±2.0
Oct, 2016	Fen	25.3±4.0	12.9±10.1	47.6±5.2
	Vicarage	5.2±.0.005	69.9±15.7	9.9±1.6
	Corfe Castle	3.1±0.1	20.7±2.8	15.8±0.3
Nov, 2016	Fen	29.5±8.1	3.4±0.6	15.4±7.0
	Vicarage	5.9±0.0	4.5±0.6	5.1±1.4
	Corfe Castle	3.32±0.1	3.0±1.0	3.5±0.3
Dec, 2016	Fen	28.8±4.2	5.5±2.6	6.8±0.03
	Vicarage	4.89±0.2	7.7±1.8	3.7±0.3
	Corfe Castle	2.9±0.04	3.6±1.9	12.0±7.9
Jan, 2017	Fen	33.1±4.8	2.4±0.09	7.4±0.47
	Vicarage	5.3±0.02	6.0±1.1	4.4±0.08
	Corfe Castle	3.3±0.02	3.0±0.04	7.9±0.4
Feb, 2017	Fen	33.7±7.8	6.6±2.8	8.9±0.1
	Vicarage	5.5±0.3	16.8±2.0	5.2±1.0
	Corfe Castle	3.4±0.05	12.3±1.2	8.3±1.5
Mar, 2017	Fen	25.4±3.8	3.6±0.4	8.6±1.9
	Vicarage	5.0±0.4	28.5±1.5	80.4±4.9
	Corfe Castle	3.4±0.05	7.0±1.1	5.3±0.1
April, 2017	Fen	27.0±5.8	2.0±1.2	7.4±0.6
	Vicarage	5.0±0.1	209.5±9.9	163.2±24.4
	Corfe Castle	3.0±0.1	182.5±52.8	47.5±7.5

A slight fluctuation in content of SOM in the Fen was shown over two years and the four representative months for the four seasons. There was higher content of SOM in three months of 2017 (July, October, and December) compared with the same months of 2016, but it was lower in the remaining month (April) (Figure 3.3). The results of statistical analysis indicated that the content of SOM did not differ significantly between years and seasons ($F_{(1, 8)} = 0.44$, $p = 0.52$ and $F_{(3, 8)} = 0.05$, $p = 0.98$, respectively). The interaction term between year and season was also not significant ($F_{(3, 8)} = 1.07$, $p = 0.41$).

The concentration of nitrate was appreciably different between the three types of ecosystem over the sampled months (Tables 3.8 and 3.9). The concentration in both farms, Vicarage and Corfe Castle was much higher than that in the Fen, except in the two months of December 2016 and December 2017 where the concentration of nitrate-N in the Fen soil was higher than that in Corfe Castle soil. The observations in 12 consecutive months from May 2016 to April 2017 in the three study habitats (Table 3.9) were tested for statistical significance. The results of the analysis demonstrated that content of nitrate-N differed significantly among ecosystems and months ($F_{(2, 36)} = 30.74$, $p < 0.001$ and $F_{(11, 36)} = 16.50$, $p < 0.001$, respectively). The interaction term between ecosystem and month was significant ($F_{(22, 36)} = 5.91$, $p < 0.001$), showing that the change in nitrate concentrations over the sampling period followed a different pattern in the three ecosystems.

A large fluctuation of nitrate concentration in the natural ecosystem over two years of 2016 and 2017 and over seasons was also shown (Figure 3.3). Four months (April, July, October, and December) of 2016 had higher nitrate concentrations in comparison to the same months in 2017. However, the results of statistical analysis showed that there were no significant main effects of year and season on nitrate concentration in the Fen ($F_{(1, 8)} = 1.80$, $p = 0.21$ and $F_{(3, 8)} = 0.97$, $p = 0.45$, respectively).

Besides determination of nitrate-N, ammonium - another type of inorganic nitrogen was also determined. The concentrations of ammonium showed clear differences among the three study sites and sampling months. In contrast with high accumulation of nitrate-N in agricultural soils, ammonium concentration in natural soil was higher than that in agricultural soils in almost all months (Tables 3.8 and 3.9). The statistical analysis showed that concentration of ammonium was significantly different among ecosystems and months ($F_{(2, 36)} = 11.36$, $p < 0.001$ and $F_{(11, 36)} = 34.32$, $p < 0.001$, respectively). The interaction effect between ecosystem and month was also significant ($F_{(22, 36)} = 28.26$, $p < 0.001$, showing significant

differences in the way ammonium concentrations changed over time amongst the three ecosystems.

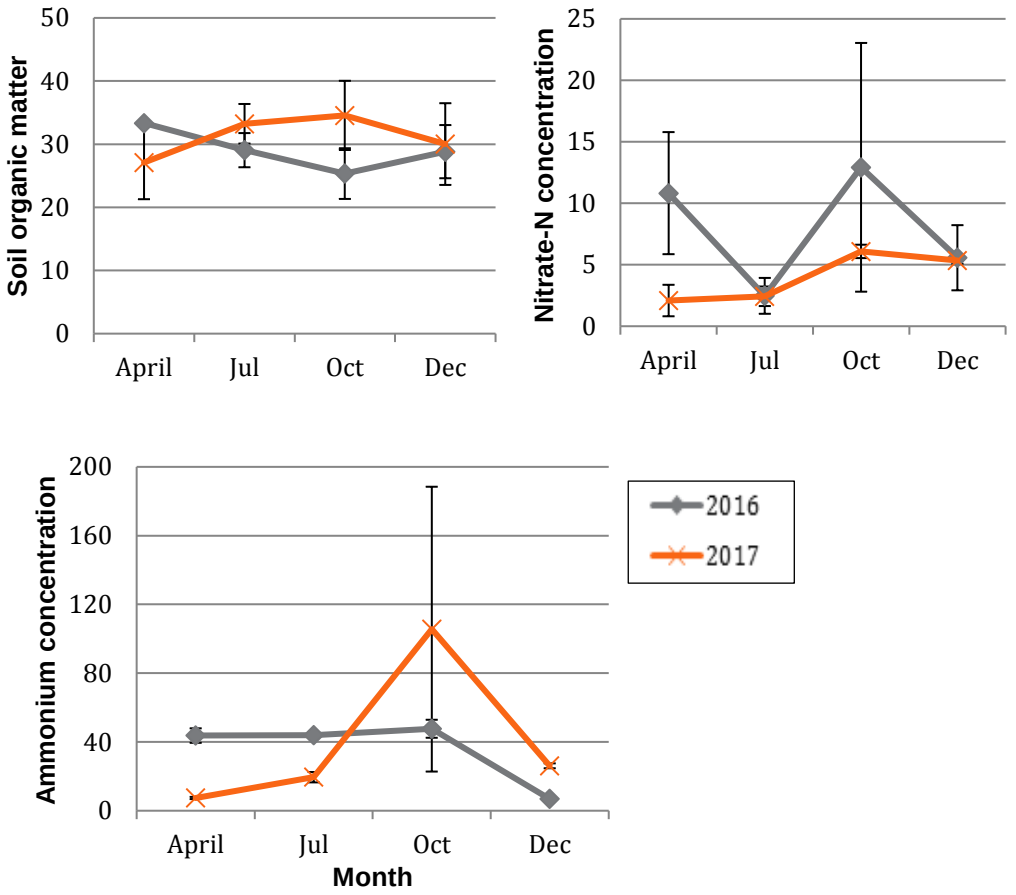


Figure 3.3 Changes (mean ± 1SE) in soil organic matter (%) and nitrate-N (mg kg^{-1}) and ammonium (mg kg^{-1}) in the natural Fen soil of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

A similar trend in the concentration of soil ammonium in the natural habitat between the two years 2016 and 2017 and among the four seasons can be seen (Figure 3.3). There was an increase of ammonium concentration from April to October in both years, but then it decreased in December. Interestingly, there was a very high peak of ammonium concentration in October 2017. However, the results of two-way ANOVA analysis showed that there were no significant differences between two years and amongst four seasons ($F_{(1, 8)} = 0.03$, $p = 0.84$ and $F_{(3, 8)} = 1.65$, $p = 0.25$, respectively) and no significant interaction term was found ($F_{(3, 8)} = 1.07$, $p = 0.41$).

3.3.4 Soil trace metals

Seven trace metal elements were investigated: Al, Cd, Cr, Cu, Ni, Pb, and Zn that normally occupy at low levels in the environment and living organisms only need

trace amounts. At high levels of these trace metals, they can become toxic for organisms (Driscoll et al. 1994). Higher total concentrations of Al were found in the two farm soils compared to the natural Fen soil in all measured months, with very high concentration of Al recorded from Vicarage Farm (Tables 3.10 and 3.11). A consequent two-way ANOVA was conducted to analyses the significance of differences between ecosystems and the 12 months period May 2016 to April 2017 (Table 3.11). The results of the analysis demonstrated that there were significant differences in total Al concentration among ecosystems and months ($F_{(2, 36)}$ = 6901.06, $p < 0.001$ and $F_{(11, 36)}$ = 27.71, $p < 0.001$, respectively). Also, there was a significant interaction effect between ecosystem and month ($F_{(22, 36)}$ = 20.18, $p < 0.001$), showing that there was a difference in the way each type of habitat responded to changes in total Al concentration by month.

Table 3.10 Mean (mean ± 1 SE) of selected trace metals (mg kg^{-1}) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Total Al	Total Cd	Total Cr
Jan, 2016	Fen	3066.3±26.8	0.14±0.07	4.4±0.2
Feb, 2016	Fen	2936.7±10.9	0.20±0.0	5.1±0.2
Mar, 2016	Fen	2392.4±368.4	0.19±0.11	4.5±0.1
April, 2016	Fen	2544.5±55.6	0.14±0.04	4.6±0.1
Jul, 2017	Fen	1453.9±96.2	0.11±0.02	3.7±0.2
	Vicarage	12414.2±177.9	1.37±0.16	17.5±0.1
	Corfe Castle	2061.1±200.8	0.13±0.002	4.5±0.2
Oct, 2017	Fen	1974.4±66.8	0.19±0.009	5.4±0.2
	Vicarage	16806.0±146.2	1.67±0.25	17.8±1.3
	Corfe Castle	2224.9±148.2	0.097±0.0004	4.4±0.1
Dec, 2017	Fen	1755.5±125.2	0.158±0.018	3.8±0.5
	Vicarage	17148.6±312.5	1.70±0.29	17.5±0.2
	Corfe Castle	2284.8±311.4	0.09±0.015	4.6±0.2

Table 3.11 Mean (mean ± 1 SE) of selected trace metals (mg kg^{-1}) in three ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	Total Al	Total Cd	Total Cr
May, 2016	Fen	1766.3±99.6	0.08±0.005	3.9±0.4
	Vicarage	13129.3±441.6	1.34±0.10	15.8±0.9
	Corfe Castle	1901.7±214.5	0.08±0.01	3.9±0.3
Jun, 2016	Fen	1748.2±88.8	0.10±0.01	3.9±0.5
	Vicarage	13662.0±463.8	1.37±0.21	10.2±0.8
	Corfe Castle	2025.9±84.6	0.13±0.0	4.1±0.1
Jul, 2016	Fen	1667.9±156.0	0.13±0.02	4.1±0.5
	Vicarage	13991.5±505.2	1.40±0.19	9.4±0.1
	Corfe Castle	1796.9±124.3	0.15±0.0	4.1±0.1
Aug, 2016	Fen	1518.0±47.5	0.075±0.005	4.0±0.4
	Vicarage	11131.7±141.0	1.75±0.43	24.1±5.3
	Corfe Castle	2004.4±207.9	0.075±0.015	4.4±0.3
Sep, 2016	Fen	1608.6±0.02	0.075±0.015	5.6±1.0
	Vicarage	10452.6±275.1	1.53±0.24	18.9±0.1
	Corfe Castle	2014.5±161.8	0.075±0.005	4.4±0.3
Oct, 2016	Fen	1581.4±73.5	0.05±0.01	4.3±0.5
	Vicarage	8967.5±129.3	1.53±0.19	16.0±0.6
	Corfe Castle	1884.2±133.9	0.09±0.0	5.1±0.05
Nov, 2016	Fen	1376.2±150.8	0.10±0.01	4.5±1.0
	Vicarage	8714.7±473.8	1.85±0.01	18.0±0.3
	Corfe Castle	1667.5±146.0	0.085±0.005	4.6±0.1
Dec, 2016	Fen	1259.7±45.2	0.085±0.005	3.7±0.1
	Vicarage	9004.7±160.7	2.08±0.35	18.3±0.5
	Corfe Castle	1683.8±125.3	0.095±0.015	4.5±0.2
Jan, 2017	Fen	1836.0±307.8	0.14±0.012	4.7±1.1
	Vicarage	12672.1±206.7	1.52±0.26	19.4±1.1
	Corfe Castle	2231.2±190.2	0.129±0.02	7.2±1.5
Feb, 2017	Fen	1778.0±75.2	0.13±0.0	4.4±0.7
	Vicarage	13576.1±374.7	1.63±0.25	22.0±2.5
	Corfe Castle	1823.5±419.6	0.11±0.019	4.5±1.2
Mar, 2017	Fen	1673.0±52.6	0.13±0.003	4.1±0.3
	Vicarage	12251.1±124.8	1.90±0.10	16.5±0.7
	Corfe Castle	2004.3±167.8	0.128±0.004	4.4±0.07
April, 2017	Fen	1557.9±122.4	0.10±0.011	4.1±0.8
	Vicarage	12191.7±335.9	1.67±0.10	17.3±0.09
	Corfe Castle	2220.6±260.3	0.129±0.001	4.6±0.2

Total Al concentration found at the Fen over two years and the four seasons are presented in Figure 3.4. Total Al accumulation decreased over the year 2016, in contrast to 2017 which showed a relative increase. Statistical analysis showed that year had no significant effect on Al concentration ($F_{(1, 8)} = 1.22$, $p = 0.30$), but there was a significant influence of season ($F_{(3, 8)} = 23.45$, $p < 0.001$). The interaction term between year and season was also significant ($F_{(3, 8)} = 23.45$, $p < 0.001$), indicating that there was an effect of year on soil total Al concentration by month

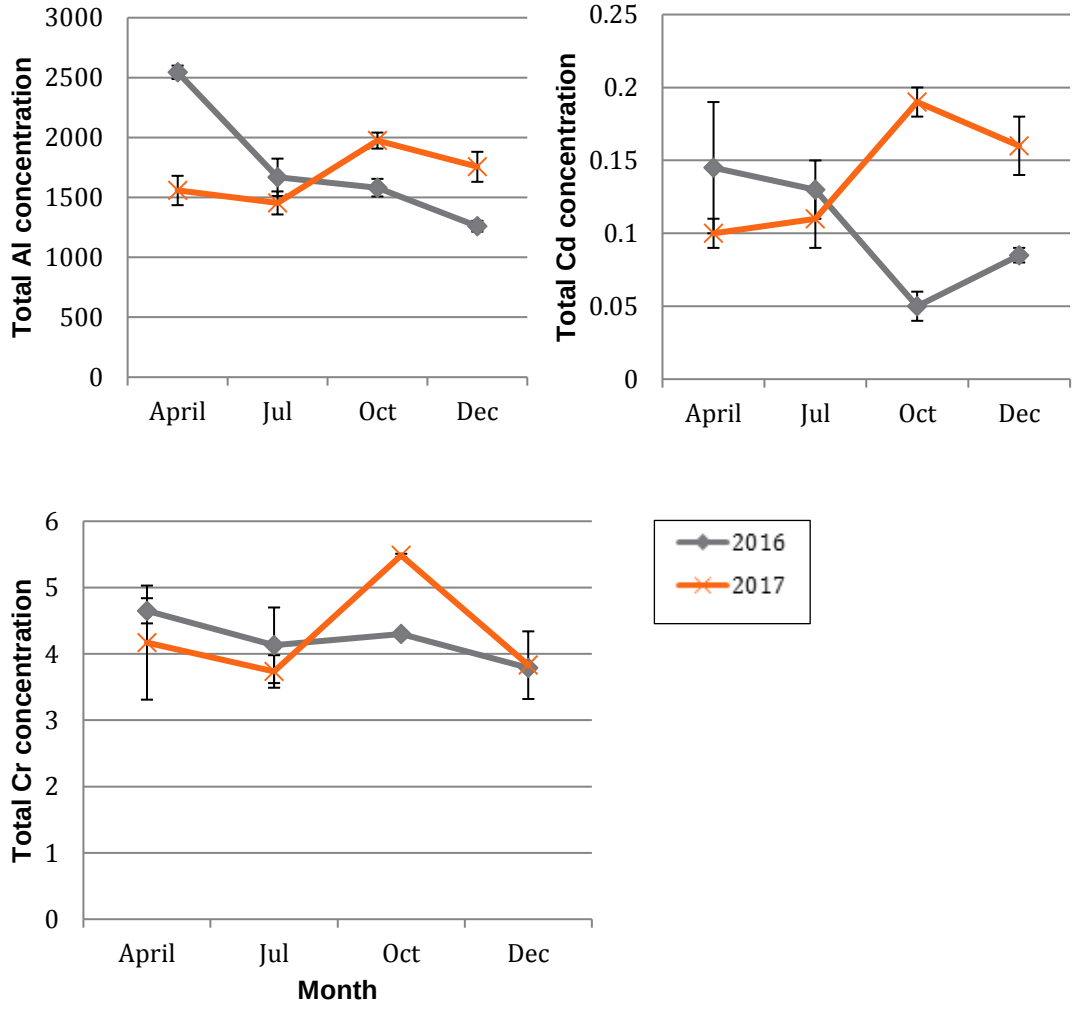


Figure 3.4 Changes (mean ± 1SE) in selected trace metals (mg kg^{-1}) in the natural Fen soil of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

While Al accumulation was very high in all study sites, low total Cd concentration was reported in the period from January 2016 to December 2017 (Tables 3. 10 and 3. 11). The concentration of Cd was always highest in Vicarage Farm in all months

sampled, whilst the concentration was relatively similar between the Fen and Corfe Castle Farm. Statistical analysis for the data set of 12 consecutive months between May 2016 to April 2017 (Table 3.11) indicated that Cd concentration differed significantly among ecosystems ($F_{(2, 36)} = 510.47$, $p < 0.001$), but not among months ($F_{(11, 36)} = 0.93$, $p = 0.52$). The interaction term between ecosystem and month was not significant ($F_{(22, 36)} = 0.97$, $p = 0.51$), showing Cd accumulation in the three ecosystems followed the same pattern over the 12 months

Changes in Cd concentration over two years and the four seasons in the natural Fen soil were found (Figure 3.4). The year 2017 showed an increase from April to October and a decrease in December; meanwhile the inverse trend was seen in 2016. The results of statistical analysis showed that Cd concentration differed significantly between two years and among the four seasons ($F_{(1, 8)} = 6.33$, $p = 0.035$ and $F_{(3, 8)} = 8.27$, $p = 0.007$, respectively). The interaction effect was significant ($F_{(3, 8)} = 8.27$, $p = 0.007$), indicating that there was different in the way year responded to changing Cd concentration by season.

Similar to Cd, the soil total concentration of Cr was the highest in Vicarage Farm and quite similar between the Fen and Corfe Castle Farm (Tables 3. 10 and 3. 11). A two-way ANOVA was also conducted, the results of which showed that Cr concentration differed significantly among ecosystems and months ($F_{(2, 36)} = 465.79$, $p < 0.001$ and $F_{(11, 36)} = 5.34$, $p < 0.001$, respectively). The interaction term was also significant ($F_{(22, 36)} = 4.3$, $p < 0.001$), showing that there was a difference in effect of ecosystem on Cr concentration among months.

In general, there were slight fluctuations in Cr concentration over two years and the four seasons, except for a rapid increase in October of 2017. Consequently, no significant difference was found for the main effects of year and season ($F_{(1, 8)} = 0.07$, $p = 0.79$ and $F_{(3, 8)} = 1.35$, $p = 0.32$, respectively).

Moving to Cu accumulation, changes in the concentration of this trace metal by ecosystems and months since January 2016 to December 2017 are presented in Tables 3.12 and 3.13. The results of a two-way ANOVA test showed that there were significant differences in Cu accumulation among ecosystems and months ($F_{(2, 36)} = 48.08$, $p < 0.001$ and $F_{(11, 36)} = 2.09$, $p = 0.047$, respectively), in which the highest concentration of Cu was at Vicarage Farm in all months sampled, followed by the Fen and Corfe Castle Farm. However, the interaction term between was not significant ($F_{(22, 36)} = 0.85$, $p = 0.64$), indicating that there was the same pattern in the way ecosystem responded to changing Cd concentration by month.

The total concentration of Cu in the natural Fen soil changed slightly over the two years and four seasons (Figure 3.5). A decrease over the year 2016 was observed, while Cu concentration in 2017 fluctuated around a value of 8 mg kg^{-1}. These observations were assessed for statistical significance by a two-way ANOVA. The results of the analysis showed that the concentration of Cu did not differ significantly for the main effects of year and season ($F_{(1, 8)} = 1.79$, $p = 0.21$ and $F_{(3, 8)} = 1.39$, $p = 0.15$, respectively), not was the interaction term significant too ($F_{(3, 8)} = 1.35$, $p = 0.35$).

Table 3.12 Mean (mean ± 1 SE) of selected trace metals (mg kg^{-1}) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Total Cu	Total Ni	Total Pb	Total Zn
Jan, 2016	Fen	7.3±1.2	3.4±0.2	31.1±7.5	23.9±8.5
Feb, 2016	Fen	8.7±0.4	4.1±0.3	32.7±7.4	31.9±8.7
Mar, 2016	Fen	9.7±0.4	3.8±0.1	29.7±12.9	33.4±16.1
April, 2016	Fen	9.7±0.6	3.5±0.04	27.2±8.8	26.6±7.9
Jul, 2017	Fen	7.5±0.4	2.2±0.1	27.4±8.9	24.6±5.0
	Vicarage	14.4±0.7	14.9±0.5	20.3±0.06	65.4±2.4
	Corfe Castle	6.6±0.3	1.1±0.1	11.1±0.6	14.8±0.7
Oct, 2017	Fen	8.8±0.5	4.7±0.03	33.4±13.9	26.3±4.6
	Vicarage	16.9±0.4	18.4±1.5	22.9±0.2	71.8±0.8
	Corfe Castle	6.4±0.4	2.8±0.2	11.2±1.4	12.6±0.7
Dec, 2017	Fen	7.6±0.1	3.5±0.02	38.5±17.6	24.3±6.3
	Vicarage	17.4±1.1	18.1±1.2	24.3±0.1	73.1±2.1
	Corfe Castle	6.3±0.4	3.0±0.3	10.4±1.1	12.8±1.0

Table 3.13 Mean (mean ± 1 SE) of selected trace metals (mg kg^{-1}) in three ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	Total Cu	Total Ni	Total Pb	Total Zn
May, 2016	Fen	8.2±0.1	3.2±0.2	31.9±12.2	24.4±5.1
	Vicarage	14.8±1.7	16.2±0.2	19.6±0.3	53.6±3.7
	Corfe Castle	5.3±0.1	1.7±0.2	10.0±0.7	12.0±0.9
Jun, 2016	Fen	7.7±0.03	3.3±0.3	31.0±11.8	25.7±3.5
	Vicarage	12.7±0.6	15.1±1.0	17.8±0.2	36.4±0.3
	Corfe Castle	6.6±0.1	1.8±0.09	11.6±0.1	13.3±0.1
Jul, 2016	Fen	8.4±0.4	3.2±0.1	29.2±9.7	26.1±5.3
	Vicarage	12.2±0.5	15.6±1.4	19.0±0.05	36.1±0.2
	Corfe Castle	5.8±0.2	1.7±0.1	11.4±0.5	12.3±0.2
Aug, 2016	Fen	10.3±2.0	3.8±0.5	32.1±13.5	25.9±3.4
	Vicarage	17.3±0.5	18.9±2.1	22.8±0.3	71.3±2.3
	Corfe Castle	7.7±0.4	2.4±0.06	12.6±0.5	10.8±0.4
Sep, 2016	Fen	12.0±3.2	4.0±0.7	32.7±11.7	24.6±4.7
	Vicarage	16.3±0.9	17.0±1.4	21.1±0.08	64.1±1.5
	Corfe Castle	7.5±0.3	2.0±0.1	12.5±0.7	9.5±1.4
Oct, 2016	Fen	8.3±0.2	3.4±0.1	32.8±11.1	22.6±7.9
	Vicarage	13.6±0.6	16.1±1.3	20.6±0.5	56.2±2.0
	Corfe Castle	7.6±0.4	2.4±0.03	12.7±1.1	10.8±0.5
Nov, 2016	Fen	6.7±0.01	5.1±0.2	35.0±10.0	34.8±7.7
	Vicarage	12.4±1.9	21.3±1.3	21.2±0.9	67.6±3.9
	Corfe Castle	5.3±0.1	2.7±0.1	12.7±1.0	16.8±0.7
Dec, 2016	Fen	7.4±1.4	4.7±0.9	36.1±15.5	30.6±1.2
	Vicarage	13.3±0.6	24.3±0.07	21.9±0.5	67.5±0.5
	Corfe Castle	5.6±0.005	3.1±0.5	13.2±0.8	16.4±0.5
Jan, 2017	Fen	8.9±1.4	3.4±0.1	33.9±6.6	33.5±9.5
	Vicarage	16.3±1.2	16.6±1.2	21.8±0.6	74.5±3.6
	Corfe Castle	7.6±0.3	2.7±0.8	12.5±0.5	18.0±0.5
Feb, 2017	Fen	19.4±10.85	3.0±0.5	33.3±10.8	32.0±3.9
	Vicarage	16.9±0.3	17.5±1.6	23.1±0.8	84.1±3.1
	Corfe Castle	6.2±1.3	1.4±0.4	10.2±2.1	15.9±3.6
Mar, 2017	Fen	8.4±0.2	2.9±0.2	29.1±10.3	33.4±8.4
	Vicarage	15.9±1.0	19.1±3.9	20.4±0.3	71.1±0.1
	Corfe Castle	6.8±0.07	1.2±0.1	11.0±0.7	16.2±0.9
April, 2017	Fen	7.5±0.01	2.4±0.3	29.6±10.7	26.2±5.6
	Vicarage	14.5±0.78	15.17±0.9	19.9±0.2	108.0±42.3
	Corfe Castle	6.9±0.1	1.3±0.1	12.2±0.3	16.3±0.7

Ni and Zn had a similar pattern in Cu accumulation. The total concentrations of Ni and Zn were the highest in Vicarage Farm and the lowest in Corfe Castle Farm. In contrast, the Fen had the highest concentration of Pb, following by Vicarage and Corfe Castle farms. There were also big changes in Ni, Pb, and Zn over the months sampled (Tables 3. 12 and 3.13).

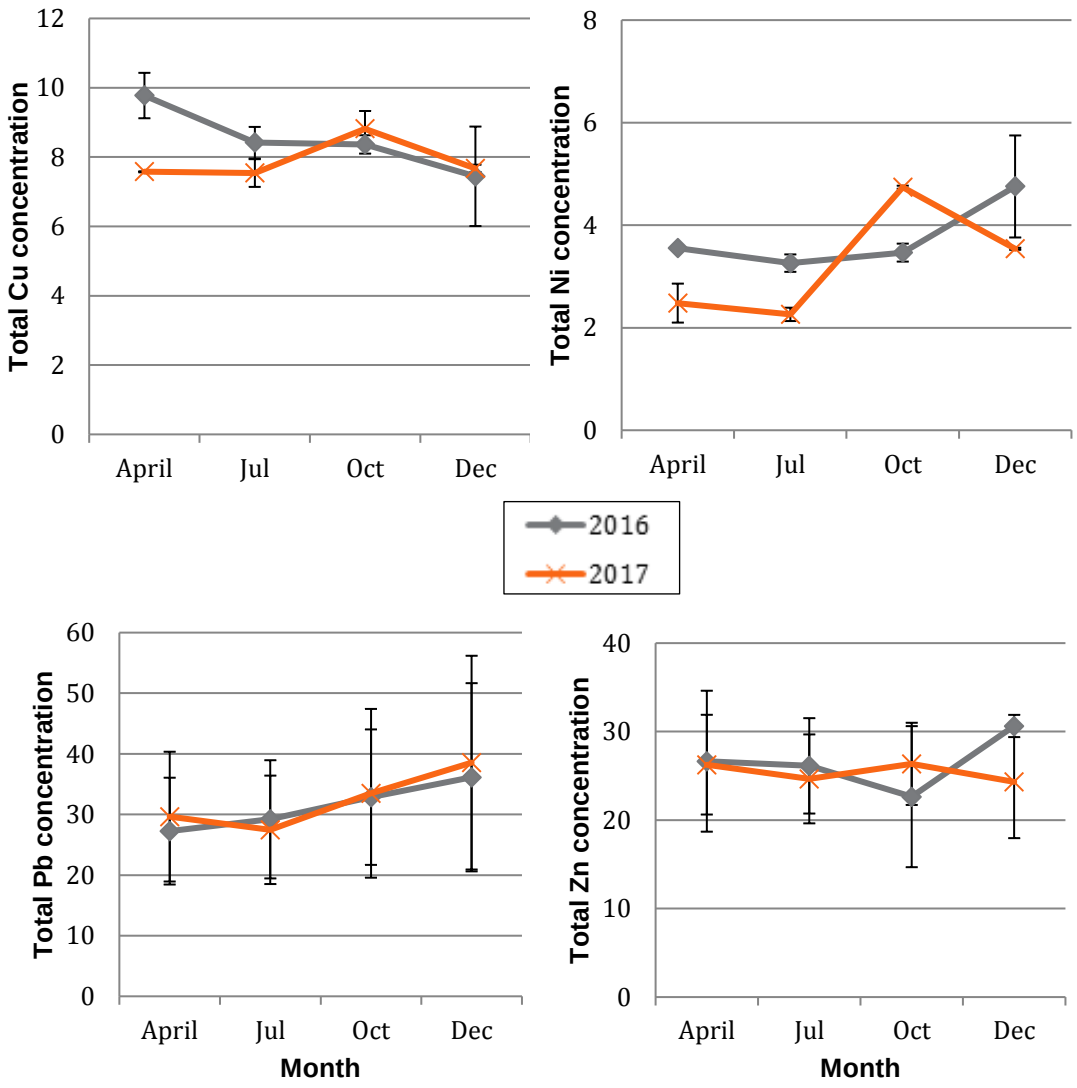


Figure 3.5 Changes (mean ± 1SE) in selected trace metals (mg kg^{-1}) in the natural Fen soil of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

Statistical analysis for the significance of differences in Ni, Pb, and Zn between three ecosystems and 12 months since May of 2016 to April of 2017 (Table 13) indicated that total Ni concentration differed significantly between habitats and months ($F_{(2, 36)} = 844.38$, $p < 0.001$ and $F_{(11, 36)} = 4.54$, $p = 0.047$, respectively), but the interaction term between ecosystem and month was not significant ($F_{(22, 36)}$ =

1.77, p = 0.64). Changes in total Zn concentration showed the same tendency as Ni, i.e., there was significant differences between ecosystems and months ($F_{(2, 36)}$ = 133.92, p < 0.001 and $F_{(11, 36)}$ = 2.82, p < 0.01, respectively), but the interaction term was not significant ($F_{(22, 36)}$ = 1.79, p = 0.057). However, total Pb concentration was only significantly different among three types of habitat ($F_{(2, 36)}$ = 28.60, p < 0.001), but not for months ($F_{(11, 36)}$ = 0.11, p = 0.99). No significant interaction effect between ecosystem and month was showed ($F_{(2, 36)}$ = 0.03, p = 1.0). Hence, there was the same way total Pb accumulated in the ecosystems over the 12 month sampling period.

In general, an increasing trend in total Ni concentration in the natural Fen soil can be seen in both years over the seasons (Figure 3.5). There was no significant difference in total Ni accumulation between years ($F_{(1, 8)}$ = 3.32, p = 0.1), but significant amongst seasons ($F_{(3, 8)}$ = 4.65, p = 0.036). The interaction term between year and season was also significant ($F_{(3, 8)}$ = 4.65, p = 0.036), indicating there was difference in the way year had effect on changing total Ni concentration in soil among seasons. Meanwhile, no significant difference in Pb accumulation was indicated between year and season ($F_{(1, 8)}$ = 0.011, p = 0.91 and $F_{(3, 8)}$ = 0.24, p = 0.86, respectively). Similarly, total Zn concentration also did not differ significantly for the main effects of year and season ($F_{(1, 8)}$ = 0.07, p = 0.79 and $F_{(3, 8)}$ = 0.09, p = 0.95, respectively). No significant interaction effects between year and month on the total concentrations of Pb and Zn were found significantly ($F_{(3, 8)}$ = 0.012, p = 0.99 and $F_{(3, 8)}$ = 0.24, p = 0.86, respectively), indicating that there were no differences in effect of year on accumulations of total Pb and Zn in soil of Fen by month.

3.3.5 Soil enzyme activity

Total microbial activity determined in the three ecosystems over the sampling period is presented in Tables 3.14 and 3.15. The results showed that the activity was much higher in the natural Fen ecosystem in all sampled months compared to the two agricultural ecosystems. Meanwhile, the concentrations of total microbial activity in two farms were only slightly different. Wide fluctuations over the sampling period were also observed in each ecosystem. The results of a two-way ANOVA analysis for data set of 12 consecutive months (Table 3.15) revealed that there was significantly higher total microbial activity in the Fen compared to the two farms amongst ecosystems and months ($F_{(2, 36)}$ = 581.56, p < 0.001 and $F_{(11, 36)}$ = 12.29, p < 0.001, respectively). A significant interaction between ecosystem and month

was also confirmed ($F_{(22, 36)} = 8.99$, $p < 0.001$), indicating that there was different in the way ecosystem responded to changing total microbial activity by month.

Differences in the concentration of total microbial activity during four distinct seasons over two years at the natural study site was also explored (Figure 3.6). In cold months (autumn and winter), higher activity was observed in 2016, in contract lower activity can be seen in warm months (spring and summer). Consequently, There was significant effect of season and year on total microbial activity ($F_{(1, 8)} = 48.30$, $p < 0.001$ and $F_{(3, 8)} = 41.69$, $p < 0.001$, respectively). A significant interaction effect between the two factors, i.e., season and year, was also indicated ($F_{(3, 8)} = 41.68$, $p < 0.001$), indicating that there was a difference in effect of year on total activity in soil of the Fen by season.

Table 3.14 Mean (mean ± 1 SE) of soil microbial activity (μg g^{-1} d.w. h^{-1}) and soil enzymes (μmol g^{-1} d.w. h^{-1}) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Microbial activity	β-Glucosidase	Acid phosphomono-Esterase
Jan, 2016	Fen	578.2±50.5	7.4±1.1	7.0±0.1
Feb, 2016	Fen	600.0±7.3	9.5±0.6	7.2±0.05
Mar, 2016	Fen	606.0±1.5	11.3±3.6	9.8±1.5
April, 2016	Fen	446.2±18.6	1.1±0.1	24.6±1.8
Jul, 2017	Fen	326.6±2.1	7.7±0.4	10.3±1.9
	Vicarage	81.3±2.4	2.6±0.01	20.5±1.5
	Corfe Castle	54.9±7.9	0.9±0.02	19.4±1.2
Oct, 2017	Fen	307.6±10.0	6.7±1.1	9.8±0.5
	Vicarage	88.4±15.4	1.8±0.2	5.7±0.7
	Corfe Castle	73.0±6.0	0.8±0.01	5.8±0.01
Dec, 2017	Fen	337.1±14.2	9.0±1.3	8.5±0.09
	Vicarage	79.6±4.9	1.9±0.2	11.6±0.01
	Corfe Castle	61.4±2.1	0.9±0.01	10.6±0.1

Table 3.15 Mean (mean ± SE) of soil microbial activity (µg g^{-1} d.w. h^{-1}) and soil enzymes (µmol g^{-1} d.w. h^{-1}) in ecosystems of 12 consecutive months from May 2016 to April 2017.

Month	Ecosystem	Microbial activity	β-Glucosidase	Acid phosphomono-sterase
May, 2016	Fen	601.3±96.1	1.16±0.20	14.6±0.8
	Vicarage	91.1±1.1	1.37±0.14	13.2±0.7
	Corfe Castle	124.2±2.8	0.88±0.01	12.5±1.1
Jun, 2016	Fen	437.3±47.2	10.81±0.25	18.8±0.9
	Vicarage	186.2±0.8	1.97±0.02	11.4±0.5
	Corfe Castle	162.2±18.9	1.25±0.13	11.4±0.7
Jul, 2016	Fen	277.8±29.4	8.16±0.21	12.2±0.3
	Vicarage	120.9±0.5	2.15±0.11	11.2±0.1
	Corfe Castle	104.6±6.5	1.26±0.06	14.3±1.2
Aug, 2016	Fen	269.7±58.1	8.88±0.72	13.4±1.4
	Vicarage	220.2±1.0	3.10±0.23	12.2±0.1
	Corfe Castle	166.2±0.3	1.24±0.03	12.6±0.7
Sep, 2016	Fen	401.7±78.8	8.39±0.77	10.6±0.6
	Vicarage	123.8±8.8	2.54±0.28	11.6±0.3
	Corfe Castle	149.9±12.1	1.07±0.02	10.3±0.01
Oct, 2016	Fen	486.5±19.1	6.66±0.38	8.4±1.0
	Vicarage	114.6±17.2	2.44±0.82	8.8±0.1
	Corfe Castle	136.7±1.9	1.12±0.03	7.8±0.5
Nov, 2016	Fen	482.6±58.0	8.80±0.92	12.6±0.1
	Vicarage	131.9±12.9	2.38±0.54	12.9±0.3
	Corfe Castle	191.2±17.3	1.18±0.11	12.3±0.07
Dec, 2016	Fen	628.7±19.3	10.04±.0.03	12.6±0.1
	Vicarage	102.3±6.5	2.42±0.49	10.2±0.2
	Corfe Castle	220.2±7.05	1.91±0.39	10.6±0.3
Jan, 2017	Fen	633.0±37.9	8.79±0.53	12.4±1.0
	Vicarage	122.2±4.9	2.55±0.39	18.0±0.1
	Corfe Castle	258.4±14.8	1.64±0.09	17.4±0.5
Feb, 2017	Fen	614.1±14.1	9.35±0.95	12.6±0.1
	Vicarage	165.9±5.1	2.20±0.26	11.8±0.5
	Corfe Castle	228.8±3.5	1.69±0.005	11.7±0.1
Mar, 2017	Fen	756.0±5.3	10.33±1.24	13.3±0.6
	Vicarage	152.1±13.7	2.36±0.02	10.1±0.1
	Corfe Castle	215.4±16.6	1.79±0.27	9.3±0.4
April, 2017	Fen	489.9±23.5	3.94±1.51	12.5±0.8
	Vicarage	147.2±17.5	1.66±0.22	11.5±0.1
	Corfe Castle	117.0±2.1	0.17±0.10	12.1±0.005

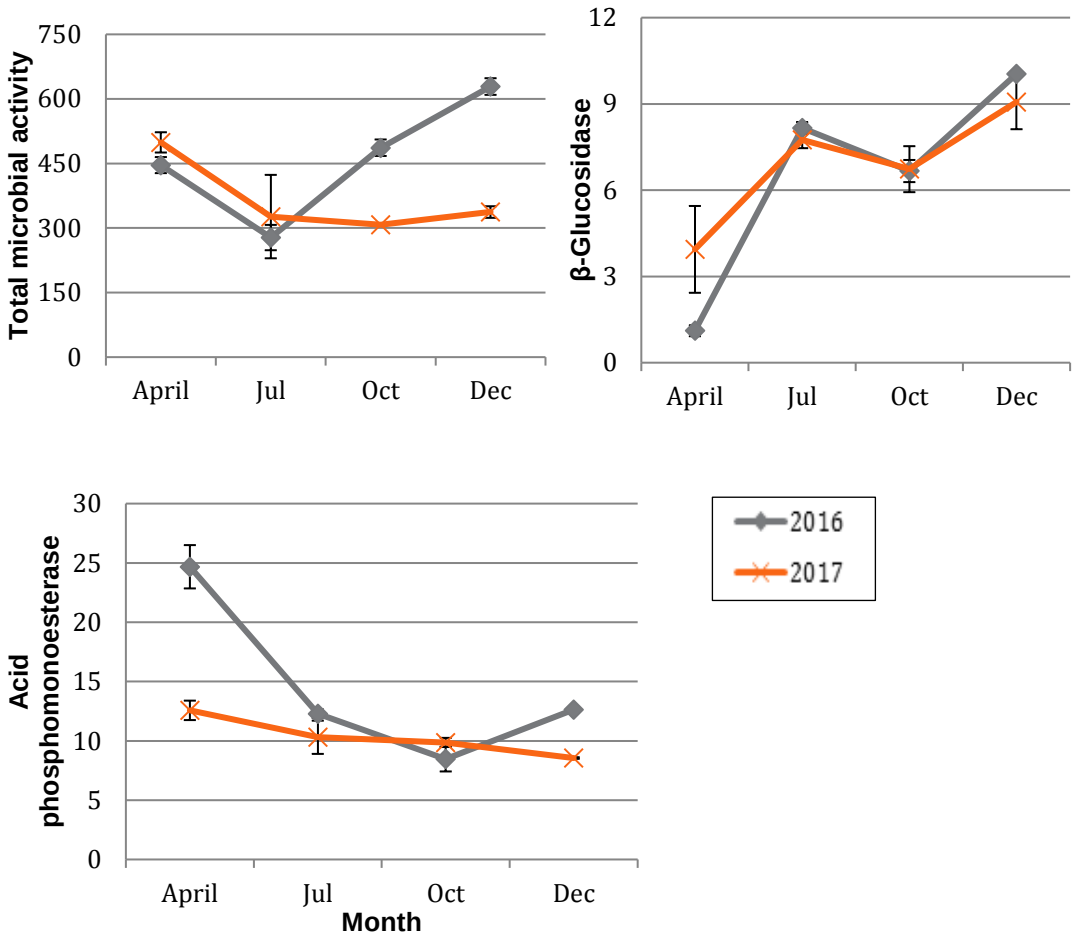


Figure 3.6 Changes (mean ± 1SE) in soil microbial activity (µg g^{-1} d.w. h^{-1}) and soil enzymes (µmol g^{-1} d.w. h^{-1}) in the natural Fen soil of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

Activity of the enzyme β-glycosidase was highest in the natural Fen soil in almost all months, except for May 2016, in which the enzyme concentration was the highest at Vicarage Farm. The concentrations of β-glucosidase in Corfe Castle Farm soil always were the lowest in all observed months (Tables 3.14 and 3.15). When taking the concentration of β-glycosidase in twelve consecutive months from May 2016 to April 2017 of the three different study sites into consideration (Table 3.15), the significance of differences in activity of β-glycosidase for both main effects of ecosystem and month was indicated ($F_{(2, 36)} = 672.26$, $p < 0.001$ and $F_{(11, 36)} = 17.35$, $p < 0.001$, respectively). The interaction term between ecosystem and month was also significant ($F_{(22, 36)} = 9.21$, $p < 0.001$), showing that there was difference in the effect of ecosystem on the enzyme activity following month.

There were changes in the concentration of β-glycosidase following the seasons over the two sampled years in Fen (Figure 3.6), but both years showed a similar

trend in enzyme activity. In spring, the activity was the lowest, and conversely the highest activity was in winter. Significant differences between season in the activity of this enzyme and insignificant differences between two years were indicated ($F_{(3, 8)} = 35.07$, $p < 0.001$ and $F_{(1, 8)} = 0.55$, $p = 0.47$, respectively). Interaction effect between season and year was also not significant ($F_{(3, 8)} = 2.76$, $p = 0.11$), indicating that there were no differences in effect of year on the activity of β-glycosidase in the natural Fen soil by season.

Concerning acid phosphomonoesterase activity in the soils, there was fluctuation between ecosystems and months. In almost all months, this enzyme showed activity that was slightly higher in the Fen than in Vicarage and Corfe Castle farms, but some months, acid phosphomonoesterase activity was much higher in the farms, such as in January, July, and December 2017 (Tables 3.14 and 3.15). Significant difference in the activity of acid phosphomonoesterase between ecosystem and month was showed ($F_{(2, 36)} = 6.9$, $p = 0.0028$ and $F_{(11, 36)} = 26.21$, $p < 0.001$, respectively). There was significant interaction between ecosystem and month ($F_{(22, 36)} = 7.53$, $p < 0.001$). Hence, there was difference in the way the activity of acid phosphomonoesterase differed among the ecosystems over the 12 months.

A similar trend in the activity of this enzyme in Fen's soil by season over two years was demonstrated (Figure 3.6). Higher activity was recorded in the spring month (April) and lower activity in months of remaining seasons over two years. Concentration of this enzyme in year 2016 was higher than in year 2017 in almost all months, except the month of October. The result of statistical analysis showed the activity of acid phosphomonoesterase was significantly affected by year and season ($F_{(1, 8)} = 38.\ 19$, $p < 0.001$ and $F_{(3, 8)} = 17.90$, $p < 0.001$, respectively). Interaction effect between two factors, i.e., season and year was also significant ($F_{(3, 8)} = 17.89$, $p < 0.001$), showing that there was difference in the way the activity of acid phosphomonoesterase changed with season over two years

3.4 Discussion

Soil moisture, temperature and pH

Soil moisture in the natural Fen soil was always much higher than in the agricultural soils, with a range of *ca.* 30-50 % compared with the range of *ca.* 11-23 % and *ca.* 9-19 % in Vicarage Farm and Corfe Castle Farm soils, respectively. This is because of the hydrology of the natural Fen and the greater content of soil organic matter in the Fen in comparison with the other soils. It is widely accepted that increased

organic matter can improve the water holding capacity of soil (Yang et al. 2014; Sujatha et al. 2016) as organic matter has the ability to absorb water well, and it is easy available for plants to uptake this water (Bauer 1974). Thus, available water holding capacity is considered as indicator of soil quality (Reynolds et al. 2002). In addition, it needs to be mentioned here that thanks to the percentage of sand, silt and clay fractions (Table 3.1) and the USDA textural classification, soils of the Fen and Corfe Castle Farm were defined as sandy loam, and soil in Vicarage Farm as silt loam. It has been shown that silt loam soil retains water better than sandy loam soil because the former contains modest organic matter, while organic matter in the latter is low (Costa et al. 2013). The results of the present study also confirmed this, i.e., Corfe Castle Farm soil was a sandy loam with low organic matter, but soil in Vicarage Farm was a silt loam with modest organic matter. Furthermore, silt and clay fractions have the ability to retain water better than sand (Hedley and Yule 2009; Sujatha et al. 2016). Hence, this explains why soil moisture in Vicarage Farm was higher than in Corfe Castle Farm. In addition, Li et al. (2016) showed that soil moisture is positively correlated with rainfall. Observations in the present study also indicated that moisture contents in the three ecosystems increased in wet months (from November to May every year) in comparison with dry months (June to October-less rain than the wet months).

Moisture contents of the natural Fen soil in the four months representing the four seasons in 2016 were higher than in 2017, except for July (Figure 3.1), because the observations in the present study indicated that on/before the sampling dates of April, October, and December 2016 there was rain, but no rain fell before the July 2016. In contrast, before the sampling date of July 2017 it rained, thus moisture of this month was higher in 2017 than in 2016. However, there was no significant difference in the moisture of the natural Fen soil between two years and among 4 seasons.

The Fen study site is a woodland area covered by many trees. Thus, in general during warm months of the year, air and soil temperatures in the Fen were lower than in the two agriculture farms and higher in cold months. In addition, Corfe Castle Farm is low lying, this made air and soil temperatures usually the highest in warm weather conditions. In contrast, owing to a higher elevation, Vicarage Farm always had lower soil and air temperatures compared with the other farm. Besides soil temperature and soil moisture are usually linked; water has the second highest heat capacity of liquids (4.18 KJ kg^{-1} K^{-1}), which means that it takes a lot of energy to heat up. Consequently, wet soils tend to be colder than dry soils. This partially

explains the observations of lower soil temperatures in the Fen and Vicarage Farm sites than in Corfe Castle Farm. Borowik and Wyszkowska (2016) found that soil temperature affected the growth of microorganisms and soil enzyme activity, and optimal soil temperature for development of bacteria and fungi was 15^{o} C. However, their work also indicated that the response of soil microorganisms and enzymes depends more on soil texture and nutrients than temperatures (Borowik and Wyszkowska 2016). Consequently, higher temperatures in summer and colder temperatures in winter would be expected to affect soil microbial enzymes and soil ciliates; this is discussed further in Chapter 4.

Soil and air temperatures in the natural Fen site in 2017 were significantly higher than in 2016 in April, July, and October, except December (Figure 3.1). This might have been affected by the weather. There was an increase in temperature in 2017 compared to 2016 for both April and October, whilst the temperature in December 2017 decreased compared to 2016 (Kendon et al. 2017; Kendon et al. 2018).

With regards to pH, it was shown that soil pH strongly influences the availability of trace metals and nutrients (Giller et al. 1998). For example, Moir and Moot (2010) showed there was sharp increase in the availability of aluminium at pHs below 5.8. In this present study, pH of the natural soil was lower than 5.0, which would be expected to noticeably increase availability of trace metals (Moir and Moot 2010; Berbecea et al. 2011) and thus may result in toxicity of trace metals to living organisms, including soil ciliated protozoa (Forge et al. 1993; Piña and Cervantes 1996; Quiroz-Vázquez et al. 2010). However, the pH of the farm soils, as is typical for agricultural soils, were adjusted to neutral or alkaline values to ensure the greatest availability of nutrients for plant's uptake, and this limits the availability of undesirable trace metals.

No significant changes in soil pH in the natural Fen soil was found between two years 2016 and 2017 and among four seasons (Figure 3.1), probably due to fewer disturbances from human or indeed nature.

Soil essential chemical elements

In all living organisms, K is a dominant macro-element in the cytoplasm. In plants, along with N and P, it is one of the major macronutrients. It plays a vital role in electrical neutralization of inorganic and organic anions and macromolecules, pH homeostasis, control of membrane electrical potential, and regulation of cell osmotic pressure (Nieves-Cordones et al. 2016). It also takes part in the activation of enzymes, protein synthesis, and metabolism of cells (Nieves-Cordones et al. 2016).

Thus, plants need to take up large amounts of K for their growth and survival, but K is generally abundant in soils. Interpretation of K relies on soil texture, for example soils with a higher clay fraction or higher organic matter content have a greater K holding capacity than sandy soils. In low pH, K ions are displaced from cation exchange sites by protons and are then more easily leached from the soil (Gourley 1999). From the results of the present study, Vicarage Farm soils had a high content of small-sized fractions, i.e., silt and clay fractions, thus it is clear why K concentrations in this farm were usually higher than Corfe Castle Farm in almost all sampling months; a range of 160-740 mg kg^{-1} was found in Vicarage Farm compared with 130-370 mg kg^{-1} at Corfe Castle Farm. This shows that available K concentrations of the two farms in general were at medium and high levels if compared with the critical value below which the element limits plant growth (Dinkins and Jones 2013), with the exception for the three months August, September, and November 2016 in Corfe Castle Farm that had low levels (less than 150 mg kg^{-1}) (Table 3.5). The decrease of K concentrations in these months may be corresponding to the end of the agricultural cycle, when crops take up K for their growth. Also, the natural Fen soil had a high content of organic matter, but soil pH at this site was very acidic, thus K may be replaced by protons on cation exchange sites. Consequently, this can partly affect the concentration of K in the natural Fen soil, which oscillated from 130-520 mg kg^{-1}. It needs to be mentioned that NPK fertiliser was applied in March or April each year in the farms. This explains why K concentrations in the farm soils of months from March to May were usually higher than in other months, except for dramatic increases of K concentration to *ca.* 700 mg kg^{-1} in October and December 2017 in Vicarage Farm. It should be noted that the content of soil organic matter in October and December 2017 was higher compared to other sampling months in Vicarage Farm. Hence, the dramatic increases of K concentrations in the two months may be related to increases in soil organic matter. In contrast, the natural Fen soil had no fertilizer input, thus K concentrations in the Fen were usually lower when compared to the farm soils.

To interpret soil chemical elements, a critical soil test value is used to show the soil concentration of any essential elements below which the element limits plant growth (Gourley et al. 2007). It was indicated that critical values for extractable K in the pastures of Australia were in the range of 80-200 mg kg^{-1}, with similar values for many crops (Gourley 1999). Critical values of K also related strongly to soil texture; sandy loam soils have lower values than in clay loam (Gourley et al. 2007). The available concentrations of K in the three ecosystems were in the range of 130 to

nearly 400 mg kg^{-1} (Table 3.5) - this level was higher than critical values of K, this showed that soil K in the three study sites here was sufficient for the growth of plants.

There were significant differences in soil available K concentration between two years (2016 and 2017) and over four seasons in the natural Fen soil. The higher available K concentrations over the four seasons in 2017 were recorded in comparison with 2016. Hunsigi (1975) showed that increasing temperature can increase soil K availability. In the present study, soil and air temperature in 2017 were significantly higher than in 2016, except December. Thus, temperature could be a contributory factor to the increased available concentration of K in 2017 compared to 2016. An increase in soil moisture also increases soil K availability due to increasing K mobility (Claassen et al. 1986). In line with this, December 2017 in this present study had higher moisture than December 2016. Thus, soil moisture was likely a factor accounting for the increase in K concentration in December 2017 when compared to December 2016.

Sulphur is a secondary nutrient element behind N, P, and K. It is an important component of some amino acids and therefore proteins, and it is needed for activation of enzymes in cells (Lewandowska and Sirko 2008). Plants use sulphur in the form of sulphate and there are several factors affecting available sulphate concentrations in soil. Accumulation of organic matter is closely related to increasing organic S (Lewis 1999). Also, mineralisation of S (linked to the organic S levels), supply by fertilizer, and atmospheric deposition affect the amount of soil inorganic S (Lewis 1999). Mineralization of S is orchestrated by micro-organisms and is in turn influenced by soil moisture, temperature and soil organic matter (Lewis 1999). Critical values of extractable S were found to be in the range of 5-10 mg kg^{-1} for pastures in Australia when extracted by two main soil S tests of calcium phosphate and KCl (Lewis 1999; Gourley et al. 2007). Although, as other nutrient elements, it is noted that there are no S critical values for all crops, critical values of extractable S for some crops such as sugarcanes, tropical legume, and Caribbean Stylo investigated in Australia fluctuated from 5 to 10 mg kg^{-1} as also shown for pastures (Lewis 1999; Gourley et al. 2007). It can be seen that S concentration in the ecosystems of this study showed wide fluctuations that changed with the months, ranging between 50-300 mg kg^{-1} or above. The exception was March 2017, when one of the farms had an S concentration of around 30 mg kg^{-1}. The range of S concentrations was much higher than S critical soil test values as shown above. This can be explained by the organic matter in the Fen supplying available S. In the

agricultural soils, changes in available S were strongly related to fertilizer/manure applications. Cardelli et al. (2008) also proved that compost and cattle manure have increased release of SO_4^{2-}-S in soils.

There were significant differences in the concentrations of available S between years (2016 and 2017) and over seasons in the natural Fen soil. In general, available S concentrations were the lowest in July and the highest was in October with a peak of 360.4 mg kg^{-1} in October of 2016. Jaggi et al. (1999) showed that rapid rate S organic mineralization was presumably related to rapid decomposition, which was potentially driven by re-moistening of dry soil in their study. In the present study, there was less rain and higher temperatures in summer, so moisture was lower in July, but then more rain occurred in October. Hence, the soil was re-wetted in October, increasing S organic mineralization, leading to a higher available S concentration in the natural Fen soil.

Calcium is also a secondary nutrient that is important for crops and soil organisms. Calcium can exchange with Na in soil, so contributes to Na removals in soil and improves water infiltration (Silvertooth and Norton 2001). High soil concentration of Na salts have decreased water penetration and infiltration. This is because that Na causes the dispersion of clay particles in soil. These dispersed clay particles can fill up pore spaces in soil which were available for water penetration and infiltration (Silvertooth and Norton 2001). In contrast to Na, Ca contributes to soil flocculation, so an adequate amount of Ca can improve water penetration and infiltration. In agricultural production, gypsum ($CaSO_4$) is commonly used to remove Na in soil, because gypsum increases the levels of Ca. Then, Ca can exchange with Na to create Na_2SO_4 (sodium sulphate) which is mobile and can be leaded from soil (Silvertooth and Norton 2001). Deficiency of Ca is rare, only occurring when soil is very acidic (Bruce 1999), but in both farms, pH is above 6.5, which should provide adequate available Ca for crops. The total concentration of Ca was highest in Vicarage Farm (range of *ca.* 3000-5700 mg kg^{-1}). This is because the soil of Vicarage Farm had a high percentage of clay fraction and so has greater adsorption and capacity to hold Ca than the soil that has a high sand content (Motto and Drake 1982). In addition, geology may also be an important factor affecting Ca concentrations. Tertiary beds which embraced Bagshot Beds are typical of Wareham area, whilst Winfrith is located in an area of Cretaceous Beds (Arkell et al. 1947). Thus, the geology and hence parent materials, underlying Corfe Castle Farm should be Ca poor Bagshot beds, whilst calcareous geology is underlying Vicarage Farm. This obviously affects Ca in overlying soil. Furthermore, higher

organic matter can have the effect of increasing of cation exchange capacity, leading to a rising attractive ability with regard to Ca (Motto and Drake 1982). This reason may explain why the concentration of Ca in the Fen soil (from 1600 to 2800 mg kg^{-1}) was higher than that of Corfe Castle Farm (usually range of 1200-2000 mg kg^{-1}). However, in some months in 2017, the concentration of Ca in Corfe Castle Farm increased rapidly (up to 4500-7500 mg kg^{-1}); this may be due to the farmer applying lime to improve soil pH, so it can be seen that pH from April to December 2017 was higher than the other months.

However, no significant changes in Ca concentrations over two years (2016 and 2017) and over seasons were found in the natural Fen soil (Figure 3.3). This may be due to no human disturbance occurring here (such as lime application) and reflect no effect of differing climate (such as temperature, rainfall) on soil Ca concentrations.

Phosphorus is one of the most abundant elements in soil (Moody and Bolland 1999) and is an essential element in all organisms. It is an important constituent of nucleic acids, cell membranes and the energy carrying compounds of all living cells , such as Adenosine triphosphate (Moody and Bolland 1999). The availability of P in soil is affected by pH, organic matter and soluble Al and cation concentrations (Ch'ng et al. 2014). In acidic soils, pH is a limiting factor of the availability of P owing to the fixation of available P into insoluble salts by Al and other ions (Ch'ng et al. 2014). Adding organic matter is considered a good way to improve the availability of P due to increased soil pH, which may decrease the linkage between Al and P (Yu et al. 2013; Ch'ng et al. 2014). This can explain the findings of the present study in which pH of the natural Fen soil was very acidic, but Plot 1 of Fen had higher pH values (~5.0) than that of Plot 2 (~4.0). Thus, the concentration of available P in Plot 1 was recorded at a much higher concentration than Plot 2, a range of 14-40 mg kg^{-1} compared with 2-13 mg kg^{-1}. In general, the available concentration of P in both farms was much lower than the Fen, despite the relatively equal total concentrations among them. However, the mineralization of organic P is an important process to supply available P for plants (Rita et al. 2013). Consequently, the very high content of organic matter in the natural Fen explains why the available P of the Fen soil was higher than agricultural soils. An index of P availability can be applied to soil (low, medium, high, excess of P). Depending on the type of test/crops/soil texture, the indexes are different. In general, Dinkins and Jones (2013) showed that an available P concentration of less than 8 mg kg^{-1} is a low level. However, in the farm soils in the current study, the concentrations of available

P were very low and fluctuated in a range of 0.15 to 3 mg kg^{-1}. This may cause deficiency of P for crops in these farms (Horneck et al. 2011). Total P concentration in Vicarage Farm was higher than in Corfe Castle Farm, oscillating from 360-970 mg kg^{-1} compared to 220-500 mg kg^{-1} in Corfe Castle Farm. This relates to soil texture, because the higher percentage clay and silt fractions a soil contains, the more P holding capacity it has (Spohn and Kuzyakov 2013). Corfe Castle was a sandy loam with higher sand content, so P holding capacity was low. Besides, although the soil in the Fen also had high sand fraction, it contained high percentage of organic matter. Therefore, high total P was recorded here with the range of 490-820 mg kg^{-1}.

There were no significant differences in total and the available concentrations of P over two years 2016 and 2017 and in the available P concentration over four seasons in the natural Fen soil, but differences were significant in the total P concentration over seasons. Although change in the available concentration of P was not significant over seasons, both available and total concentrations in the present study decreased in July compared with April, October, and December in both two years, except December 2017. Geng et al. (2017) found that the total and available concentration of P reduced from cold to warm sites in Chinese grasslands. Temperature can partly explain the finding of reduction in the total and available concentrations of P in the present study because temperature in July was higher than other months in both years. In addition, P supply depends more on organic inputs and physiochemical processes of soils (Geng et al. 2017), for example increases in soil organic matter and the mineralization of organic P have increased P concentrations in soil (Rita et al. 2013). In the present study, July had less rain and higher temperature, thus moisture was decreased. This can affect degradation of soil organic matter and therefore P mineralisation (Sierra et al. 2015), so lower P concentrations in this month were recorded.

Soil organic matter, nitrate Nitrogen and ammonium

As discussed above, soil organic matter (SOM) or organic carbon plays a vital role in increasing nutrients and water holding capacity and improving soil pH. Also, SOM supplies nutrient for soil organisms and plants due to the soil mineralization process driven by microorganisms because it contains reserves of the essential elements C, H, O, N, P and S. Thus, there is no doubt that SOM relates strongly to fertilizers, productivity and sustainability of terrestrial ecosystems (Craswell and Lefroy 2001). In this study, the natural soil was shown to contain the highest SOM content (range of 25-35 %) in comparison with the two farms because this was a woodland area

and therefore had a higher input of organic material from plants. However, in agricultural soils, SOM accumulation was higher in Vicarage (with 4.5-7.5 %) than that in Corfe Castle (with 3.0-3.8 %); this can be related to soil texture or manure application in Vicarage in March every year (based on practical observations and reports from the farmer). Soil with a high clay fraction also has a higher content of SOM, due to slower decomposition of organic matter (Azlan et al. 2012).

It is believed that a threshold value of soil organic carbon (SOC) is 2 % (*ca.* 3.4 % of SOM), below which there can be the potential for soil quality to decline seriously (Loveland and Webb 2003). Loveland and Webb (2003) reviewed the effects of a reduction in SOC/SOM contents in the soils of England and Wales and noted marked effects on crop yield. Furthermore, Loveland and Webb (2003) showed that agricultural systems require the mineralization of SOM to maintain crop yields, and decreases of SOC/SOM below a certain level may lead to insufficient nutrient release, but this is unlikely to be a problem in temperate agricultural systems where adequate nutrient supply is assured with fertilizers. However, Musinguzi et al. (2016) proved that a Ferralsol with SOC of more than 1.2 % (*ca.* 2 % of SOM) had the highest yield response to applied N. Consequently, if the critical value of 2 % of SOC (*ca.* 3.4 % of SOM) is applied, SOM content at both farms in the present study was equivalent to or even higher than this value. This shows that Vicarage Farm and Corfe Castle Farm soils had normal SOM.

There were no significant differences in the content of SOM between two years (2016 and 2017) and seasons at the natural Fen soil. This shows consistent content of SOM due to annual supply from the deciduous trees and other plants in the area.

Moving to inorganic nitrogen, plants are generally assumed to mainly absorb nitrogen in the inorganic forms of NH_4^+-N and NO_3^--N (Gaines and Gaines 1994). Soluble nitrate-N is easy to lose though leaching, and then it is moved to groundwater. Soil texture affects the loss of NO_3^-N and it has been shown that the presence of silt and clay fractions in the soil was significantly and positively correlated to the accumulation of NO_3^--N (Gaines and Gaines 1994). This can explain in part why nitrate concentrations in Vicarage Farm soil were higher than in Corfe Castle Farm.

In soil nitrification, the process happens when NH_4^+ is oxidised to NO_2^-, and then oxidised again to NO_3^- by soil nitrifying biota. Soil pH has an influence on speed of reactions of nitrification (Sahrawat 2008). Optimum pH for the nitrification process to take place is around 8.5. In acidic pH (less than 5) nitrification is decelerated or

even stopped (Dancer et al. 1973; Sahrawat 2008). Thus, the accumulation of ammonium is high in this type of soil and nitrate-N low. Soil at the Fen was so acidic (pH range of 4 - 5) that very slow or no nitrification would be expected. Therefore, it is clear why the concentrations of nitrate-N were very low (1.4-16 mg kg^{-1}) in this site, but ammonium was much higher (7-105 mg kg^{-1}) in comparison to the neutral/alkaline farm soils. In addition, in cold months, concentrations of NH_4^+ in the Fen were low, probably due to decreased N mineralization rate caused by low temperature (Guntiñas et al. 2012).

A range of temperature from 25-35 ^oC was reported as optimal for nitrifying bacteria (Myers 1975; Sahrawat 2008). The observations from the study sites in this study also showed that in warm months (except for July, 2017) the concentration of nitrate-N was higher than cold months. The concentration of nitrate-N and ammonium were very high in both farms in April, 2017 and the concentration of ammonium in March, 2017 of Vicarage was high. This can be related to fertilizer application as part of the normal agricultural cycle, because the information from the farmer indicated that nitrogen fertilizers were annually applied from April to June in both farms and manure in Vicarage in March.

As discussed above, ammonium does not accumulate in soil if moisture, pH, and temperature is appropriate for soil nitrification process. Soil ammonium concentrations of 2-10 mg kg^{-1} are typical, but are exceed 10 mg kg^{-1}, possible due to cold/extremely wet soil or applied fertilizer (Marx et al. 1996). In this present study, in some months when fertilizer or manure was applied, the concentrations of ammonium exceeded the level of 10 mg kg^{-1}, but in other months from October to February of both years (2016 and 2017), the concentration of ammonium oscillated in the range of typical values. These show the agricultural soils in the present study are typical in ammonium. Similarly, soil nitrate-N concentration is low if it is less than 10 mg kg^{-1} and is excessive if above 30 mg kg^{-1} (Marx et al. 1996). In months where fertilizers/manure was applied or close to application time, the concentrations of nitrate-N were very high (up to *ca.* 209 mg kg^{-1} at Vicarage Farm, and *ca.* 184 mg kg^{-1} at Corfe Castle Farm). However, in other months, nitrate concentrations decreased to less than 10 mg kg^{-1}. Consequently, in both farms of this present study, nitrate may be deficient in cultivated months if fertilizer is not applied.

Although, there were no significant differences in nitrate-N and ammonium in the natural Fen soil over two years and seasons, in general the concentrations of nitrate-N and ammonium were the highest in October of both years in comparison with other months. Guntiñas et al. (2012) also showed that optimal soil moisture for

nitrogen mineralization was from 80 to 100 % soil water holding capacity in the field. Temperatures October were not too cold to inhibit nitrogen mineralization in soil. Also, moisture increased after the drier months of summer, which facilitated the mineralization process. Hence, the increase of nitrate-N and ammonium concentrations in October can be related to moisture and temperature factors.

Soil metals

Concentrations of all selected trace metals in the analysis of this project were in 95 % percentile of rural soils investigated in the United Kingdom (Barraclough 2007) and were therefore representative of rural soils. The concentration of trace metals analysed in this study, namely Cd, Cr, Cu, Ni, Pb and Zn were compared to the standards set in the Finnish legislation for contaminated soil (Ministry of the Environment 2007). The standard values in Finland approximately relate to the mean values of different national systems in Europe (Carlon et al. 2007). All trace metal concentrations analysed were lower than the standard value, with exception of Cd concentration in Vicarage Farm. The standard value for Cd is lower than 1 mg kg^{-1}, but Cd accumulation was slight higher in this habitat with fluctuation at a range of 1.3 to 2.0 mg kg^{-1}. This may relate to manure application in this farm. In addition, Dorset area (UK) has Cd hotpots where soil Cd concentrations can be up to the range of 0.819-1.23 mg kg^{-1} (http://www.ukso.org/nis/Cadmium.html). Hence, either manure application or geology can be factors accounting for high total Cd concentration in Vicarage Farm. Regarding Al, this is most common metallic element, forming up to 8% of the Earth's crust. The typical range of Al concentration is from 10,000-300,000 mg kg^{-1} (EPA 2003). This shows that soils in the natural and agricultural ecosystems in this study meet the requirements for the standard concentration of metals and that the agricultural soils are suitable for agricultural production.

There is no doubting that pH is a main factor affecting the availability of metals. A low pH tends to increase the available trace metal concentration (Rieuwerts 2007; Takac et al. 2009). Xian (1989) indicated that exchangeable Pb, Cd and Zn increased when pH decreased. Soil pH was also strongly related to available Al, with a sharp rise in availability of Al noted below pH 5.8 (Moir and Moot 2010). It was also shown that Al is only considered a contaminant of potential concern for ecological risk assessment if soil pH is less than 5.5 (EPA 2003). Thus, Al was selected for analysis in this study to assess Al effects on ciliates because of its presence at a relatively high concentration in the study sites and the pH of the natural Fen soil, which was lower than 5.0. At acidic pHs, the increased availability

of Al can affect soil living organisms (Moir and Moot 2010). Although in this Chapter, just total concentration of the seven metals was presented, the available metal concentrations were also recorded. Available concentrations (data shown in Appendices) of selected metals were usually determinable in the Fen only (levels were below detection levels in the farm soils) and Plot 2 always showed higher concentration in all selected metals than that in Plot 1. This can be related to soil pH, as soil pH in the Fen was so acidic and pH in Plot 2 was lower than Plot 1.

In addition to pH, soil texture and soil organic matter also have an impact on soil metal accumulation. SOM has a positive correlation with the accumulation of soil trace metals, especially for Pb, which is accumulated in rich-organic soil (Rieuwerts 2007). Consequently, soil total Pb concentration was observed to be the highest at the natural Fen soil, despite there being no source of Pb contamination in the Fen. Furthermore, total concentrations of all other selected metals were higher in the natural Fen soil than in Corfe Castle Farm soil. Yao et al. (2015) found that decreasing sediment particle size increased the concentration of trace metals, because high levels of silt and clay increases the surface area for metal sorption. Accordingly, total concentrations of all selected metals, except for Pb, were highest in all sampling months in the Vicarage Farm soil. In addition, manure was applied in two years in Vicarage Farm. This could be a factor contributing to high levels of total trace metals in this farm because composted manure is a significant metal source (Vukobratovic et al. 2014).

The total concentrations of Cr, Cu, Pb, and Zn in the natural Fen soil did not differ over two years (2016 and 2017) and or during the seasons. However, there were significant differences in total Al and Ni concentrations at the Fen site over the seasons (not between two years). Total Ni concentrations increased in autumn and winter compared to spring and summer, meanwhile total Al concentrations in autumn and winter of 2017 were higher than two other seasons, but a reversed trend was seen in 2016. It may be due to the increased moisture in October after the drier season, which would have facilitated decomposition of organic matter, so trace metal returned to soil and increased metal concentration in October and December. In addition, the several times before/on the sampling dates of October and December there was rain in 2016, but no rain occurred before the sampling dates of 2017. Increased rainfall may have leached metals from the topsoil layer, accounting for the reduction in total Al and Ni concentration in October and December of 2016. In contrast, total Cd concentrations differed significantly over two years and during the seasons. A significant decrease in total Cd was seen in

October and December 2016 compared with April and July 2016, but 2017 had an inverse pattern compared with 2016; total Cd in October and December 2017 was higher in other two months. It is noted that Cd concentrations were very low at the Fen (less than 0.2 mg kg^{-1}) (Figure 3.4), Similarly, rains in several dates before/on the sampling dates of October and December of 2016 can dilute Cd concentration or leach for this metal, thus total Cd concentration decreased in October and December of 2016.

Soil enzyme activity

Soil microbial enzyme activity can be used as a bio-indicator of nutrient dynamics and changes in soil functioning due to agricultural management and ecological factors (Nannipieri et al. 2003). This activity informs the nutrient state and soil health.

The microbial biomass, consisting of bacteria and fungi, plays an important role in decomposition of organic matter. It was reported that total microbial activity had a strong positive correlation with soil organic matter in agricultural soil (Schnürer et al. 1985). In the present study, total microbial activity measured by fluorescein release in the natural Fen had a range of *ca.* 270-620 µg g^{-1} h^{-1}, which was much higher than in the agricultural soils in all sampling months. This may be due to the SOM/nutrient concentration being higher in the natural soil than in the agricultural soils. In the case of the agricultural soils, although SOM content in Vicarage Farm was higher than in Corfe Castle Farm in many months (from September, 2016 to March, 2017), total microbial activity in the latter (from *ca.* 55-260 µg g^{-1} h^{-1}) was greater than in the former (from *ca.* 80-220 µg g^{-1} h^{-1}). It was reported that fine-textured soil has pores with a smaller size than coarse-textured soil, so it is difficult for bacterial to access organic matter (Van Veen and Kuikman 1990). Accordingly, it may be that the high content of silt and clay fractions in the Vicarage Farm soil limited the access of microorganisms to SOM, resulting in lower total microbial activity. In addition, herbicides and fungicides were used in the both farms in this present study. Information obtained from the farmers indicated that the use of these agro-chemicals at Corfe Castle Farm was usually from May to July every year, with some use in September. By contrast, Vicarage Farm used herbicides and fungicides more than at Corfe Castle Farm, applying them annually from February to November. Thus, this could have partly inhibited total microbial activity in the agricultural soils in this present study. Son et al. (2006) detected total microbial activity in the range of 60-300 µg g^{-1} h^{-1} and 60-420 µg g^{-1} h^{-1} during the year at field crop conversion to shrub and at indigenous forest in Korea, respectively. The total

microbial activity in the natural Fen soil was much greater than the results of Son et al. (2006), and the activity in the agricultural soils (Vicarage and Corfe Castle farms) fluctuated around the activity of field crop conversion to shrub. These show that the soils of both natural and agricultural sites had high total microbial activity.

There was a significant effect of season and year on total microbial activity in the natural Fen soil. Total microbial activity in 2016 was the lowest in July, increasing in October and December. This could be due to the dryness in the summer, but October and December had more rain, so soil moisture and hence conditions for microorganisms were improved. It has been shown that the decomposition of soil organic matter increased with increasing soil moisture (Wang et al. 2016), and this means greater total microbial activity. However, total microbial activity in 2017 was lower in October and December compared to these months in 2016. Wang et al. (2016) also showed that the increase of soil temperature stimulated the decomposition of soil organic matter. The reduction of total microbial activity in 2017 may partly relate to a decrease in temperature during the winter.

Similarly, β-glucosidase is also sensitive to changes in SOM, pH, temperature, moisture, and soil management practices (Adetunji et al. 2017). Activity of soil β-glucosidase was higher in the natural soil than in the agricultural soils, and Vicarage Farm had a higher activity than Corfe Castle Farm. This can also be related to SOM content. This was also indicated in the study of Wang and Lu (2006) where β-glucosidase activity was strongly and positively correlated to organic matter content. However, soil pH has a negative correlation to β-glucosidase (Eivazi and Tabatabai 1990; Wang and Lu 2006). Soil pH in natural Fen soil was acidic, so would be expected to be a factor influencing the significant difference in activity of β-glucosidase. The activity of β-glucosidase in the natural Fen soil ranged from *ca.* 1.1 to 9.5 μmol g^{-1} h^{-1}; meanwhile this enzyme oscillated from *ca.*1.6 to 3 and *ca.* 0.17-2 μmol g^{-1} h^{-1} in Vicarage Farm and Corfe Castle Farm soils, respectively. Compared with the results of previous studies, which refer to productive agricultural soils (0.19-2.1 μmol g^{-1} h^{-1}) (Eivazi and Tabatabai 1988), olive orchard soil (0.34 μmol g^{-1} h^{-1}) (Sofo et al. 2014), or to active natural soil (0.29-0.65 μmol g^{-1} h^{-1}) (Rastin et al. 1988), the activities of β-glucosidase in the three sites in the present study were greater, and they were in the reliable index for a productive soil.

It is also noted that activity of β-glucosidase decreased sharply in the Fen in April of both years (2016 and 2017), and May 2016. In addition, there was a significant difference in the activity of this enzyme over the four seasons in the natural Fen site, and the same pattern in seasonal variations was seen in both years (2016 and

2017), in which the lowest activity of β-glucosidase was observed in spring (April) (Figure 3.6). The seasonal variations of soil β-glucosidase were shown in previous work (Steinweg et al. 2013; Ali et al. 2015). Soil moisture and temperature are two factors accounting for seasonal changes in enzyme activities (Steinweg et al. 2013). In this present study, these factors did not clearly reveal their role in seasonal variations in the activity of β-glucosidase. However, it is indicated that more carbon availability through roots or root exudates may explain differences in the activity of β-glucosidase (Hargreaves and Hofmockel 2014; Ali et al. 2015). In addition, β-glucosidase is a catalyst for the hydrolysis of glycosides to release glucose, so β-glucosidase activity may be used to indicate the availability of simple sugar (C source) for growth of microorganisms (Adetunji et al. 2017). In this present study, there are annually strong growths of bluebell plants (*Hyacinthoides non-scripta*) in April and May. It may be that If more C input to the soil occurs via exudates from the roots of the rapidly growing plants, then microorganism will limit excretion of β-glucosidase as it will not be needed to obtain C. Hence, the reduction in the activity of β-glucosidase in this period in the natural Fen soil was found.

In contrast to total microbial activity and of β-glucosidase, the activity of acid phosphomonoesterase in the natural Fen soil was not always significantly higher than that in agricultural soils. The range of activity was of *ca.* 7-24 μmol g^{-1} h^{-1} in the natural Fen soil compared to *ca.* 6 -20 μmol g^{-1} h^{-1} and *ca.* 6-19 μmol g^{-1} h^{-1} in Vicarage Farm and Corfe Castle Farm soils, respectively. In several months, activity of this enzyme was higher in the agricultural soils, and for some months it was relatively equal between the three ecosystems. It is reported that activity of phosphatases correlates positively to the addition of organic matter (Joner and Jakobsen 1995). This provides an explanation for the high activity of this enzyme in the natural soil. However, it was also found that activity of phosphatase increases the availability of P (Adetunji et al. 2017). Thus, if this essential element is limited, the microbial community will excrete more phosphatases to release P from organic matter (Adetunji et al. 2017). In the present study, available P in agricultural farms was low, so this would be expected to stimulate an excretion of extracellular phosphatase to release P for the growth of microorganisms. If compared to the range of 0.37-1.4 μmol g^{-1} h^{-1} in the study of Eivazi and Tabatabai (1977) and the range of 1.3-2.22 μmol g^{-1} h^{-1} in soils found by Debnath et al. (2015), the acid phosphatase activities which detected here are much greater. This shows that soils at all three study sites in this present study are very active biologically.

The activity of acid phosphomonoesterase was significantly affected by year and season in the natural Fen soil. The highest activity of this enzyme was seen in spring (April) of both 2016 and 2017 (Figure 3.6). Seasonal variation in the activity of acid phosphomonoesterase was shown in beech forest soil (Rastin et al. 1988). Rastin et al. (1988) showed that the highest activity of this enzyme was in spring and summer, but lower in autumn and winter. Furthermore, Steinweg et al. (2013) proved that increasing temperature increased the activity of this enzyme. Moreover, the reduction of enzyme production in drought conditions is considered a strategy of resource allocation for survival (Schimel et al. 2007; Ali et al. 2015). Consequently, in the present study, the lower activity of acid phosphomonoesterase in winter and autumn may relate to low temperature, and in summer is due to dryness of soil.

Interpretation of the health of the soils

As discussed above, soil pH in the natural Fen soil was very acidic (pH ~ 4-5), but pH in the agricultural soils was greater than 6.5 to facilitate the greatest nutrient availability. The concentrations of essential element such as K, S, and Ca were sufficient for plant growth. However, concentrations of available K were very low in the agricultural soils, thus fertilizer application would be necessary during crop growth. Also, the SOM content was high in the natural Fen soil and relatively low in the agricultural soils, but the SOM values in the agricultural sites in the present study in general meet the threshold value of soil organic carbon (*ca.* 3.4 % of SOM). In addition, concentrations of nitrate-N and ammonium in the both farms met the critical values of crops because they was added from fertilizer application. The concentration of ammonium in the natural Fen soil were much higher than nitrate-N, this characteristic is typical of an acid soil, because low pH is a known inhibiting factor for the soil nitrification process (Dancer et al. 1973; Sahrawat 2008). Furthermore, concentrations of trace metals in the three habitats analysed in this study had lower values than the standard values, with exception of Cd concentration in Vicarage Farm (Ministry of the Environment 2007). Although total concentrations of selected trace metals were higher in the agricultural soils than in the natural Fen soil, due to the neutral to alkaline pH, available concentrations of almost selected metals were not detected in Vicarage and Corfe Castle farms. Analysis and comparison of enzyme activities of total microbial activity, β-glucosidase, and acid phosphomonoesterase with previous studies (Eivazi and Tabatabai 1977, 1988; Rastin et al. 1988; Son et al. 2006) showed that soils in the Fen, Vicarage Farm, and Corfe Castle Farm were very active biologically. It should be noted that soil health is a combination of multi factors, including macronutrients

such as P, N, and K, water, organic matter contents, presence of contaminants such as trace metals, soil organisms such as bacteria, fungi, protozoa and worms (De La Rosa and Sobral 2008). In addition, the accepted concept of soil quality is in regards to agricultural production (Nortcliff 2002). Therefore, the soil properties in the three ecosystems in the present study, especially in term of farmland soils, meet standard values of a healthy soil in terms of macronutrients, soil organic matter, pH, and high biological activity, at least with regards to agricultural production.

3.5 Conclusion

There were significant differences in the selected soil properties investigated amongst the three ecosystems and sampling months. There were significant interaction terms between ecosystems and months for air and soil temperatures, available concentrations of K and S, nitrate-N, ammonium, total concentrations of Al, Cr and Pb, total microbial activity, β-glucosidase and acid phosphomonoesterase, indicating there were differences in the effect of ecosystem on changes in soil characteristics over the year. In general, content of nutrients, including available P, SOM and accumulation of ammonium, enzyme activities of β-glucosidase, total microbial activity and soil moisture were significantly higher in the natural soil than in the agricultural soils. Also due to difference in pH (acidic pH at the natural Fen soil, and neutral/alkaline in Vicarage and Corfe Castle farms), although total concentrations of almost all selected metals in Vicarage Farm were the highest, available concentrations of the almost all selected metals were detected and much higher in the natural Fen soil compared with agricultural soils. The exception was for available concentrations of Cr, which were detected in the three ecosystems. Furthermore, availabilities of some metals, i.e., Al, Pb, and Cd in the farm soils were too low to detect. The results showed that the total concentrations of selected trace metals in the three ecosystems where within safe limits, meeting the requirements regarding metal concentrations in soil that is good for agricultural production.

Nevertheless, no interaction terms were indicated for pH, moisture, total and available concentrations of P, total concentration of Ca, SOM, and total concentrations of Cd, Cu, Ni and Zn, showing that there were no differences in the effect of ecosystem on the way these soil properties changed over the months, but they were significant differences amongst three ecosystems.

In conclusion, the Fen is typical for a natural ecosystem with high content of nutrients, high moisture, while Vicarage Farm and Corfe Castle Farm are typical of agro-ecosystems and justify requirements of soil quality in cultivated production.

Trends in the soil parameters in the natural Fen soil over two years and four seasons was shown. There were significant differences in soil and air temperatures, the available concentrations of K, S, the total concentration of Cd, and activity of acid phosphomonoesterase and total microbial activity, but not for the total concentrations of Cr, Cu, Pb and Zn, total concentrations of P and Ca, accumulation of nitrate-N and ammonium, soil moisture, and soil pH. In addition, β-glucosidase, the total concentration of Al and Ni, and available P only changed significantly over the four seasons. In general, the natural Fen site was relatively consistent in almost all soil properties over the two years of the study, because this natural site suffered less impact of changes in environment, and human disturbance.

Chapter 4. DETERMINATION OF THE ABUNDANCE AND SPECIES RICHNESS OF SOIL CILIATED PROTOZOA IN NATURAL AND AGRICULTURAL ECOSYSTEMS AND CORRELATIONS BETWEEN THEM AND SOIL PROPERTIES

Abstract

Ciliates are single-celled eukaryotes that play important roles in food webs by controlling smaller microorganisms and recycling organic matter. They can also be affected by soil properties. In this Chapter, the investigation and comparison of the abundance and species richness of soil ciliates in the natural and agricultural soils investigated were carried out in order to establish the relationships between ciliates and soil properties, and to find out whether ciliates can be bio-indicators of soil quality. The abundance of soil ciliates was significantly higher in the natural soil than in the two agricultural soils. Changes in ciliate abundance showed a clear temporal pattern in the natural habitat. In addition, the abundance and species richness of soil ciliates had strong correlations with several soil properties, both when considering the data from all the three ecosystems combined together and the natural Fen habitat separately. Soil moisture, available concentrations of phosphorus, soil organic matter, total microbial activity and β-glycosidase were positively correlated with the abundance of ciliate after both 4 days and 10 days of incubation, but a negative correlation was found between soil pH and ciliate abundance in the three ecosystems together. Furthermore, there was a positive effect of available phosphorus and soil pH on the richness of soil ciliate species in the natural habitat. Interestingly, the available concentrations of measured metals including Al, Cd, Cu, Ni, Pb, and Zn had strong negative correlations with ciliate species richness in the natural Fen soil, showing toxic effects of metals on ciliate species.

4.1 Introduction

Protozoa are the most abundant phagotrophic organisms in soils (Esteban et al. 2006) and there are almost certainly new ciliate species to be discovered (Foissner 2016), although many are possibly physiological variants of known species (Esteban et al. 2001b). Protozoa in general, and ciliates in particular are considered to have a cosmopolitan distribution (Finlay 2002; Finlay and Esteban 2013), but some researchers claim that many species have endemicity and more restricted distributions (Foissner et al. 2005; Chao et al. 2006).

Foissner (1997c) concluded from the literature that the diversity of protozoa in agricultural ecosystems was sparse. Up to now, studies in this field are still rare. The author stated that testate amoebae were the most important indicator group for agro-ecosystems because of their distinctly lower species richness in this type of soil in comparison with natural biotopes. However, Foissner (1997c) also indicated that amoebae are more difficult to identify than ciliates, hampering their use as bio-indicators. Moreover, ciliates were indicated to be more sensitive to pesticides than amoebae (Petz and Foissner 1989). Ciliates were also sensitive to trace metals (Díaz et al. 2006). However, further research into the changes in abundance and species richness of ciliates is needed to determine if this group may prove useful as bio-indicators. Furthermore, detailed analysis into specific ciliate species that may indicate certain soil parameters should also be considered more greatly, because the current knowledge on this is lacking.

Soil ciliates are very sensitive to changes in environmental conditions (Foissner 1987, 1997c). For example, soil ciliated protozoa have been shown to have strong correlations with soil properties such as pH, moisture, total phosphorus, total carbon, and total potassium (Foissner et al. 2005; Li et al. 2010a; Li et al. 2010b). The impact of metal toxicity on soil ciliate species has also been investigated (Díaz et al. 2006). It therefore seems surprising that little research has taken place on the role of ciliates as bio-indicators of soil quality. However, research on the abundance of ciliates in different soil types is unfortunately scant, which limits the potential use of soil ciliates as bio-indicators. Consequently, the aim of this chapter is to determine the abundance and species richness of soil ciliates in natural and agricultural soils and to determine the correlation between them and soil physical, chemical and biological properties. This investigation provides the opportunity to improve our understanding of ciliate biodiversity in different soil habitats, and to assess their potential as bio-indicators of soil quality. It is hypothesised that there are differences in soil ciliate abundance and species richness between natural and agricultural soils and seasonal and annual changes in the abundance and species richness of soil ciliates in the natural soil.

The objectives of the work presented in this chapter are:

i) to investigate the abundance and species richness of ciliates in a natural soil habitat;
ii) to investigate the abundance and species richness of ciliates in two different agricultural soil habitats;

iii) to determine if and/or how abundance and species richness of ciliates differ between natural and agricultural soils;

iv) to find out whether there are ciliate species typical for each study habitat;

v) to determine the correlation between abundance and species richness of soil ciliates and soil properties in all three ecosystems and in the natural Fen habitat separately.

4.2 Material and methods

Three study sites were selected to investigate the abundance and species richness of soil ciliated protozoa: one nature reserve site (East Stoke Fen, East Stoke, Wareham, and Dorset) and two agricultural sites (i.e., Vicarage Farm, Winfrith Newburgh, and Corfe Castle Farm, Corfe Castle, Wareham, Dorset). At each study site, two plots were designed to analyses. Each study site Sampling was carried out once a month at all study sites. The detailed descriptions of the study sites, sampling methods and investigation of soil ciliates can be found in Chapter 2, sections 2.1, 2.2 and 2.3. Each ciliate species has excystment in different periods of time (Finlay et al. 2000). It means that each species will be observed after different days of incubation. Therefore, the total species richness used in this part of the present study refers to the total number of ciliate species found at 4 days and 10 days of incubation together, not separately.

A two-way ANOVA was used for all statistical analyses in this Chapter to find differences in soil ciliates between natural and agricultural ecosystems and to determine if these changes over the year were different between the two habitats. Statistical analyses were conducted using R vs. 3.4.2 (Team 2017) and significance was determined by $p < 0.05$ in all instances. Data sets were analysed for homogeneity of variance with Levene's test. When this assumption was not met, data was subject to adjustment using the Heteroscedasticity-Consistent Covariance Matrix Estimator HC3 described by MacKinnon and White (1985) using the R car package (Fox and Weisberg 2011).

Correlations between soil parameters and the abundance and species richness of soil ciliates were determined by Spearman's rank order method, conducted by using SPSS vs.20 software and significance was determined by $p < 0.05$ in all cases.

As described in Chapter 3, because of differences in sampling time between agricultural and natural soils, the data sets were divided into different data groups to be analysed:

i) Differences in soil ciliate abundance and species richness between natural and agricultural ecosystems were determined using the data set collected over twelve consecutive months between May 2016 and April 2017;

ii) The natural habitat is more appropriate to assess seasonal and annual changes on ciliate communities than the agricultural habitats because inter-year comparisons are made difficult by the impacts of agricultural production, such as tillage and changes in practice from year to year caused by crop rotation (i.e., different crops were grown between years and fertiliser/pesticide applications were altered correspondingly). Hence, only one model, that of the natural habitat, was analysed to determine differences in ciliate communities between seasons and over two years 2016 and 2017 by using data from four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter;

iii) Correlations between ciliates and soil properties were conducted by using data sets from 15 sampled months from May 2016 to December 2017 in all three ecosystems together.

iv) Due to wide differences of soil properties and the abundance and richness of soil ciliates in Plot 1 and Plot 2 at the natural Fen site, correlations between ciliates and soil properties were conducted by using data sets from the 19 sampled months from January 2016 to December 2017 in the natural Fen ecosystem separately.

4.3 Results

4.3.1 The abundance of soil ciliated protozoa after 4 days of incubation

There were wide changes in the abundance of soil ciliated protozoa after 4 days of incubation in the natural Fen site from January 2016 to December 2017, and in the two farms from May 2016 to December 2017 (Table 4.1 and Figure 4.1). It can be seen that the abundance of ciliates in the natural Fen site was clearly higher than that of the two farms during the study periods. Interestingly, there was big difference in ciliate abundance and in measured soil properties as well between Plot 1 and Plot 2 in the natural Fen habitat. For example, a large difference in ciliate abundance after 4 days was shown between Plot 1 and Plot 2 in May 2016, 1755 ciliates cells were recorded in Plot 1, but only 511 ciliates cell were found in Plot 2 (the distance of differences between Plot 1 and Plot 2 at the Fen are shown through the values of SE in Table 4.1 and Figure 4.1 and in Chapter 3), in which Plot 1 usually had much higher number of ciliates than Plot 2, but in three months, including July, August, September 2016 more ciliate cells were observed in Plot 2.

However, a slight difference in the abundance of soil ciliates after 4 days of incubation was recorded between the two agricultural habitats. In Vicarage Farm, at the start of the sampling process, the abundance of soil ciliates was much higher than in Corfe Castle Farm, but thereafter the number of ciliates increased in Corfe Castle Farm and decreased in the other farm. This led to the number of soil ciliates being very similar between the two farms, even though in some months it was higher in Corfe Castle. In the last three months of sampling, i.e., July, October, and December 2017, there was an apparent increase in the abundance of soil ciliates in Vicarage Farm (Table 4.1 and Figure 4.1)

Table 4.1 The abundance of soil ciliated protozoa estimated mean number of two plots per gram dry weight after 4 days of incubation (mean ± 1SE) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Ciliate abundance after 4d
Jan, 2016	Fen	614±496
Feb, 2016	Fen	924±607
Mar, 2016	Fen	2767±2451
April, 2016	Fen	4487±1148
Jul, 2017	Fen	377±32
	Vicarage	280±13
	Corfe Castle	111±27
Oct, 2017	Fen	415±21
	Vicarage	225±121
	Corfe Castle	98±25
Dec, 2017	Fen	496±107
	Vicarage	488±53
	Corfe Castle	168±28

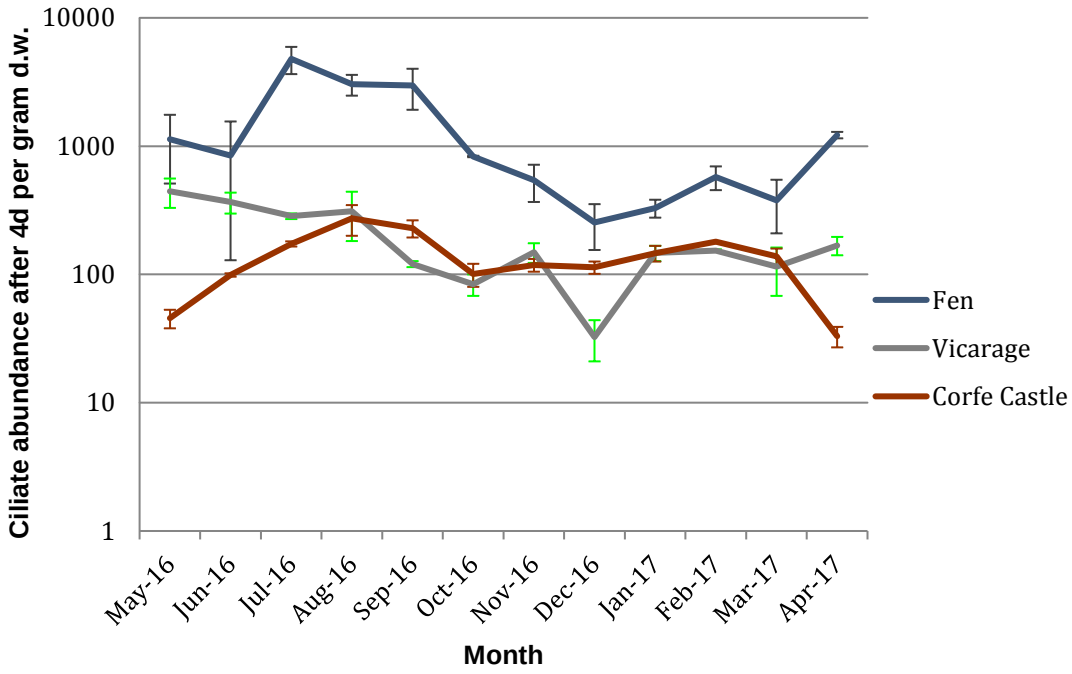


Figure 4.1 Changes in the abundance of soil ciliated protozoa - estimated mean number of two plots per gram dry weight after 4 days of incubation (mean ± 1SE) in three ecosystems from May 2016 to April 2017.

A two-way ANOVA was conducted to determine the significance of difference in the abundance of soil ciliates between the three ecosystems and twelve months from May 2016 to April 2017. The results of the analysis indicated that ciliate abundance after 4 days significantly differed between three ecosystems and months ($F_{(2, 36)}$ = 59.07, p < 0.001 and $F_{(11, 36)}$ = 7.2, p < 0.001, respectively). The interaction between ecosystem and month was statistically significant ($F_{(22, 36)}$ = 6.17, p < 0.001), showing that there was a difference in ciliate abundance between ecosystems by month. To test the difference amongst groups of ecosystems, the Games-Howell in *post-hoc* test in one-way ANOVA was used. The results of this analysis showed that there was significantly higher abundance of ciliates after 4 days of incubation in the natural habitat than in the two agricultural habitats, but no difference between Corfe Caste and Vicarage farms.

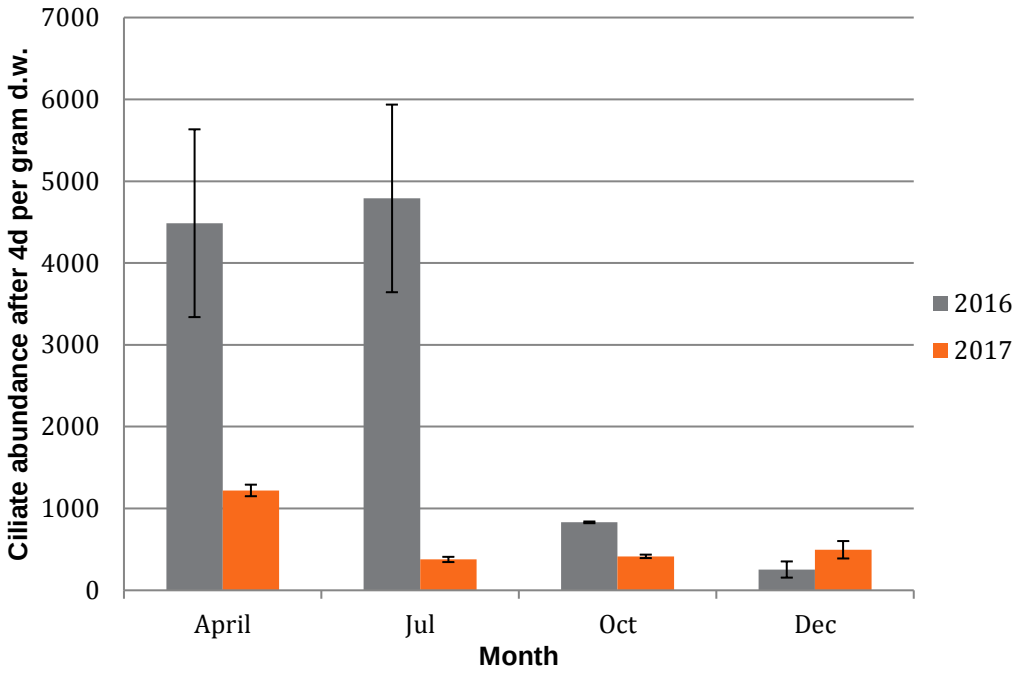


Figure 4.2 Changes in the abundance of soil ciliated protozoa-estimated mean number of two plots per gram dry weight after 4 days of incubation (mean ± 1SE) in the natural ecosystem of four representative months for four seasons: April represents spring, July for summer, October for autumn, and December for winter over two years 2016 and 2017.

It is clear that there were big changes in the abundance of soil ciliates in the natural Fen soil over two years and four seasons (Figure 4.2). The number of ciliates in 2016 was much higher than in 2017 in three seasons, but lower in winter. In general, there was a dramatic decrease in abundance of ciliates in 2017 compared to 2016. Consequently, a two-way ANOVA conducted to explore changes in ciliate abundance by year and season indicated that the number of ciliates after 4 days of incubation in the natural habitat (the Fen) differed significantly between years and seasons ($F_{(1, 8)} = 23.19$, $p < 0.001$ and $F_{(3, 8)} = 7.5$, $p = 0.01$, respectively). The interaction term between year and season was also significant ($F_{(3, 8)} = 7.49$, $p = 0.01$), indicating that there were differences in effect of year on the number of ciliates by season.

4.3.2 The abundance of soil ciliated protozoa after 10 days of incubation

The changes in the abundance of soil ciliates in the three ecosystems after 10 days of incubation are shown in Table 4.2 and Figure 4.3. In general, an increase in the number of ciliates was seen after 10 days in comparison to 4 days in almost all months. Similar to after 4 days, there were large differences in abundance of soil

ciliates between the Plot 1 and Plot 2 of the natural ecosystem. For example, a large difference in ciliate abundance after 10 days was shown between Plot 1 and Plot 2 in May 2016, 3435 ciliates cells were found in Plot 1, but only 1432 ciliates cell were found in Plot 2 (the distance of differences between Plot 1 and Plot 2 at the Fen are also shown through the values of SE in Table 4.2 and Figure 4.3). In 2016, the abundance was higher in Plot 1 than in Plot 2, except in August and September, but this trend was reversed in months of 2017, except December.

Meanwhile, the abundance of soil ciliates in agricultural soils decreased apparently after 10 days in comparison to after 4 days (Table 4.2 and Figure 4.3). In both farms, very low ciliate abundance was recorded in almost all months. The number of cells was lower than 100 cells, even less than 10 cells per gram soil dry weight in some sampling months (Figure 4.3), except an observation of 304 ciliate cells per gram Vicarage Farm in May.

Table 4.2 The abundance of soil ciliated protozoa estimated mean number of two plots per gram dry weight after 10 days of incubation (mean ± 1SE) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Ciliate abundance after 10d
Jan, 2016	Fen	1063±786
Feb, 2016	Fen	1619±880
Mar, 2016	Fen	3324±1112
April, 2016	Fen	4854±1020
Jul, 2017	Fen	994±141
	Vicarage	84±36
	Corfe Castle	29±3
Oct, 2017	Fen	694±303
	Vicarage	112±97
	Corfe Castle	25±4
Dec, 2017	Fen	349±23
	Vicarage	159±93
	Corfe Castle	42±4

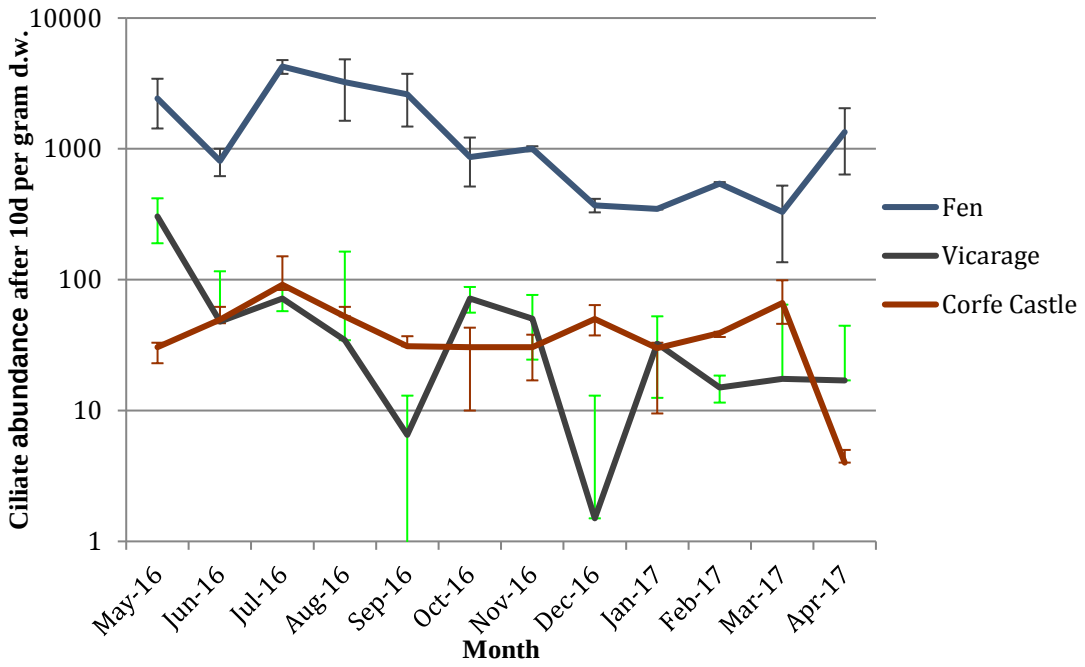


Figure 4.3 Changes the abundance of soil ciliated protozoa- estimated mean number of two plots per gram dry weight after 10 days of incubation (mean ± 1SE) in three ecosystems from May 2016 and April 2017.

The abundance of soil ciliates in natural soils after 10 days of incubation was also much higher than that in agricultural soils in all months (Table 4.4 and Figure 4.3). These observations (from May 2016 to April 2017) were tested for statistical significance by a two-way ANOVA in which the dependent variables were the abundance of soil ciliates after 10 days of incubation and the independent variables were the type of ecosystem and the period of sampling time (month). The results of this analysis indicated that the main effects of the type of ecosystem and month were significant on the abundance of soil ciliates ($F_{(2, 36)} = 52.81$, $p < 0.001$ and $F_{(11, 36)} = 3.42$, $p = 0.001$, respectively). The interaction term between ecosystem and month was also statistically significant ($F_{(22, 36)} = 3.42$, $p < 0.001$), showing that the abundance of ciliates after 10 days of incubation as the months changed differed with the type of ecosystem. The significance of differences in abundance was shown between natural soil and agricultural soils, but not between the two farms by Games-Howell in *post-hoc* test in one-way ANOVA.

There were also big differences in the abundance of soil ciliates after 10 days of incubation between the two studied years and the four seasons in natural soil (Figure 4.4). There is a decrease in the number of ciliates during cold months, with the lowest abundance being observed in winter (December) of both years. However, the overall abundance decreased in 2017 (Figure 4.4).

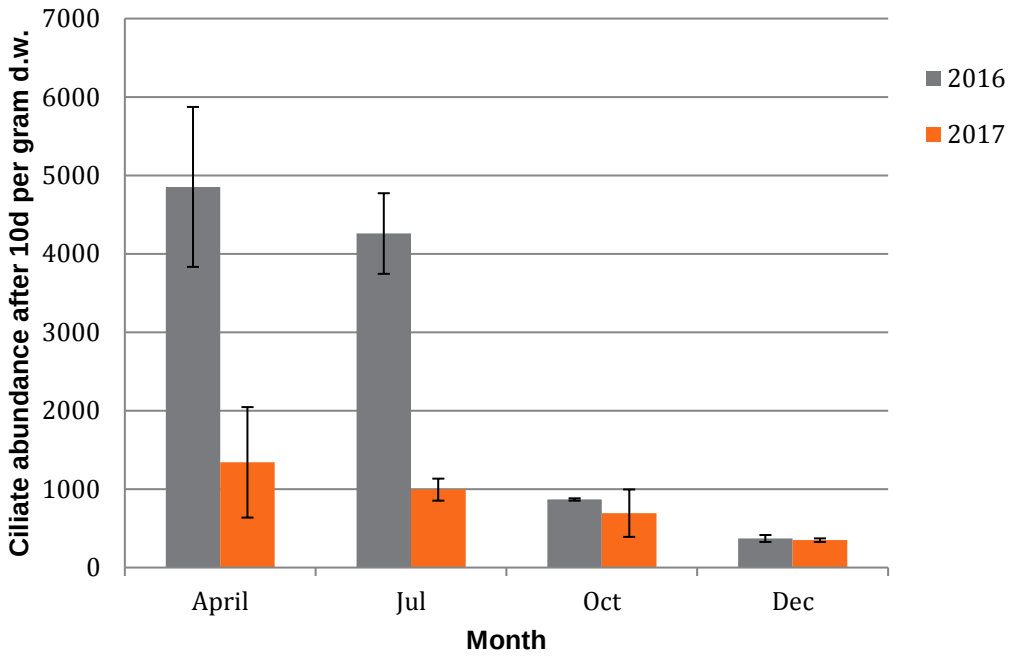


Figure 4.4 Changes in the abundance of soil ciliated protozoa-estimated mean number of two plots per gram dry weight after 10 days of incubation (mean ± 1SE) in the natural ecosystem of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

Analysis of variances by a two-way ANOVA was conducted to explore the effect of year and season on the abundance of ciliates in natural ecosystem. The results of the analysis indicated that there was significant difference in ciliate abundance after 10 days of natural soil for either main effects of year or season ($F_{(1, 8)}$ = 25.41, p = 0.001 and $F_{(3, 8)}$ = 7.57, p = 0.01, respectively). The interaction term between year and season was also significant ($F_{(3, 8)}$ = 7.57, p = 0.01), indicating that there was a difference in response of ciliates between two years by season.

4.3.3 The species richness of soil ciliated protozoa

While the number of ciliates was much higher in the natural ecosystem than in the two agricultural ecosystems, differences in the species richness were unapparent between them (Table 4.3 and Figure 4.5). Within the natural habitat, observations in species richness of soil ciliates in the 19 months from January 2016 to December 2017 showed that the number of species was always higher in Plot 1 than in Plot 2, for example, 34 different ciliate species recorded in Plot 1 *vs.* only 23 species found in Plot 2 in May 2016 (the distance of differences between Plot 1 and Plot 2 at the Fen are also shown through the values of SE in Table 4.3 and Figure 4.5).

Comparing natural *vs*. farm soils, species richness of ciliates in the Fen (natural habitat) and Corfe Castle Farm was higher than in the Vicarage Farm in almost all months (Figure 4.5, Table 4.3). At the starting point of sampling (May 2016) until August 2016, the number of species in Vicarage was relative high, but they dropped to about 20 species or even lower from September 2016 to February 2016, thereafter they increased slightly again in the following months. Meanwhile, Fen and Corfe Castle habitats shared the first place in the number of ciliate species. There was a slight different between these two habitats. Interestingly, in cold months, ciliate species richness was slightly greater in comparison with some warm months.

To determine the influence of the type of habitat and month on the number of ciliate species, a two-way ANOVA was conducted to analyse the data set of twelve months from May 2016 to April 2017 in three natural and agricultural ecosystems. The results of this analysis demonstrated that the type of habitat had significant effect on ciliate species richness, but month did not ($F_{(2, 36)} = 7.90$, $p = 0.001$ and $F_{(11, 36)} = 0.8$, $p = 0.63$, respectively). The interaction term between habitat and month was not statistically significant ($F_{(22, 36)} = 1.38$, $p = 0.18$), showing that there was no response of soil ciliate species to ecosystem when sampling months were changed. The Tukey HSD in *post-hoc* test in one-way ANOVA indicated that differences in ciliate species richness were significant between Vicarage Farm and two other habitats, but no different between the Fen and Corfe Caste Farm.

Table 4.3 The species richness of terrestrial ciliated protozoa- estimated mean number of two plots (mean ± 1SE) in three ecosystems from Jan to April 2016 and Jul to Dec 2017.

Month	Ecosystem	Ciliate species richness
Jan, 2016	Fen	29.5±5.5
Feb, 2016	Fen	33.5±5.5
Mar, 2016	Fen	30.0±9.0
April, 2016	Fen	26.5±5.5
Jul, 2017	Fen	22.0±1.0
	Vicarage	24.5±0.5
	Corfe Castle	27.0±1.0
Oct, 2017	Fen	27.0±3.0
	Vicarage	22.5±2.5
	Corfe Castle	24.5±0.5
Dec, 2017	Fen	22.5±4.5
	Vicarage	28.0±1.0
	Corfe Castle	28.0±1.0

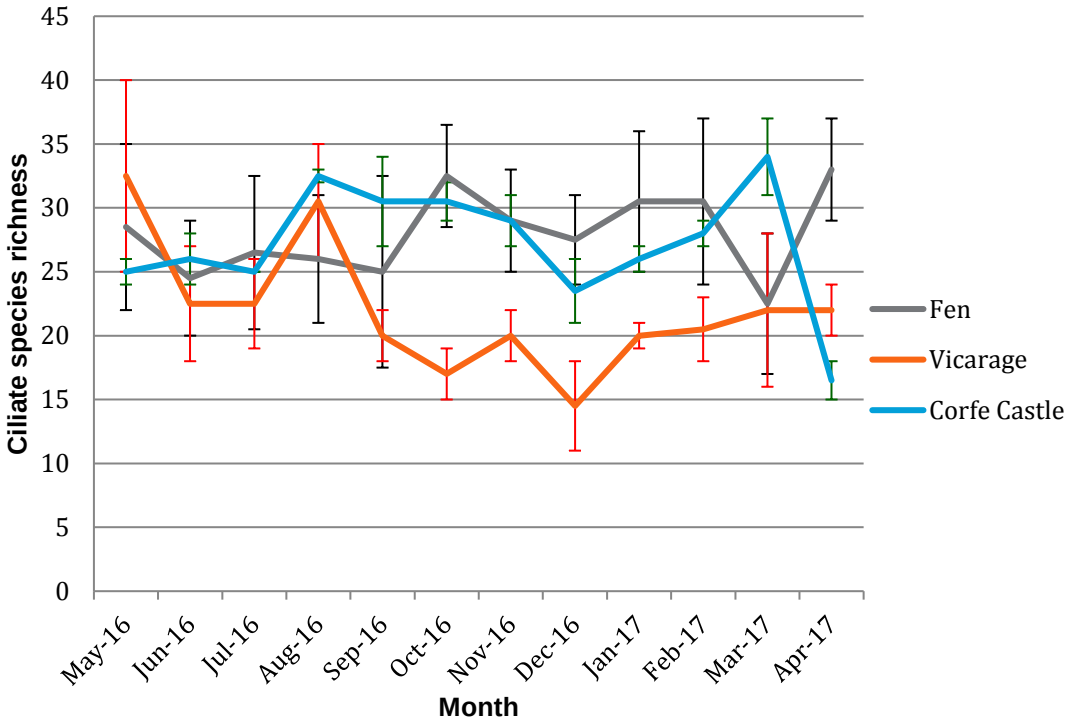


Figure 4.5 Changes in the species richness of soil ciliated protozoa- estimated mean number of two plots (mean ± 1SE) in three ecosystems from May 2016 and April 2017.

There were changes in the species richness of ciliates in the natural habitat over two years and four seasons (Figure 4.6). The number of species was slightly greater in 2016 than that in 2017 in almost all seasons, except spring, where reversed trend was shown. Specially, in both years, the number of ciliate species was the lowest in the summer season. A two-way ANOVA showed that there was no significant difference in the species richness of soil ciliates in the natural soil for either main effects of year or season ($F_{(1, 8)} = 0.43$, $p = 0.52$ and $F_{(3, 8)} = 0.85$, $p = 0.50$, respectively). The interaction term between year and season was also not significant ($F_{(3, 8)} = 0.80$, $p = 0.52$), indicating that there was no difference in effect of year on the presence of total ciliate species by season.

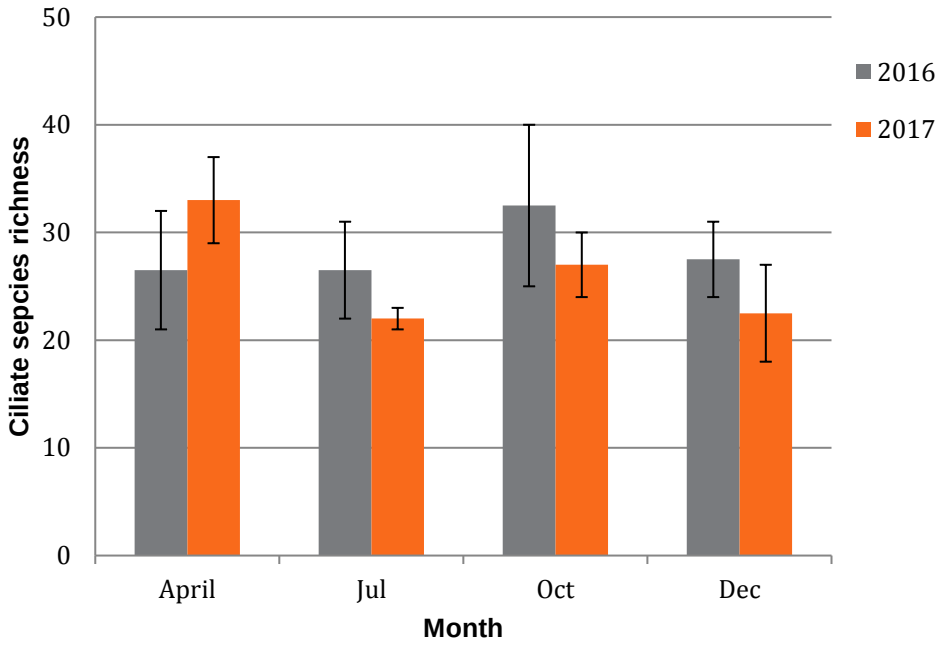


Figure 4.6 Changes in the species richness of soil ciliated protozoa- estimated mean number of two plots (mean ± 1SE) in the natural ecosystem of four representative months for four seasons: April represents spring, July represents summer, October represents autumn, and December represents winter over two years 2016 and 2017.

4.3.4 Correlation between the abundance and species richness of soil ciliates and soil selected properties in a natural habitat

Due to the differences observed in ciliate abundance, ciliate species richness and soil properties between Plot 1 and Plot 2 in the Fen site (see above and Chapter 3), thus correlation between soil ciliates and soil characteristics in this site were separately analysed by using the data collected from January 2016 to December 2017. Correlations between ciliates and soil properties were determined by Spearman's rank order method.

The results are shown in Tables 4.4 to 4.9. The analyses indicated that pH had a strong positive correlation with total ciliate species richness; moisture content correlated negatively with ciliate abundance after 4 days (Table 4.4). However, no correlations between soil and air temperatures and the abundance of soil ciliates and total ciliate species richness were shown (Table 4.4).

Table 4.4 Correlations between the abundance after 4 days and 10 days of incubation and total species richness of soil ciliates and soil properties of the natural ecosystem collected over 19 months from January 2016 to December 2017.

Soil ciliates	pH	Moisture	Soil temperature	Air temperature
Abundance after 4d	0.108	-0.404*	0.18	0.232
Abundance after 10d	-0.161	-0.127	0.218	0.23
Species richness	0.779**	-0.233	-0.270	-0.228

*- $p<0.05$, **- $p<0.01$

The abundance of ciliates from natural soil after 4 days and 10 days of incubation correlated negatively to available concentration of potassium; ciliate species richness was strongly positively correlated to concentration of available and total P, and total Ca (Table 4.5).

Table 4.5 Correlations between the abundance after 4 days and 10 days of incubation and total species richness of soil ciliates and soil essential elements of natural ecosystem collected over 19 months from January 2016 to December 2017. K: Potassium; P: Phosphorus; Ca: Calcium; S: Sulfur.

Soil ciliates	Available K	Available P	Available S	Total Ca	Total P
Abundance after 4d	-0.409*	0.239	0.072	0.257	-0.095
Abundance after 10d	-0.461**	0.004	0.141	-0.037	-0.239
Species richness	0.101	0.752**	-0.07	0.803**	0.492**

*- $p<0.05$, **- $p<0.01$

It is noted that data on **available** concentrations of trace metals is only given in appendices A and B of this study because only some trace metals were detected in very low concentrations, especially in agricultural soils. However, a correlation is analysed to clarify partly how trace metals affect the abundance and species richness of soil ciliates.

Correlation coefficients between soil ciliates and **total and available concentration** of selected soil trace metals in the natural ecosystem investigated are shown in

Tables 4.6 and 4.7. Because available concentration of Cr was too low in natural soil during a large part of the sampling period it could not be detected by the method used in this project – hence the absence of Cr results in Table 4.7.

Total concentration of Cr correlated positively with species richness, but not with ciliate abundance (Table 4.6). Total Cu had positive correlation to ciliate abundance after 4 days and 10 days, but total Pb had a negative correlation coefficient with the number of ciliates after 4 days. Besides, ciliate species richness correlated negatively with total Pb, and positively with total Cr and Zn (Table 4.6).

Table 4.6 Correlations between the abundance after 4 days and 10 days of incubation and total species richness of soil ciliates and **total** concentration of soil trace metals of natural ecosystem collected over 19 months from January 2016 to December 2017.

Soil ciliates	Total Al	Total Cd	Total Cr	Total Cu	Total Ni	Total Pb	Total Zn
Abundance after 4d	0.04	0.02	0.24	0.383*	-0.1	-0.357*	0.205
Abundance after 10d	0.07	-0.03	0.13	0.431*	0.09	-0.11	-0.051
Species richness	0.02	0.12	0.54**	-0.051	0.12	-0.649**	0.778**

*- $p<0.05$, **- $p<0.01$

As our records in available concentrations of trace metals, availability of all six types of trace metal was much higher at Plot 2 than at Plot 1 in the natural Fen soil (Appendix A), meanwhile the number of soil ciliate species at Plot 1 was higher than Plot 2 (as shown in section 3.3 of this chapter). Consequently, strong negative correlations between total ciliate species richness and **available** concentration of all measured metals were indicated. The abundance of ciliates after 4 days of incubation also correlated with Ni and Pb (Table 4.7).

Table 4.7 Correlations between the abundance after 4 days and 10 days of incubation and total species richness of soil ciliates and **available** concentration of soil trace metals of natural ecosystem collected over 19 months from January 2016 to December 2017.

Soil ciliates	Available Al	Available Cd	Available Cu	Available Ni	Available Pb	Available Zn
Abundance after 4d	-0.317	-0.149	-0.091	-0.341*	-0.342*	-0.28
Abundance after 10d	-0.066	0.132	0.148	0.066	-0.011	0.014
Species richness	-0.732**	-0.518**	-0.475**	-0.685**	-0.797**	-0.54**

*- $p<0.05$, **- $p<0.01$

Concerning correlation between ciliates and soil organic matter (SOM) and two types of inorganic nitrogen as well in Fen soil, the abundance of ciliates after 4 days and 10 days of incubation correlated to ammonium, but not with nitrate-N. Meanwhile, positive correlation was shown between species richness and nitrate-nitrogen. Interestingly, SOM had negative correlation coefficient with the number of ciliate species (Table 4.8).

Table 4.8 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and soil inorganic nitrogen and Soil Organic Matter (SOM) of natural ecosystem collected over 19 months from January 2016 to December 2017.

Soil ciliates	SOM	Nitrate-N	Ammonium
Abundance after 4d	-0.039	0.207	0.394*
Abundance after 10d	0.221	0.101	0.568**
Species richness	-0.544**	0.583**	0.109

*- $p<0.05$, **- $p<0.01$

Towards soil enzyme activity, ciliate abundance after 4 days and 10 days of incubation only had negative correlation with total microbial activity. The species richness of ciliates did not indicate any relationship to measured enzyme activity (Table 4.9)

Table 4.9 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and soil enzyme activity of natural ecosystem collected over 19 months from January 2016 to December 2017.

Soil ciliates	Total microbial activity	β-glycosidase	Acid-phosphomonoesterase
Abundance after 4d	-0.414**	-0.172	0.231
Abundance after 10d	-0.349*	-0.308	0.14
Species richness	0.242	0.066	0.015

*- $p<0.05$, **- $p<0.01$

4.3.5 Correlation between the abundance and species richness of soil ciliates and selected properties in natural and farm soils

To determine Spearman's correlation coefficient between ciliates and soil properties in all study habitats, a data set of fifteen months (from May 2016 to December 2017) in the natural and agricultural ecosystems was used. The results of analysing correlation between ciliates and some measured characteristics indicated that there was strongly negative correlation between soil pH and ciliate abundance after 4 days and 10 days of incubation, but not with ciliate species richness. Meanwhile, the number of ciliates and was shown as positive correlation with moisture. The abundance and species richness of soil ciliates was not totally correlated to both soil and air temperatures (Table 4.10).

Table 4.10 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and soil property of three study ecosystems collected over 15 months from May 2016 to December 2017.

Soil ciliates	pH	Moisture	Soil temperature	Air temperature
Abundance after 4d	-0.603**	0.522**	0.006	0.057
Abundance after 10d	-0.709**	0.595**	-0.023	-0.007
Species richness	-0.116	-0.037	0.061	0.073

*- $p<0.05$, **- $p<0.01$

Interestingly, available concentration of phosphorus had strongly positive correlation with both ciliate abundance and species richness, but total concentration of this element was weakly correlated to the abundance of soil ciliate after 4 days only.

However, total concentration of Calcium was shown as a negative correlation with ciliate abundance after 4 days and 10 days and ciliate species had correlation with available concentration of K.

Table 4.11 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and soil essential elements of three study ecosystems collected over 15 months from May 2016 to December 2017.

Soil ciliates	Available K	Available P	Available S	Total Ca	Total P
Abundance after 4d	-0,064	0.615**	0.022	-0.238*	0.242*
Abundance after 10d	-0.031	0.665**	-0.088	-0.444**	0.131
Species richness	-0.21*	0.254*	-0.086	0.072	-0.148

*- p<0.05, **- p<0.01

While almost all selected trace metals i.e., 'total' Cd, Cu, Ni, Pb, and Zn were negatively correlated with species richness of ciliates, 'total' Pb had strongly positive correlation to ciliate abundance after 4 days and 10 days. Also, aluminium was correlated negatively with ciliate abundance after 4 days and 10 days, and Cadmium and Chromium had correlations to the number of ciliates after 10 days (Table 4.12).

Table 4.12 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and **total** concentration of soil trace metals of three study ecosystem collected over 15 months from May 2016 to December 2017.

Soil ciliates	Total Al	Total Cd	Total Cr	Total Cu	Total Ni	Total Pb	Total Zn
Abundance after 4d	-0.29*	-0.20	-0.19	0.20	0.10	0.46**	0.15
Abundance after 10d	-0.56*	-0.36**	-0.46*	-0.04	-0.06	0.42**	-0.03
Species richness	-0.19	-0.37**	-0.14	-0.31**	-0.34**	-0.29**	-0.25*

*- p<0.05, **- p<0.01

In contrast to total concentration, availability of three types of trace metal including Cu, Ni and Zn which were recorded in the three ecosystems had strongly positive correlation coefficients with both ciliate abundance after 4 days and 10 days, but totally no correlation with ciliate species richness (Table 4.13).

Table 4.13 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and **available** concentration of trace metals of three study ecosystems collected over 15 months from May 2016 to December 2017.

Soil ciliates	Available Cu	Available Ni	Available Zn
Abundance after 4d	0.598**	0.566**	0.548**
Abundance after 10d	0.665**	0.622**	0.666**
Species richness	0.035	-0.018	0.164

*- $p<0.05$, **- $p<0.01$

Owing to higher percentage of SOM and higher accumulation of NH_4^+ in natural soil, the content of SOM had strongly positive correlation with ciliate abundance after 4 days and 10 days, and ammonium showed the same tendency. In contrast, negative correlation coefficients were observed between ciliate abundance and accumulation of nitrate-nitrogen (Table 4.13).

Moving to soil biological property, total microbial activity was strongly corrected with ciliate abundance and species richness. Activity of enzyme β-glycosidase also had positive correlation coefficients with the abundance of ciliates after 4 days and 10 days. However, soil enzyme acid-phosphomonoesterase had not any relationships with soil ciliated protozoa at all (Table 4.14).

Table 4.13 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and soil inorganic nitrogen and Soil Organic Matter (SOM) of three study ecosystems collected over 15 months from May 2016 to December 2017.

Soil ciliates	SOM	Nitrate-N	Ammonium
Abundance after 4d	0.657**	-0.33**	0.292**
Abundance after 10d	0.663**	-0.532**	0.217*
Species richness	-0.108	-0.021	0.035

*- $p<0.05$, **- $p<0.01$

Table 4.14 Correlations between the abundance after 4 days and 10 days of incubation and species richness of soil ciliates and soil enzyme activity of three study ecosystems collected over 15 months from May 2016 to December 2017.

Soil ciliates	Total microbial activity	β-glycosidase	Acid-phosphomonoesterase
Abundance after 4d	0.551**	0.57**	0.177
Abundance after 10d	0.617**	0.579**	0.119
Species richness	0.251*	-0.034	0.079

*- $p<0.05$, **- $p<0.01$

4.3.6 Ciliate species richness for each study habitat

The abundance of each ciliate species was determined from April 2016 to December 2017.

In the Fen natural habitat's Plot 1 and Plot 2 the most abundant ciliates were bacterial feeders (Tables 4.15 and 4.16). The abundance of ciliates in natural habitat after 4 days of incubation was contributed mostly by the Colpodids, which had the same species composition at Plot 1 and Plot 2 (Table 4.15). Species composition after 10 days of incubation was different between both Plots; *Sathrophilus muscorum* and *Drepasomonas revoluta* were abundant at Plot 1 in almost all sampling months, but they were hardly recorded Plot 2. In contrast, more species of the Hypotrichia group were found in Plot 2 (Table 4.15, 4.16).

Table 4.15 Species that contributed most to the abundance of soil ciliates after 4 days and 10 days of incubation in Plot 1 of the natural Fen soil.

After 4d of incubation	Food pref.[a]	After 10d of incubation	Food pref.[a]
Nivaliella plana	Fungi/yeasts	*Lepthopharynx costatus*	Bacteria
Pseudoplatyophrya nana	Bacteria	*Sathrophilus muscorum*	Bacteria
Grossglockneria acuta	Fungi/yeasts	*Drepasomonas revoluta*	Bacteria
Colpoda group	Bacteria	*Cyclidium mucicola*	Bacteria

a: Food preferences (Foissner 1987)

Table 4.16 Species that contributed mostly to the abundance of soil ciliates after 4 days and 10 days of incubation in Plot 2 of the natural Fen soil.

After 4d of incubation	Food pref.[a]	After 10d of incubation	Food pref.[a]
Nivaliella plana	Fungi/yeasts	*Lepthopharynx costatus*	Bacteria
Pseudoplatyophrya nana	Bacteria	*Cyclidium mucicola*	Bacteria
Grossglockneria acuta	Fungi/yeasts	Hypotrichia group	Bacteria
Colpoda spp.	Bacteria	*Colpoda* spp.	Bacteria

a: Food preferences (Foissner 1987)

These species groups as shown in Tables 4.15 and 4.16 were also observed in the two agricultural study sites but the abundance was either very low or they are absent altogether.

In agricultural soils, the majority of soil ciliate abundance after 4 days of incubation was contributed by the species of *Homologastra setosa* in all months and in both study farms, but this species was not found in the natural Fen soil. In months where a higher number of ciliates was found in agricultural soils, this was mostly because of a higher number of this species. In addition, the species *Drepasomonas sphagni* was found in all sampling months in Corfe Castle Farm soil only (Table 4.17).

Table 4.17 Species that present and contributed most to the abundance of soil ciliates in the farm soils.

Vicarage Farm	Food pref.[a]	Corfe Castle Farm	Food pref.[a]
Homalogastra setosa	Bacteria	*Homalogastra setosa*	Bacteria
No		*Drepasomonas sphagni*	Bacteria

a: Food preferences (Foissner 1987)

4.4 Discussion

The abundance and richness of soil ciliated protozoa in the three study habitats

The abundance of soil ciliate

Foissner (1999b) indicated that meadow and arable land contain very few active ciliates, usually lower than 100 individuals per gram of dry soil, while the abundance of ciliates reach up to 1000 individuals per gram of dry soil in the uppermost litter

layer of natural soils. The results of the present study showed a similar trend. In particular, the abundance of soil ciliates in natural soil was permanently much higher than in agricultural soils in all months investigated. The biggest difference in the abundance of soil ciliates between natural soil and agricultural soils was found in July 2016 after both 4 days and 10 days of incubation. It could be speculated that the natural soils had higher concentrations of nutrients and microbial biomass, which facilitate the growth of soil ciliates (Li et al. 2010b). In the results from analysing soil properties presented in Chapter 3, it showed that the enzyme activity, including β-glucosidase and total microbial activity and concentrations of essential nutrient element such as 'available' phosphorus were significantly higher in natural soil than in agricultural soils. Moreover, the highest percentage of soil organic matter (SOM) was recorded in the natural soils of the Fen. Soil organic matter is a source of nutrients (Craswell and Lefroy 2001) and also retains water (Sujatha et al. 2016) in the soil. Plant litter would not only have supplied sufficient organic matter, humus and other nutrients to soil ciliates, but also allowed greater ciliate mobility by elevating soil water content. Consequently, SOM content as shown above can account for the significantly higher abundance of soil ciliates in the natural Fen soil.

The result from a two-way ANOVA analysis indicated that there was a temporal pattern in the abundance of ciliates after 4 days and 10 days of incubation in the three ecosystems investigated over the 12 sampling month period (May 2016 to April 2017). Finlay et al. (2000) found that there was no obvious temporal pattern in the abundance of soil ciliates in their study (i.e., the potential abundance of soil ciliates seems to be independent of season), but other work observed seasonal dynamics in soil ciliate abundance (Li et al. 2010a). This was explained due to effect of temperature. Ciliates are reported to be most abundant at temperatures in the range15-26 ^{o}C (Darby et al. 2006) and Ning and Shen (1998) also showed that moderate temperatures (22-28 ^{o}C) are favourable for most soil protozoa (see in Li et al. 2010a). Buitkamp (1979) and Petz et al. (1985) also showed soil ciliate species adapted to the local temperature. Although no correlation was found between soil and air temperatures and soil ciliates in this present research (Table 4.10), it can be seen that soil and air temperatures at the Fen site were usually lower during the warm seasons and higher in cold season than in agricultural sites (Tables 3.1 and 3.2). In addition, the Fen site was covered by the canopy formed from the substantial presence of trees growing on the site, which might result in less temperature variation. It can also be seen that the temporal pattern was clearer in

the natural site than in the agricultural sites (Figures 4.1 and 4.3). Temperature, in part, can be a factor affecting ciliate abundance here.

Furthermore, statistical analysis showed that there were significant differences in ciliate abundance measured after 4 days and 10 days of incubation between the two years (2016 and 2017) and among four seasons (April, July, October, and December) in the natural habitat (Figure 4.2 and 4.4). It is clear from this that in the present study; temporal patterns had an influence on ciliate abundance. In warmer months, the abundance of soil ciliates increased, and *vice versa* - it decreased in colder months. The abundance of ciliates in natural soils decreased in 2017 compared to 2016; this is particularly evident in April and July. This could be related to changes in temperature. The results in this study show that temperatures in spring, summer and autumn were higher in 2017, but lower in winter (Figure 3.1). However, the impact of temperature on ciliate abundance is not clear. One suggestion is that because both soil and air temperatures in the study sites were only recorded at the time of sampling, the effect of inter-year variation in temperature could have been missed. Besides temperature, a change in inorganic nitrogen was also indicated over two years (Figure 3.3). In general, concentrations of nitrate-N and ammonia-N decreased strongly in 2017 compared to 2016, with an exception for an unusually high concentration of ammonium in the Plot 2 of the natural habitat. Particularly, strong decreases in ammonium were indicated in April and July in 2017. This could also be a causal factor to the sudden reduction in ciliate abundance in this both months. Acosta-Mercado (2004) demonstrated that total inorganic nitrogen was the best predictor for variation in the abundance and species richness of soil ciliates. Further, Li et al. (2010a) found that ciliate abundance was positively correlated with the concentrations of ammonia-N in acidic soil. Consequently, the factors of temperature and ammonium concentration most likely accounted for the decrease in ciliate abundance in 2017.

It is also important to note that a significant increase in ciliate abundance occurred in the Vicarage Farm soil in December 2017 when compared to December 2016. An investigation into the farming practice showed that the farmer refreshed old top soil (0-10 cm deep) through turning the soil by ploughing in December 2017. Foissner (1987) concluded from a review that protozoa are usually most abundant in the top 0-10 cm of soil, but he also pointed out that ciliates did not show pronounced changes because of top soil removal five years before the investigation. Therefore, the impact on soil ciliates of topsoil removal is not clear in the review of Foissner (1987). This time period limits our understanding of the

effects of topsoil disturbance. However, Foissner (1987) indicated that lime was applied to the soil used in his study to improve pH to neutral levels possibly allowed an increase in microbial activity and decomposition, which ciliates benefit from (Foissner 1987). Cao et al. (2016) showed that pH was strongly positively correlated to soil enzyme activity. In addition, in the review of Foissner (1987) another finding was that enzyme activity and CO_2 release was reduced, but the change in pH was not recorded. In the present study, although ciliate abundance increased after ploughing, total microbial activity did not increase and was even lower than that in other months. The cause in this case is difficult to determine, but lower total microbial activity in this month is likely to be related to low temperature. Soil pH in December 2017 increased compared with other months and it can be a factor stimulating bacterial activity, but this activity was not clearly shown here due to no control with the same conditions but having acidic pH to compare with. Therefore, pH could be a factor behind the increase in ciliate abundance in this month. Furthermore, there may have been many cysts and more ciliate species in the lower layer of the soil. When this layer was turned to the surface, these cysts grew in more favoured conditions, such as un-compacted soil. Hence, pH increase and un-compacted soil condition may account for the increase in the abundance of soil ciliates in December 2017 compared to December 2016.

Interestingly, sometimes the greater abundance of ciliates was found in soil from the natural habitat after 4 days of incubation, but the abundance was usually higher after 10 days of incubation throughout the whole study period. Finlay et al. (2000) also noted that ciliate abundance in grassland soils after 4 days of incubation was, in some sampling occasions, greater than after 10 days and *vice versa*. This complies with the findings from the natural soil of this present research. However, ciliate abundance in the agricultural soils was always higher after 4 days of incubation, and after 10 days of incubation active ciliate numbers were very low. This can possibly be related to poor nutrient conditions in the soil slurry after 10 days of incubation or possibly due to the species comprising the community being different, so almost all ciliate species was come out of their cysts after 4 days. Each ciliate species appears at different times after soil is rewetted (Foissner 1987; Finlay et al. 2000). Overall, it is confirmed that to determine the abundance of soil ciliates in natural habitats, counts should be made after 4 days and 10 days of incubation as Finlay et al. (2000) indicated, but in agricultural habitats, it is suggested that ciliate abundance can be investigated after 4 days of incubation only.

The total ciliate species richness

The results of the present study also demonstrated that there was a significant difference in ciliate species richness between Vicarage Farm and the other two (i.e., Fen natural soils and Corfe Castle Farm). Soil texture can be a factor limiting the dispersal of protozoan species (Ekelund and Rønn 1994). The soil of Vicarage Farm had a fine texture, while the Fen and Corfe Castle soils were coarse textured (Table 3.1 in Chapter 3). Thus, it is possible that a fine textured soil reduced the movement of ciliate species amongst soil particles. This is consistent with previous studies that have shown higher densities of nematodes and amoeba in coarse soil compared to fine soil (Elliott et al. 1980) because greater pore sizes in coarse soil increases the freedom of movement of soil organisms. It is also noted that ciliate movement was affected by the size of water-filled pore necks in soil (Darbyshire 1976). However, there was no significant difference between the Fen and Corfe Castle Farm. Foissner (1997c) found similar results when investigating ciliate species richness in agro-ecosystems and in natural habitats. This can be explained through the ability of ciliates to form resting cysts if unfavourable conditions develop, and to their random dispersal in soil (Foissner 1987, 1994, 1999b), meaning that differences in soil properties between natural and agricultural soils are not a permanent limiting factor for ciliates. Furthermore, in a review Geisen et al. (2018) concluded that it was well documented that the diversity of soil protozoa usually decreases under low pH conditions. Thus pH will have partly limited the diversity of soil ciliate species in Fen despite the site being a nutrient rich environment.

Correlation between the abundance and species richness of soil ciliated protozoa and soil property

Soil pH, temperature, and moisture

Soil pH had a strongly negative correlation with the abundance of soil ciliates after 4 days and 10 days of incubation (Table 4.10). A negative correlation was also observed in a previous study (Li et al. 2010a). This can be due to an increase in nutrient absorption of ciliates at acid pH (Li et al. 2010b). Although in a review it was concluded that the species richness was lower in acidic soils (Geisen et al. 2018), but many ciliate species had optimum growth at acidic pH. Weisse and Peter (2006) proved that some freshwater ciliate species were most abundance in acidic conditions. Similarly, in the present study, pH at Plot 2 in the natural Fen site was extremely acidic (pH~4), but ciliates were abundant. This was contributed by only some specific species such as *Cyclidium mucicola*, *Grossglockneria acuta*, and *Pseudoplatyophrya nana*. In contrast to abundance, a strong positive correlation

between species richness and pH was found in the natural Fen soil. This was also indicated by a study where many ciliate species were absent in strongly acidic soil environments (Foissner et al. 2005). As mentioned above, the pH of Plot 2 in the Fen was very acidic (pH~4), whilst Plot 1 had a less acidic pH (pH~5). The absence of some ciliate species in the soil habitat with very low pH suggests that is an extreme habitat for these species, hence the reason why the total species richness of soil ciliates was positively correlated to soil pH.

In the present study, both soil and air temperature had no correlation with the abundance of soil ciliates when considering correlations in all habitats combined together and the natural habitat separately. There is no doubt that soil temperature and soil moisture are usually linked. Water has the second highest heat capacity of liquids, which means that it takes a lot of energy to heat up. Consequently, wet soils tend to be colder than dry soils, so ciliates could be responding to soil moisture rather than to soil temperature in this study. Also, as discussed above, soil ciliates can adapt to a large range of temperatures and to temperature conditions of the local habitat (Buitkamp 1979; Darby et al. 2006). Some species also show adaption to low temperature (at 4^{o}C) (Buitkamp 1979; Petz et al. 1985). Therefore, it is clear why ciliate abundance after 4 days and 10 days of incubation in all ecosystems was positively correlated to soil moisture. Previous studies also demonstrated a positive correlation between soil moisture and ciliate abundance at Baiyun Mountain and Mangrove forest in China (Li et al. 2010a; Li et al. 2010b). However, as explained above, although, no correlation between measured temperatures and ciliate abundance was found, cooler temperature in summer and less cold in winter may, in part, contribute to ciliate abundance in the natural Fen soil. This is because wet soil cools slowly thanks to its heat storage capacity. Thus, higher soil moisture decreases differences in soil temperature between day-time and night-time (Al-Kayssi et al. 1990). In the present study, the Fen soil having higher moisture had less temperature variations than the agricultural soils with lower moistures during the sampling period (Table 3.2 and 3.3). Consequently, the Fen provide stable temperature conditions than the other sites. In addition, the impact of temperature on soil microorganisms is clear. The optimal temperature for bioactivity is 10-28 ^{o}C, lower temperatures decrease microbial activity (onwuka and Mang 2018), whilst ciliates favour temperatures in the range 15-26 ^{o}C (Darby et al. 2006). Temperature fluctuations between day and night are more prevalent in the spring when day-time is usually relatively hot and night-time is cold. This may lead to ciliates in the natural Fen soil suffering less stress due to temperature fluctuations than in the agricultural

soils. This study's findings confirmed this in that the abundance of soil ciliates in Corfe Castle Farm soil, which had lowest moistures and bigger temperature variations (Tables 3.2 and 3.3) during the sampling period, decreased strongly in April-May 2016 and April 2017 (Table 4.1 and Figure 4.1).

In contrast, a negative correlation between moisture and ciliate abundance after 4 days was shown for the natural habitat. As discussed above, high nutrient content and pH are factors that partly account for the abundance of soil ciliates. Moreover, the disparity in soil moisture between Plot 1 and Plot 2 in the natural habitat is not great (Tables 3.2 and 3.3 in Chapter 3). Hence, lower soil moisture in this case may not account for the decrease in soil ciliate abundance. It could be integrated by other soil factors such as temperature as discussed above.

Soil essential elements

Moving to relationships between ciliates and selected essential elements, it has been shown that total potassium (K) had a negative correlation with ciliate abundance (Li et al. 2010a; Li et al. 2010b). The present study found that this also applies to available potassium in natural soils, finding a negative correlation between available potassium and ciliate abundance after 4 days and 10 days of incubation in the natural Fen soil. A weakly negative correlation between ciliate species richness and available potassium was also found when considering all three ecosystems together. Although a negative correlation between ciliate abundance and potassium has been reported previously, no worker has been able to postulate an explanation for the interaction. In general, potassium is a base cation, so it has tendency to partake in reactions that make soil pH more alkaline (Brady and Weil 2008). It is expected that the concentration of available K in Plot 1 of the natural Fen site was higher than Plot 2, but the results of the analysis showed that concentrations of K in the both Plots was similar. Thus, the role of K in increasing soil pH is not clear here. Also, the requirement for K by plants is very high (Bidari and Hebsur 2011), but soil micro-organisms which are prey for ciliates only need a small quantity of K for their growth in comparison (Moro et al. 2014). Therefore, it is assumed that these negative correlations between ciliate abundance after 4 days and 10 days in the natural Fen site were controlled by other soil parameters, not K. In addition, weakly negative correlation between the species richness of soil ciliates and available K was found in the three ecosystems together. The most likely reason why there was a negative correlation is the fact that high available K concentration may reflect agriculture. Thus, it is relationship between agricultural practice and species richness, and not K with species richness.

Similar to K, calcium was negatively correlated with ciliate abundance after 4 days and 10 days of incubation in all three ecosystems together, but only strongly positively correlated with ciliate species richness in the natural Fen habitat. Lime is applied to increase pH in agro-ecosystems (Goulding 2016), explaining why concentrations of Ca were high in agricultural soil, and related to these negative correlations. It may be the relationship between agricultural practice and the abundance of ciliates, not between Ca and ciliates in this case. Therefore, it may be concluded that there was an integration of other soil factors controlling the abundances of soil ciliates rather than the impact of calcium on them. It was found that total concentrations of Ca in Plot 1 (~2500 mg kg^{-1}) of the natural Fen habitat were much higher than that in Plot 2 (~1000 mg kg^{-1}). Thus, higher pH value in Plot 1 may have contributed to by Ca, which is another important base cation. As discussed above, the very acidic pH of Plot 2 (pH~4) limited the diversity of soil ciliates (Foissner et al. 2005). Consequently, a strong positive correlation between Ca and ciliate species richness in natural habitat was recorded, but it is the effect of Ca on soil pH which is the important factor.

Availability of phosphorus is suggested as one of the limiting factors for growth of microflora, because adding P to old-growth forest increased the microbial biomass and the diversity of community (Liu et al. 2012). In the natural Fen habitat, ciliate species richness was strongly positively correlated with both total and available concentrations of phosphorus. In addition, positive correlations between ciliate abundance after 4 days and 10 days and species richness and available concentrations of P were found in all three habitats together. Also, the abundance of soil ciliates after 4 days weakly but positively correlated with total concentration of P in all three habitats. A positive correlation between total P and ciliate abundance was indicated in previous studies (Li et al. 2010a; Li et al. 2010b) and the present study confirms this relationship. The effect of phosphorus on soil ciliates is indirect. It affects ciliate abundance and species richness through stimulating the growth of the soil microbial community (Liu et al. 2012), which are then predated by ciliates (Finlay and Esteban 2013). This can be seen clearly in the natural Fen habitat, ciliate abundances were mainly contributed by the rapid increase of bacteria-feeding species (Tables 4.15 and 4.16). Furthermore, in the Plot 1 of the Fen, almost all species recorded were bacterial feeding species. Thus, soil phosphorus has an important positive role in determining species number and abundance, particularly for bacteriophagous ciliates. Moreover, in the present study, agricultural habitats had low ciliate abundance, even though P fertilizer was used.

This was because there were low P concentrations in the agricultural lands as determined in Chapter 3 (Tables 3.4 and 3.5)

Metals

There is no doubt about the potential toxicity of metals to soil ciliates (Forge et al. 1993; Díaz et al. 2006). To consider the effect of metals on soil ciliates, correlations between the two were analysed in the natural Fen habitat separately and amongst the three ecosystems together with both total and available concentrations of metals considered. The number of ciliate species decreased significantly with an increase in available concentration of Al in natural Fen soil. The availability of Al increases under acidic pH condition, especially below pH 5 (Piña and Cervantes 1996; Moir and Moot 2010). In general, it is acknowledged that metal solubility tends to increase when pH decreases and *vice versa* (Rieuwerts et al. 1998; Berbecea et al. 2011). There is no information about effect of Al on soil ciliates. Nevertheless, Al was toxic to a freshwater algae species (Quiroz-Vázquez et al. 2010) and effects of Al on bacteria and fungi have also been reported (Piña and Cervantes 1996). Quiroz-Vázquez et al. (2010) reviewed mechanisms of Al toxicity in algae and concluded that Al can directly damage cell membranes or cell walls; it can interfere in the uptake and transport of other cations; alter the concentration of important cations such as Ca, Mg, K, and Fe in cells or bind to intracellular enzymes. Piña and Cervantes (1996) also showed that similar mechanisms create toxic effects in microbes. Besides causing direct toxicity, Al affects organisms owing to its ability to bind with phosphorus, which leads to reduced bioavailability of this essential element (Vrba et al. 2006). It is very likely these mechanisms can happen in ciliates.

In the present study, available Al concentrations at Plot 2 in the natural Fen site were much higher than in Plot 1; the range of Al concentrations oscillated from ~10 to ~25 mg kg^{-1} in Plot 2 compared with the range of ~0.1 to ~3 mg kg^{-1} in Plot 1. This is because pH in natural Fen soil was strongly acidic and Site 2 had lower pH than Site 1; this would have increased the bioavailability of Al in Plot 2 compared to Plot 1. Therefore, higher available Al concentrations in Plot 2 were highly likely to be an important factor excluding the presence of many ciliate species. This provides an explanation for the strong negative correlation between the number of soil ciliate species and available Al concentration. One finding that needs discussion is that although total concentration of Al was very high in the farms, especially in Vicarage Farm, in almost all farm samples available Al was not detected by ICP-OES during analysis. This is due to neural/alkaline pH in the farm soils markedly decreasing the

bioavailability of Al. This is more evidence of marked effects of acidic pH on potential toxicity of Al on soil microorganisms.

Similar to Al, Cd was negatively correlated to the species richness of soil ciliates in the natural Fen soil. Cd is usually found in co-existence with Zn (Díaz et al. 2006). Zn is an essential micro-nutrient and is component of 300 enzymes in living cells, but Cd is usually not found in living cells and it has no known biological function (Díaz et al. 2006), excepting enzymes of two marine diatom species *Thalassiosira weissflogii* and *Thalassiosira pseudonana* which are found in Zn limited conditions (Lane and Morel 2000; Park et al. 2007). Cadmium is ranked as a highly toxic metal in the environment (Díaz et al. 2006). Cd was indicated as the most toxic metal in laboratory tests on some ciliate species, in which LC_{50} for *Colpoda steinii* (strain FM1-isolated from a compost sample in Spain) was 0.5 mg L^{-1} (Díaz et al. 2006), but other tests indicated that the order of toxicity of trace metals depends on ciliate species used (Martín-González et al. 2006). Rachlin and Grosso (1991) proved that low pH conditions increased Cd toxicity to the algae *Chlorella vulgaris*. Forge et al. (1993) found that a Cd concentration of 0.22 mg L^{-1} reduced the growth of *Colpoda steinii* by 50 % after 24 h. In the same study, it was also shown that the numbers of *C. steinii* did not significantly decrease compared to the control in soil solution extracted from soil and contaminated with 0.01 mg L^{-1} of Cd (Forge et al. 1993). In the present study, available Cd concentrations were recorded in the range of 0.01-0.05 mg kg^{-1} in Plot 1, and 0.02-0.07 mg kg^{-1} in Plot 2. Consequently, Cd may have been at a sufficient level to have caused toxicity to soil ciliates. In addition, the acidic pH in Plot 2 of the natural Fen habitat (pH~4) would have further increase the potential of Cd to cause toxicity to the ciliates. Thus, Cd may have limited the presence of some ciliate species in Plot 2. Dorset (UK) has Cd hotpots where soil Cd concentrations can be up to the range of 0.819-1.23 mg kg^{-1} (http://www.ukso.org/nis/Cadmium.html), potentially of geological origin as no anthropogenic sources are evident. Hence, the areas where acidic conditions, like the natural Fen site, increase the risks for soil ciliate species due to increase in the toxic potential of Cd.

By contrast, available concentrations of Cu, Ni, and Zn were detected in all three ecosystems; the available concentration of them all was the highest in the natural soil habitat compared to two farms, despite total concentrations being greatest in Vicarage Farm. While the available concentration of Zn was in the range of 4-8 mg kg^{-1} in the natural Fen soil, the concentration of this metal in agricultural soils was only 0.1 mg kg^{-1}. The available concentrations of Cu and Ni were lower than 0.5 mg

kg^{-1} in the three ecosystems. In addition, the concentration of the three metals in Plot 2 of the natural Fen was permanently greater than in Plot 1. Consequently, negative correlation between ciliate species richness and available concentration of Cu, Ni, and Zn was found in the natural Fen (Table 4.7), and positive between ciliates abundance after 4 days and 10 days of incubation and the available concentrations of the three metals in all three ecosystems together were determined (Table 4.11). Cu is more toxic than Zn for some ciliate species (Forge et al. 1993; Díaz et al. 2006; Martín-González et al. 2006). Forge et al. (1993) demonstrated that soil solution extracted from soil contaminated by sludge with measured Ni and Zn concentrations of 0.859 mg mL^{-1} and 2.693 mg mL^{-1} respectively decreased the number of *Colpoda steinii* after 24h of incubation. Clearly, toxicity of trace metal on ciliates depends on the type of trace metal and ciliate species. In the present study, it likely that pH is the dominant factor, probably along with Al, Cd, Cu, Ni and Zn to have an effect on ciliate species because pH also controls their availability, thus lower ciliate species richness was seen in Plot 2 of the natural habitat. Nevertheless, micro-nutrients play an important role in living organisms. For example, Zn is a required cofactor in 300 metabolic enzymes and so Zn is a particularly important for ciliate growth. In addition, ciliate species have an ability to resist trace metals (Díaz et al. 2006; Martín-González et al. 2006). Díaz et al. (2006) also detected an antagonism between Cd and Zn in colpodid ciliates: in presence of Zn, Cd toxicity decreased in soil ciliate species. The available concentrations of soil trace metals in the study sites were low, so this concentration cannot be sufficient to have a toxic effect on many soil ciliate species in the present study. However, chronic levels of metal toxic to ciliates are unknown. It could be speculated that there was some chronic toxic effects, but is this likely in the natural system. Hence, it is possible that the available concentrations may be low enough? that deficiency may have been a problem and areas with higher concentrations may have stimulated the growth of some ciliate species as listed in Tables 4.15 and 4.16. This explains why positive correlations between ciliates abundance after 4 days and 10 days of incubation and the available concentrations of Cu, Ni and Zn at all three ecosystems together were found (Table 4.11).

The available concentration of Cr was not detected in any of the three study sites for most of the study period, except for only a very low concentration (0.003-0.008 mg kg^{-1}) recorded in the natural soils. Chromium is a highly toxic and non-essential metal for micro-organisms and plant (Cervantes et al. 2001), but algae and microbes have resistance to Cr (Cervantes et al. 2001). Forge et al. (1993) also

found that there was no significant reduction of *Colpoda steinii* in soil solution extracted from soil and contaminated 0.209 mg mL^{-1} of Cr compared with control, which contained less than 0.005 mg mL^{-1}. It is therefore highly unlikely that Cr may affect ciliates in the present study.

Similarly, Pb has negative influence on living organisms. A biological role for Pb is unknown, and it is toxic to micro-organisms (Abraham et al. 2017b). Janssen et al. (1995) showed that Pb inhibited the population of the soil ciliate species *Colpoda cucullus* after 7 days of incubation, in which a Pb concentration of 2.07 mg L^{-1} reduced by 50 % the population of this species. In this present study, the available concentrations of Pb at Plot 1 and Plot 2 the Fen were only in the range of 0.01-0.2 mg kg^{-1} and 0.15-0.7 mg kg^{-1}, respectively, which are much lower than the Pb concentration in the experiment of Janssen et al. (1995). Nevertheless, toxicity of trace metal on ciliates depends on the type of ciliate species. As chronic levels of metal toxicity to ciliates are unknown, a remote possibility remains that Pb was a factor negatively affecting ciliate abundance after 4 days of incubation and ciliate richness in the natural Fen soil

All in all, total concentrations of almost all metals were the highest in Vicarage Farm, except for lead. Nevertheless, bioavailable concentrations of selected metals were highest in the natural soil. Four of the seven selected metals were not detectable in the agricultural soils. Bioavailability of trace metal decides their toxicity to organisms (Rieuwerts et al. 1998) and this is in part controlled by pH (Moir and Moot 2010; Berbecea et al. 2011). Low pH causes increases in bioavailability. Trace metals in agricultural soils were in high concentration, but in insoluble forms. Thus, toxic effects on soil ciliates in neutral and alkaline pH condition are unlikely unless contamination is high, but this finding warns of potential toxicity if pH is decreased. Furthermore, Bowers et al. (1997) evaluated toxicity of soil solutions extracted from 25 contaminated soil sites on soil ciliate *Colpoda inflata*. The authors showed that levels of some trace metals in their study were below values identified as acutely toxic in other studies. They also found at contaminated soils with pH values less than 3.5, and suggested that either pH had a direct impact, reducing the number of *C.inflata* cells, or that there was an interaction of two or more metals being responsible for the observed toxicity to *C. inflata*. In the present study, pH at Plot 2 of the natural Fen site was very low, around 4.0. Thereby, in part, pH could be the main factor controlling ciliate richness in the natural Fen soil, but interactions between metals may also have caused negative correlations between ciliate richness and metals at the Fen. Therefore, a supposition is proposed that negative

correlations between total Al concentration and total concentrations of all other selected trace metals, i.e., Cd, Cr, Cu, Ni, Pb and Zn, found in the Fen separately and in the three ecosystems together (Tables 4.6 and 4.12) was integrated by the impact of other important soil factors such as soil pH or available metal concentrations. Hence, total concentrations of selected trace metals in fact were not causal to affect negatively on soil ciliates in the present study.

Soil organic matter (SOM), nitrate-N and ammonium

Ciliate abundances after 4 days and 10 days indicated that a significant increase occurred with an increase in SOM in the three ecosystems. This agrees with previous studies that ciliate abundance has positive correlation to the percentage of SOM (Li et al. 2010a; Li et al. 2010b). It was suggested that growth of bacteria and fungi was positively affected by increase of organic matter (Schnürer et al. 1985; Nakhro and Dkhar 2010). This in turn leads to a high abundance of the bacterivorous ciliate *Colpoda aspera*, which was largely responsible for the significant increase of ciliates after organic fertiliser application (Verhoeven 2001). In the present study, the abundance of ciliates was attributed to some bacterial feeding species in the natural Fen soil, which was high in SOM (Tables 4.15 and 4. 16). However, a negative correlation between ciliate species richness and SOM was found in natural Fen habitat. Plot 2 had a higher accumulation of humus than Plot 1, but pH was lower. Thus, here soil pH could be the limiting factor on ciliate species composition rather than content of SOM.

The correlation between nitrate-N and ciliate species richness in the natural Fen soil was significantly positive. Concentration of nitrate-N in Plot 1 of the Fen was much higher than in Plot 2, which is typical for the effect of low pH on nitrification (Dancer et al. 1973). Therefore, in this case, it is assumed that pH may be the factor underlying a positive correlation between nitrate-N and ciliate species richness rather than the concentration of nitrate-N. Interestingly, there were significant negative correlations between ciliate abundance after 4 days and 10 days of incubation and the concentration of nitrate-N in the three ecosystems together. Foissner (1987) showed in a review that applying mineral fertilizer in a cotton field at application rates of 200 kg ha^{-1} N and 150 kg ha^{-1} K decreased the protozoan biomass by 25-30 % and application of urea reduced the numbers of species and biomass of testate amoeba by two-thirds. Nitrate application in the hilly red soil region in China decreased the biomass of bacteria, fungi, and soil enzyme activity (Geng et al. 2017). The agricultural sites in the present study also received nitrogen fertiliser (usually fertilizer application occurred from April to June every year) and

this led to high concentrations of nitrate in many months. However, the negative correlations in the present study can be integrated by other factors than nitrate concentration alone. The obvious reason why there may be a negative correlation between ciliate abundance and nitrate-N is that high nitrate-concentrations in some months reflect agriculture. Hence, it is the relationship between agricultural practice and ciliate abundance and not nitrate-N concentration that is likely to cause the negative relationship in this case.

Li et al. (2010a) found a positive correlation between ciliate abundance and ammonia-N in Baiyun Mountain in a strongly acidic soil, but not with nitrate-N, and it was suggested that soil ciliates may have resistence to ammonia-N. Geng et al. (2017) found that ammonium decreased the soil microbial biomass and enzyme activity, but also showed that bacteria are resistant to ammonium toxicity and they can use ammonium as a nitrogen source (Müller et al. 2006). Furthermore, it was shown that some ciliate species from acitvated sludge and marine enviroments have the capacity for acclimatise to high ammonia (Xu et al. 2004; Puigagut et al. 2005). In the present study, ciliate abundance after 4 days and 10 days in the natural Fen soil and in the three ecosystems together was also positively correlated with ammonium concentration. This could be that soil ciliates acclimatised to ammonium, thus the positive relationship was shown in the present study.

Microbial activity

Regarding interactions between soil biological properties and ciliates, there were significant positive correlations between the abundance of soil ciliates after 4 days and 10 days of incubation with total microbial activity and β-glycosidase activity in the three habitats together, but total microbial activity was weakly positively correlated with species richness.

The total microbial activity is related to the density of soil bacterial and fungi, to a certain extent (Söderström 1977; Lundgren 1981). Soil microbial organisms are food resources for soil ciliates (Esteban et al. 2006). Thus, the higher total microbial activity means greater food availability for soil ciliates. Similarly, glucosidases catalyse the breakdown of low molecular weight carbohydrates to produce sugars, and soil micro-organisms use these products as their main energy source, with β-glycosidase as the most predominant glycosidase in soil (Eivazi and Tabatabai 1988). Consequently, this may explain why the abundance of soil ciliates was positively correlated with soil total microbial activity and β-glycosidase in all three habitats. However, negative correlations between total microbial activity and ciliate

abundance after 4 days and 10 days of incubation were found in natural Fen soil. In this case, it is assumed that in natural conditions, when soil moisture was favourable for soil ciliates, the abundant ciliates may have reduced the density of soil microbial community by their feeding, and thereby reduced microbial activity. In a review, Pussard et al. (1994) also showed the relationship between predator (protozoa) and prey (bacteria) in soil was such that the abundance of protozoa increased, bacterial population reduced.

No correlation between the activity of acid-phosphomonoesterase enzymes and soil ciliates was indicated by the results of the present study. It was suggested in a previous study that activity of acid-phosphomonoesterase increases when phosphorus is deficient in micro-organisms (Kunito et al. 2012). The available concentrations of P were low in the agricultural soils, but measured concentrations of phosphomonoesterase were high. Hence, P deficiency in the agricultural soils may have stimulated micro-organisms to secrete more acid-phosphomonoesterase. This explains why activity of acid-phosphomonoesterase was high in the agricultural soils and no correlation between activity of this enzyme and soil ciliates.

The typical species for each study habitat

The species shown in tables 15 and 16 are common species in soils (Foissner 1993; Finlay et al. 2000). Based on an in-depth review, Foissner (1987) stated that soil humus or organic matter content plays an important role in determining the abundance of each species and species richness of soil protozoa. Verhoeven (2001) found that there was an increase in big populations of *Colpoda aspera* and *Cyrtolophosis elongata* when the content of soil organic matter increased. Therefore, in the present study, it may be that groups of these ciliate species were abundant due to the high content of organic matter determined in the natural soil habitat. Also, a high density of two species *Sathrophilus muscorum* and *Drepasomonas revoluta* was determined in Plot 1, but very few were found in Plot 2 of natural Fen soil. This can be related to low pH of Plot 2 and suggests that the acidic pH in Plot 2 inhibited the growth of *S. muscorum* and *D. revoluta*.

Homalogastra setosa is a common ciliate species found in soil, fresh waters, and marine habitats (Foissner 1987; Petz and Foissner 1989; Esteban and Finlay 2004). This species is easy to recognize through its shape and movement behaviour. Foissner (1987) found this species in the soils of wheat and cereal fields, alpine grassland, and spruce forests with pH ranging from acidic to alkaline. It was also found in grassland in Scotland with a soil pH~4.5-4.8 (Esteban et al. 2006), but the

abundance of *H. setosa* was not reported. However, *H. setosa* could not be detected in very acidic (pH < 5) soils in other studies (Foissner et al. 2008; Li et al. 2010b). In the present study, *H. setosa* was much more abundant compared to other species in agricultural soils at neutral/alkaline pH, but absent from the natural Fen soil at more acidic pHs (range of ~4 to ~5). Therefore pH associated with one of other factors such as available Al or the available measured trace metals would seem to limit growth of this ciliate species, although Foissner (1987) recorded it from acidic soils (pH~2.8-6). Thus, more research on the presence and abundance as well of *H. setosa* in soil ecosystems is needed to prove pH is a limiting factor if this species could potentially be used as bio-indicator of soil habitats that don't have an acidic pH.

In the present study, *Drepasomonas sphagni* was found in Corfe Castle Farm only, where the soil is coarse textured. This species has also been recorded in previous studies (Foissner 1987; Alekperov and Sadikhova 2006; Foissner 2008), The soils investigated by previous authors were: central alps in Austria, beech forests, meadow (Foissner 1987), Pirgulian State Reserve in Azerbaijan (Alekperov and Sadikhova 2006), and granitic rocks in rain forest in Singapore (Foissner et al. 2008). Unfortunately, no soil texture data is provided in the literature, making a proper comparison with this present research difficult. Thus, *D sphagni* may potentially be an indicator of coarse textured soils, but more research into its habitat is still needed.

4.5 Conclusion

The overall abundance of soil ciliates after 4 days and 10 days of incubation were significantly higher in natural soils than in the two agricultural soils investigated. In the natural ecosystem, the highest abundance was recorded after 10 days of incubation in almost all sampling months, but in agricultural habitats, the highest number of ciliates was recorded after 4 days of incubation in all observed months. Furthermore, a change in abundance with a temporal pattern was seen clearly in the natural Fen habitat. There was greater ciliate abundance in warm months than in colder months.

The results of these analyses demonstrated that the abundance and richness of soil ciliates had strong correlations with many soil properties in the three ecosystems considered together and in the natural Fen habitat separately. Soil moisture, SOM, and available concentrations of phosphorus were positively correlated with the abundance of ciliates after 4 days and 10 days of incubation at the three

ecosystems together, but negative correlations were found between soil pH and soil species richness in the natural Fen soil and between soil pH and soil ciliate abundance after 4 days and 10 days of incubation at the three ecosystems combined. Also, there was positive effect of available phosphorus on the number of soil ciliate species in the natural habitat.

Available concentrations of measured trace metals in the natural Fen soil had negative correlations with ciliate species richness, whilst available concentrations of Cu, Ni and Zn were positively correlated with ciliate abundance after 4 days and 10 days of incubation in the three habitats combined.

The level of ammonium had positive influences on ciliate abundance in three ecosystems combined and in the Fen on its own, but correlations between nitrate-N and soil ciliates in the natural Fen habitat or in the three habitats combined together can be explained by other factors such as soil pH or practices in agricultural production.

Similarly, total microbial activity and β-glycosidase were strongly and positively correlated to the abundance of soil ciliates, but acid-phosphomonoesterase showed no correlation in the three habitats combined.

A group of common ciliate species, i.e., *Cyclidium mucicola*, *Grosglockneria acuta*, *Lepthopharyx coastatus*, *Drepasomonas revoluta*, *Colpoda spp.* (*Colpoda steinii*, *Colpoda iflata*, and *Colpoda cucullus*), *Sathrophilus muscorum*, *Nivaliella plana*, *Pseudoplatyophrya nana*, and Hypotrichia group (such as *Gonostomum affina*, *Tachysoma pellionellum*, *Psilotricha silvicola* sp. nov.) was very abundant in the natural Fen habitat. This group can potentially indicate soil habitats which have rich content of organic matter. In addition, the abundance of *Homalogastra setosa* may be a potential indicator for agro-ecosystems and pH. Finally, *Drepasomonas sphagni* was found in coarse textured soil with low organic matter. Potentially, this type of soil texture is the favoured habitat of this species.

Chapter 5. TAXONOMY OF SOIL CILIATES

Abstract

The number of free-living ciliates may be up to 30,000 species. Many soil ciliate species have been recently discovered, and many species remain undiscovered. Studies comparing ciliate species richness between natural and agricultural soils are sparse. Here we investigated the species richness of soil ciliates in the natural and agricultural soils. The results showed that there was a similarity in ciliate species richness between the natural and agricultural soils, with 82 species found in the natural soil compared to 88 and 84 species in each farm investigated. One new species within the genus *Psilotricha*, and two other potential new species within the genera *Pseudoholophrya* and *Oxytricha* were found after intensive analysis of the soils samples.

5.1 Introduction

5.1.1 General introduction

The presence of each soil ciliates or total ciliate species richness can be indicators for environmental status where they live (Li et al. 2010a) or for the impact of human or natural disturbances (Foissner 1987; Petz and Foissner 1989; Foissner et al. 2005). These have been tested in the previous Chapters. As mentioned in Chapter 4 and Chapter 6, some species have potential indicators of soil characteristics (pH, soil texture, and the contaminated status of metal, herbicide, and insecticide). In addition, recording the presence of each species also gives information that the species is rare or abundant through comparison of rarity or abundance at a global scale (Finlay et al. 2001).

Based on advances in molecular phylogeny, Burki et al. (2008) suggested that the SAR super-group, i.e., Stramenophile, Alveolates and Rhizaria, in which ciliates (phylum Ciliophora) along with dinoflagellata, apicomplexa and protalveolata are comprised within the Alveolates group (Adl et al. 2005; Adl et al. 2012). The Phylum Ciliophora is divided into two sub-phyla the Postciliodesmatophora, which is characterised by members dividing their macronuclei with extramarconuclear microtubules or non-division of their macronuclei and the Intramacronucleata, which divide their macronuclei with intranuclear microtubules (Lynn 2008; Gao and Katz 2014).

Ciliates have primarily been identified and grouped at morphological level by using microscopic observations of living cells (Lynn 2008). However, with the advent of

molecular technology, sequences of ciliate ribosomal RNA genes have been used to support their taxonomic classification based on infraciliature (Lynn 2003).

Soil ciliates are ubiquitous free-living microscopic organisms living in soil habitats (Esteban et al. 2006). Many new soil ciliate species have been described and it is claimed that many remain undescribed and undetected (Chao et al. 2006; Foissner et al. 2008; Foissner 2016). Thus, investigating the species richness of different ecosystems is necessary in order to further advance our knowledge and understanding of the biodiversity of soil ciliates.

5.1.2 Background information for *Psilotricha silvicola* nov. sp.

Psilotricha acuminata was described as the type species of the hypotrich genus *Psilotricha* in sub-phylum Intramacronucleata by Stein in 1859 (Stein 1859a, 1859b) but its infraciliature remained unknown until 2001, when Esteban et al. (2001b) re-discovered the species in grassland soils from Scotland (UK). Unfortunately, no molecular data are available for this species. Stein (1859b) had proposed the inclusion of the genus *Psilotricha* within the family Oxytrichidae, which was then corroborated by Esteban et al. (2001a) based on their morphological and morphogenetic findings in *P. acuminata*: having two rows of marginal cirri (one left and one right), reduced number of transverse cirri, presence of caudal cirri, and having long primary primordia in the early stages of the morphogenesis. Lynn (2008) and Heber et al. (2014) placed the genus in the family Psilotrichidae Bütschli, 1889, along with *Urospinul*a, *Hemiholosticha*, and *Psilotrichides* based on the diagnostic characters of absence of caudal cirri, even though *Psilotricha* as described by Stein (1859a) and Esteban et al. (2001a) does have three caudal cirri. The phylogenetic analysis performed by Heber et al. (2014) placed *Urospinula succisa*, the only sequenced species within the family Psilotrichidae at that time, in a clade containing species from the families Oxytrichidae, Kahliellidae and Halteriidae, although neither the position of *Urospinula* nor the position of its clade members was statistically supported. Thereby, the branching of Psilotrichidae still remains elusive in phylogenetic analyses (Heber et al. 2014).

Here, we describe a new species within the genus *Psilotricha* Stein, 1859, similar to, but distinct from *P. acuminata*, and we provide for the first time the 18S rRNA sequence of a species in the genus *Psilotricha.* Our findings provide further evidence that the genus *Psilotricha* belongs to the Oxytrichidae family, as established by Stein (1859a, b) and Esteban et al. (2001a).

The overall aim of this Chapter is to present the results of the ciliate species richness found in the natural and agricultural soils investigated in this present research.

The objectives of the work are:

i) To catalogue the ciliate species found in the three types of soil habitats, including the list of potential undescribed species;

ii) To describe the morphology and phylogeny of new species found.

5.2 Materials and Methods

5.2.1 General methods

To identify soil ciliate species, the method described in section 2.3.2 of Chapter 2 was followed. The silver-impregnation techniques used included silver-carbonate impregnation (Fernández-Galiano 1994) and protargol (Foissner 2014) methods, which are essential for species identification (see Chapter 2).

The richness of ciliate species is separately recorded and presented for both Plot 1 and Plot 2 of three study sites.

5.2.2 Materials and methods to describe and classify *Psilotricha silvicola* nov. sp.

Sampling site and culture methods

Soil samples were collected at Plot 2 in the East Stoke Fen Nature Reserve (Wareham, Dorset, UK; grid reference SY 86531 86614) for nineteen months (January 2016 to December 2017). The soil contained ~ 30 % of organic matter, and the texture is representative of sandy loam with soil pH (H_2O) ranging from 3.8 to 4.5 and soil moisture oscillating from 36-55 %.

The experimental plot within the Fen was 1 x 2 m. Samples were taken with a 4 cm-diameter stainless steel corer from the topsoil to a depth of 5 cm, which includes the litter layer. The overall method to collect and process the soil samples followed Finlay et al. (2000). On each sampling occasion, eight core samples were taken randomly from within the experimental plot to ensure samples were representative of the plot. The soil collected was removed from the corer and placed in a labelled plastic bag to form a bulk sample for that plot and transferred immediately to the laboratory. The content of the bag was mixed well in a 30 cm diameter glass bowl. A sub-sample of soil was then taken and spread out as a layer in a 15 cm-diameter glass Petri dish, and dried at room temperature (18-22 ^{o}C) for six days. Five grams

of the air-dried soil was rewetted according to Finlay et al. (2000) with filtered rain water to stimulate population growth of ciliates including *Psilotricha silvicola* nov. sp.

Morphological methods

Morphometric measurements were performed on living cells at 40-400 x and on impregnated specimens at 1,250 x. To reveal the infraciliature, the protargol method as described by Foissner (2014) was followed. Drawings of impregnated cells were based on free-hand sketches and micrographs.

Terminology is according to Berger (1999), Esteban et al. (2001a) and Lynn (2008).

Molecular methods

P. silvicola nov. sp. was isolated from enriched soil cultures and washed in PCR water using a hand-drawn micropipette. Cells were put in PCR tubes, frozen at -30^{o}C until they were sequenced.

The 18S rRNA from ciliate cells was amplified using the KOD Hot Start DNA Polymerase kit (Merck-Millipore, Burlington, USA) following the manufacturer's standard protocol and the primers for the eukaryote SUU rRNA sequences described by (Embley et al. 1992) (forward 5'-AYCTGGTTGATYYTGCCAG-3´; reverse 5'-TGATCCATCTGCAGGTTCACCT-3´).

The PCR product was purified from a 1 % agarose gel using a QIAquick Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany), ligated into pJET 1.2 plasmids, and cloned using a CloneJET PCR Cloning Kit (Life Technologies, Carlsbad, USA) into DH5α competent *E. coli* cells. Plasmids were purified from overnight cultures using a QIAprep Spin Miniprep Kit (QIAGEN GmbH) and three clones for the PCR product were Sanger sequenced in both directions by GATC Biotech using plasmid-specific sequencing primers provided in the cloning kit. Sequencing reads were assembled using the program Sequencher 5.4.6 (Gene Codes Corporation).

Phylogenetic analyses

The SSU 18rRNA gene sequence of *P. silvicola* nov. sp. was aligned with gene sequences downloaded from the GenBank database using Muscle (Edgar 2004) implemented in MEGA 7 software (Kumar et al. 2016) under the default parameters. Accession numbers are provided in Figure 5.15. Ambiguous regions and gaps were identified and then removed by Gblocks 0.91b with all three options checked for a less stringent selection (Castresana 2000; Talavera and Castresana 2007), leaving 1,745 unambiguous positions. The Bayesian inference (BI) analysis was performed with MrBayes v.3.2.5 (Ronquist and Huelsenbeck 2003) using the GTR + *I* + *G* as

the best model selected by the jModel test v.2.1.10 software (Darriba et al. 2012) using Akaike Information Criterion with correction (AICc). Two Markov Chain Monte Carlo (MCMC) simulations were run for 10,000,000 generations with a sampling frequency of every 100 generations and a burn-in of 25,000 trees (25%). The maximum likelihood (ML) analysis was computed by MEGA 7 (Kumar et al. 2016) using 1000 bootstrap replicates. Phylogenetic trees were visualized using the free software package FigTree v1.4.3 by A. Rambaut (http://tree.bio.ed.ac.uk/software/figtree/).

DNA amplification and sequencing and polygenetic analyses was conducted by collaboration with Dr. Kacpa Sendra, Institute for Cell and Molecular Biosciences, The Medical School, Newcastle University, United Kingdom and Dr. Pablo Quintela-Alonso in Madrid, Spain.

5.3 Results

5.3.1 The list of soil ciliate species

The list of different ciliate species identified in the three ecosystems is presented in Table 5.1. In addition, photomicrographs of the ciliate species recorded are presented in Figures 5.1 to 5.8.

In general, variation in the number of ciliate species among the three ecosystems was low. The richness of ciliate species was slightly higher in the agricultural soils, i.e., 88 species in the Vicarage Farm and 84 species in the Corfe Castle Farm *vs.* 82 species in East Stoke Fen Nature Reserve (Fen).

Of the 82 species recorded in the Fen, 70 species belonged to 46 genera were recorded from Plot 1, and 57 species of 38 distinct genera were recorded from Plot 2, respectively. Meanwhile, the Vicarage Farm yielded 88 species in total, 74 species belonging to 44 genera from Plot 1, and 66 species belonging to 38 genera from Plot 2. Similarly, Corfe Castle Farm showed slightly less number of species in comparison to Vicarage Farm with 84 species in total, 63 species (38 genera) of which were recorded from Plot 1, and 73 species (45 genera) from Plot 2.

One new species *Psilotricha silvicola* nov. sp. was found in Plot 2 in the Fen (Wareham, UK) in all sampling occasions from January 2016 to December 2017. Detailed description of this species is presented below.

Two other potential new ciliate species were also discovered. One potential species possibly of the genus *Pseudoholophrya* was 40-50 µm in length and elongated in shape that found in all Plots of three study sites (Figure 5.7). The other potential

new species discovered at both Plots of Vicarage Farm possess zoochlorellae and it feeds on diatoms (Figure 5.8). Furthermore, several of the ciliate species recorded in this research are first recorded for UK such as *Colpoda minima* (Figure 5.2), *Holosticha adami* (Figure 5.6-B), *Nassulla terricola*-Complex (Figure 5.3), and *Stylonychia vorax* (Figure 5.6-A).

Table 5.1 Taxonomic and ecological summary of soil ciliated protozoa species (*: unidentified due to very low number of cell present).

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Acineria incurvata Dujardin, 1841	+	-	-	-	-	+
Amphisiella sp.	+	-	-	-	-	-
Amphsiella binucleata (Hemberger, 1985) Foissner, 1988	-	-	-	-	+	+
Apospathidium terricola	-	-	-	+	-	-
Arcuospathidium sp. 70x	-	-	-	-	+	+
Arcuospathidium vermiformis Foissner, 1984	-	-	+	+	+	+
Aspidisca cicada (Müller,1786) Claparède & Lachmann, 1858	+	-	-	-	-	-
Aspidisca turrita Ehrenberg, 1831) Claparède & Lachmann, 1858	+	+	-	-	-	-
Avestina sp.	-	-	-	+	-	-
Bardeliella pulchra Foissner, 1983	+	+	+	-	-	+
Blepharisma hyalinum Perty, 1849	+	+	+	+	+	+
Blepharisma steinii Kahl, 1932	+	+	-	-	-	+
Bresslaua vorax Kahl, 1931	-	-	+	-	-	-
Bryometopus pseudochilodon Kahl, 1932	+	+	-	+	+	+

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Bryometopus pseudochilodon Foissner, 1993	+	-	-	-	+	+
Bryophyllus sp.	-	-	+	-	-	-
Chaena sp.	+	+	-	-	-	+
Chaena stricta Dujardin, 1841	-	+	-	-	-	+
Chilodonella ucinata (Ehrenberg,1838) Strand, 1928	+	+	+	+	+	+
Chilodontopsis muscorum Kahl, 1931	+	-	-	-	-	-
Cinetochilum margaritaceum (Ehrenberg, 1831) Perty, 1849	-	-	+	+	+	-
Cirrophrya terricola Foissner, 1987	-	-	-	+	-	-
Colpoda augustini Foissner, 1987	+	-	-	-	-	-
Colpoda cucullus (Miiller, 1773) Gmelin, 1790	+	+	+	+	+	+
Colpoda henneguyi Fabre-Domergue, 1889	+	+	+	+	+	+
Colpoda inflata (Stokes, 1884) Kahl, 1931	+	+	+	+	+	+
Colpoda maupasi Enriques, 1908	+	-	+	+	+	+
Colpoda minima (Alekperov, 1985) Foissner, 1993	-	-	-	-	-	+

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Colpoda steinii Maupas, 1883	+	+	+	+	+	+
Colpoda variabilis Foissner, 1980	-	-	+	-	-	-
Colpodidium caudatum Wilbert, 1982	-	-	-	-	-	+
Cosmocolpoda sp.	-	-	-	+	-	-
Colpodea sp.1*	+	-	-	-	-	-
Colpodes sp.2*	-	-	+	+	-	-
Cyclidium muscicola Kahl, 1931	+	+	+	+	+	+
Cyrtohymena quadrinucleata (Dragesco & Njine, 1971) Foissner 1989	-	-	-	+	-	-
Cyrtohymena tetracirrata (Gellert, 1942) Foissner, 1989	-	-	+	-	-	-
Cyrtohymena muscorum (Kahl, 1932) Foissner, 1989	+	+	-	-	-	-
Cyrtohymena sp.	-	-	+	-	-	-
Cyrtolophosis elongate (Schewiakoff, 1892) Kahl, 1931	-	-	+	+	+	+
Cyrtolophosis mucicola Stokes, 1885	+	+	+	+	+	+
Deviata abbrevescans Eigner, 1995	-	-	-	-	-	+

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Dileptus mucronatus Penard, 1922	-	-	-	-	+	+
Dimacrocaryon amphileptoides (Kahl, 1931) Jankowski, 1967	+	+	-	-	-	-
Drepanomonas exigua Penard, 1922	+	+	-	-	-	-
Drepanomonas obtusa Penard, 1922	+	+	-	+	-	-
Drepanomonas pauciciliata Foissner, 1987	+	-	-	-	-	-
Drepanomonas revoluta Penard, 1922	+	+	+	+	-	-
Drepanomonas sphagni Kahl, 1931	-	-	-	-	+	+
Dileptus sp.	-	+	-	-	-	-
Enchelys multinucleata Alekperov, 1993	-	-	-	-	-	+
Enchelys sp.1	+	-	-	-	-	-
Enchelys sp.2	-	-	+	-	-	-
Epispathidium amphoriforme (Greeff, 1888) Foissner, 1984	-	-	+	+	+	-
Epispathidium ascendens	-	-	-	-	-	+
Epispathidium sp. 1	-	-	-	+	-	-

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Epispathidium sp.	-	-	+	-	-	-
Epispathidium sp.	-	-	-	-	+	+
Epispathidium terricola Foissner, 1987	-	-	+	+	+	+
Euplotes mucicola Kahl, 1932	-	-	+	+	-	-
Frontonia depressa (Stokes, 1886) Kahl, 1931	+	-	-	-	-	-
Fuscheria terricola Berger, Foissner & Adam, 1983	-	-	-	-	-	+
Gonostomum affine (Stein, 1859) Sterki, 1878	+	+	+	+	+	+
Grossglockneria acuta Foissner, 1980	+	+	+	+	+	+
Grossglockneria hyaline Foissner, 1985	+	-	+	+	-	-
Halteria grandinella (Muller, 1773) Dujardin, 1841	+	+	+	+	+	+
Hemisincirra filiformis (Foissner, 1982)	-	-	+	+	+	-
Hemisincirra gellerti (Foissner, 1982) Foissner, 1984	+	+	+	+	+	+
Hemisincirra gracilis (Foissner, 1982) Foissner, 1984	-	-	-	-	+	+
Hemisincirra interupta (Foissner, 1982) Foissner, 1984	+	+	+	+	+	+

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Hemisincirra similis (Foissner, 1982) Foissner, 1984	-	-	-	-	+	+
Holophrya sp.	-	-	+	-	-	-
Holophryidea sp. 1	+	-	+	-	+	+
Holophryidea sp.2	-	-	-	+	-	-
Holosticha adami Foissner, 1982	+	+	-	-	-	-
Holosticha monilata Kahl, 1928	-	+	-	-	-	-
Holosticha sigmoidea Foissner, 1982	+	+	+	+	-	+
Holosticha sp.	-	-	+	-	-	-
Holosticha sylvatica Foissner, 1982	+	-	-	-	-	-
Holostricha multistilata Kahl, 1928	-	-	+	+	-	-
Homalogastra setosa Kahl, 1926	-	-	+	+	+	+
Hypotrich sp.1*	-	+	-	-	-	-
Hypotrich sp.2*	+	-	-	-	-	-
Hypotrich sp.3*	-	-	-	-	-	+

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Kahlilembus attenuates (Smith, 1897) Foissner, Berger & Kohmann, 1994	+	-	+	+	+	+
Kreyella minuta Foissner, 1979	+	+	+	-	+	+
Lepthopharynx costatus Mermod, 1914	+	+	+	+	+	+
Metanophrys sp.	+	+	+	-	-	-
Metopus hasei Sondheim, 1929	-	-	-	-	-	+
Microdiaphanosoma arcuatum (Grandori & Grandori, 1934) Wenzel, 1953	+	+	+	-	+	+
Nassulla terricola-complex Foissner, 1989	-	-	+	+	+	+
Nivaliella plana Foissner, 1980	+	+	-	-	+	-
Opercularia sp. 1	-	+	-	-	-	-
Opercularia sp. 2	-	-	-	+	-	+
Oxytricha chlorelligera Kahl, 1932	-	-	-	-	+	+
Oxytricha longa Gelei & Szabados, 1950	+	-	-	-	-	-
Oxytricha setigera Stokes, 1891	+	+	+	-	+	+
Oxytricha similis Engelmann, 1862	-	-	+	-	-	-

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Oxytricha sp. with algae symbiont	-	-	+	+	-	-
Oxytricha sp.1	+	+	-	-	-	-
Oxytricha sp.2	+	+	-	-	+	+
Oxytricha sp.3	+	-	-	-	-	-
Oxytricha sp.4	-	-	+	+	-	-
Oxytricha sp.*5*	-	-	-	-	-	+
Oxytricha sp.6	-	-	-	-	+	+
Oxytricha sp.7	-	-	+	+	-	-
Oxytricha sp.8	+	+	-	-	-	-
Oxytricha sp.9	-	-	+	+	-	-
Oxytricha sp.10	-	-	-	-	+	-
Paragonostomum binucleatum Foissner, Agatha & Berger, 2002	-	-	-	-	+	+
Paragonostomum caudatum Foissner, Agatha & Berger, 2002	-	-	-	-	+	+
Phialina binucleata Berger, Foissner & Adam, 1984	+	+	-	-	-	-

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Phialina jankowskii Foissner, 1984	-	-	-	+	-	-
Phialina terricola Foissner, 1984	-	-	+	+	+	+
Platyophrya macrostoma Foissner, 1980	+	-	-	+	+	-
Platyophrya spumacola Kahl, 1927	+	+	+	+	+	+
Platyophrya vovax Kahl, 1926	+	+	+	+	+	-
Pleuroplites australis Foissner, 1988	+	-	+	-	+	-
Protocyclidium terricola Kahl, 1931	-	-	-	-	+	-
Protospathidium bonneti (Buitkamp, 1977) Foissner, 1981	+	-	+	+	+	+
Pseudoholophrya sp.	+	+	+	+	+	+
Pseudoholophrya terricola Berger, Foissner &. Adam, 1984	-	-	+	+	+	+
Pseudoplatyophrya nana (Kahl, 1926) Foissner, 1980	+	+	+	+	+	+
Pseudoplatyophrya terricola Foissner, 1985	+	+	+	+	+	+
Psilotricha silvicola nov. sp.	-	+	-	-	-	-
Sathrophilus muscorum (Kahl, 1931) Corliss, 1960	+	+	+	+	+	+

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Spathidium longcaudatum (Buitkamp & Wilbert,1974)	-	-	-	+	-	+
Spathidium procelum Kahl, 1930	-	-	+	-	+	+
Spathidium sp.1	-	+	+	+	-	+
Spathidium sp.	-	-	+	+	-	-
Spathidium spathula (Muller, 1773) Moody, 1912	+	+	+	+	+	-
Sphaerophrya sp.	+	-	-	-	-	-
Stammeridium kahli (Wenzel, 1953) Wenzel, 1969	+	+	-	-	-	-
Sterkiella cavicola Kahl, 1935	-	-	+	+	-	-
Sterkiella histriomuscorum Foissner et al., 1991	-	-	+	+	+	+
Stichotricha sp.	+	-	-	-	-	-
Stylonychia sp.	-	-	+	-	-	-
Stylonychia vorax Stokes, 1885	-	+	-	-	-	-
Tachysoma pellionellum (Müller, 1773) Borror, 1972	-	+	-	-	-	-
Tachysoma sp.1	-	-	+	-	-	-

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Tetrahymena sp.	-	-	+	-	-	-
Unknown sp.1 *	-	+	-	-	-	-
Unknown sp.2 *	-	-	-	+	-	+
Unknown sp.3 *	-	-	-	-	-	+
Uroleptus lepisma (Wenzel, 1953) Foissner, 1998	-	+	-	-	-	-
Urosoma cienkowski Kowalewski, 1882	-	-	-	-	-	+
Urosomoida agiliformis Foissner, 1982	+	+	+	-	+	-
Urosomoida agilis (Engelmann, 1862) Hemberger, 1985	-	-	+	+	+	+
Urosomoida sp.	+	+	-	-	-	-
Urotricha globose Schewiakoff, 1892	+	-	-	-	-	-
Vorticella astyliformis Foissner, 1981	+	-	-	-	+	+
Vorticella infusionum Dujardin, 1841	-	+	+	+	-	-
Vorticella similis Stokes, 1887	-	-	+	+	+	+
Vorticella sp.	-	-	+	-	-	-

Table 5.1 continued.

Species	Habitat type					
	East Stoke Fen		Vicarage Farm		Corfe Castle Farm	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Total number species in each Plot	70	57	74	65	63	73
Total number species in each study habitat	82		88		84	
The common species in both Plots of each study habitat	39		51		47	

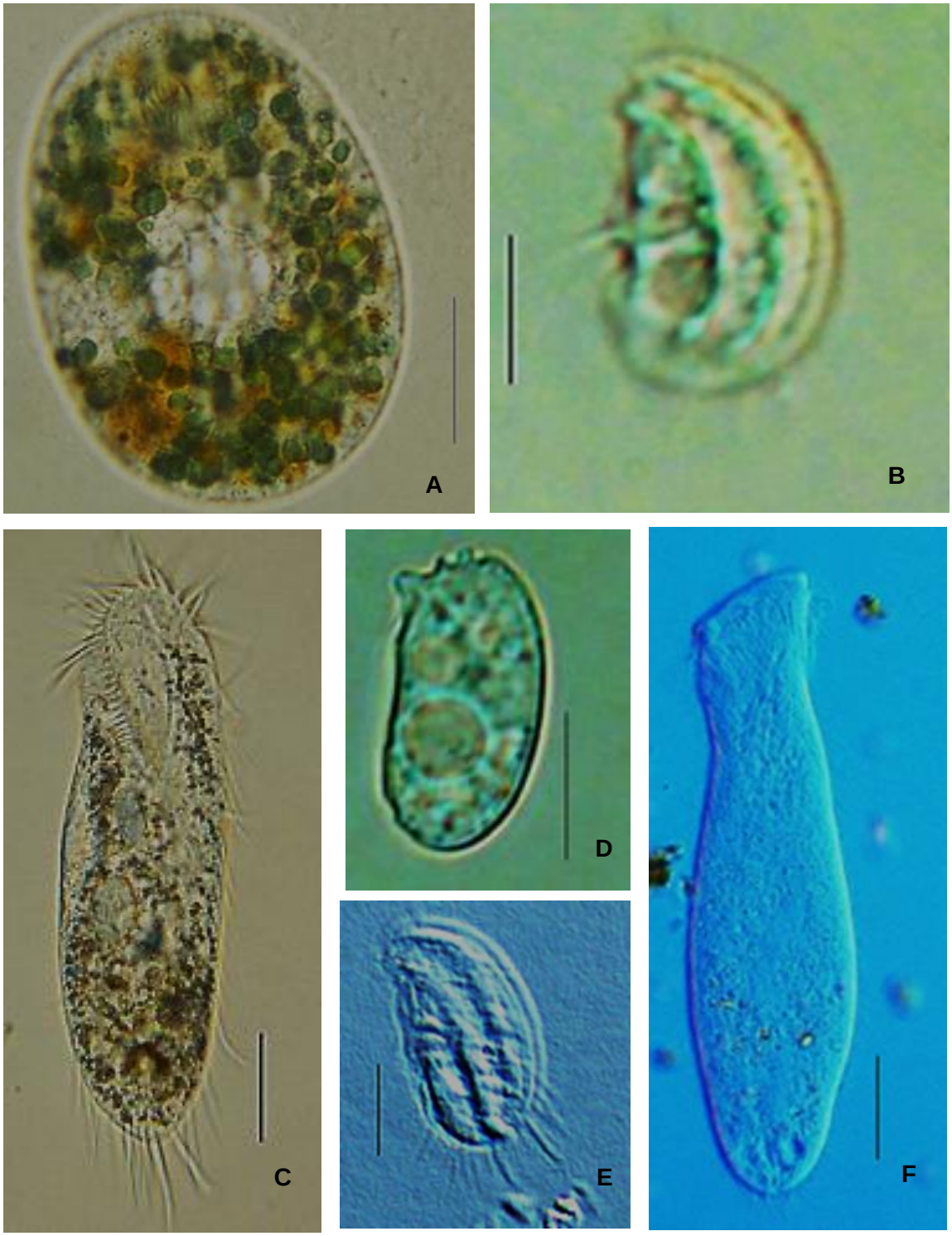


Figure 5.1 Soil ciliates, living specimens. **A**. *Nassula terricola* Complex. **B**. *Drepanomonas exigua*. **C**. *Cyrtohymena muscicola*. **D**. *Stammeridium kahli*. **E**. *Drepanomonas obtusa*. **F**. *Epispathidium terricola*. Scale bars = 30 µm in A, C, F; Scale bars = 10 µm in B, D, E. A, F from Plot 1 in Vicarage Farm; B, D, E from Plot 1 in the Fen; C from Plot 2 in the Fen.

Figure 5.2 Soil ciliate, silver-impregnated specimens of *Colpoda minima* (Alekperov, 1985) Foissner, 1993 from Plot 2 in Corfe Castle Farm. Scale bar = 40 µm.

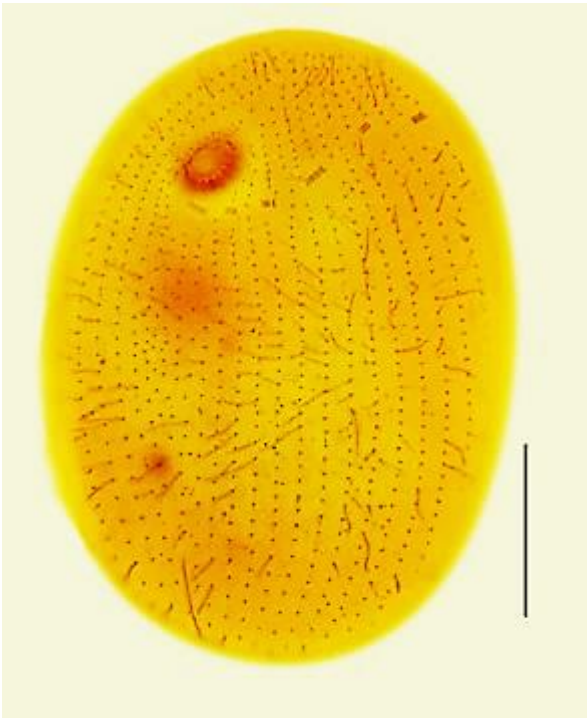


Figure 5.3 Soil ciliate, silver-impregnated specimens of *Nassulla terricola*-Complex Foissner, 1989 from Plot 1 in Vicarage Farm. Scale bar = 40 µm.

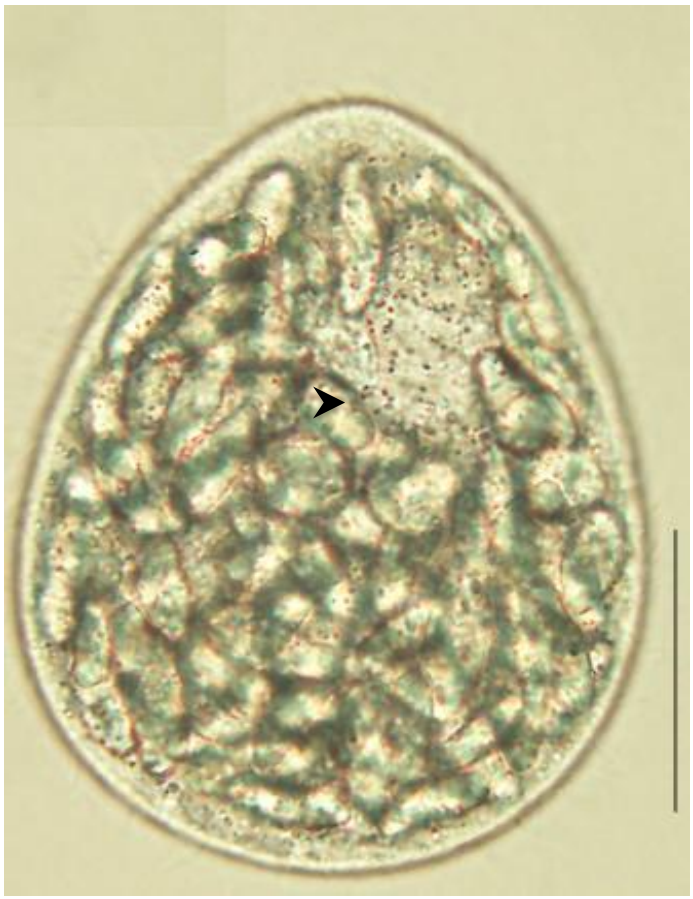
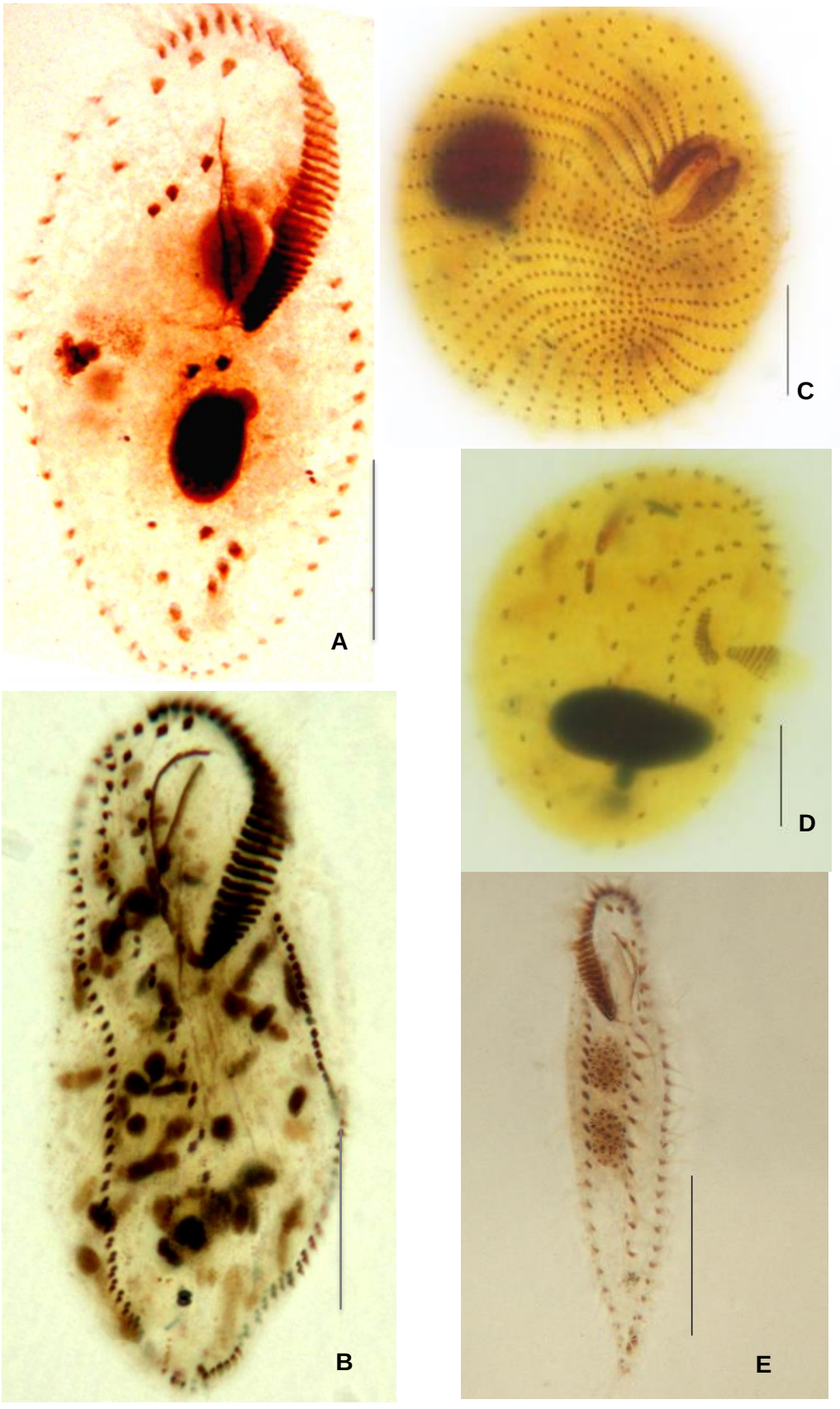


Figure 5.4 Soil ciliate**, l**iving specimen *of Bresslaura vorrax* fed with one hundred cells of *Homalogastra setosa* (arrowhead) from Plot 1 in Vicarage Farm. Scale bar = 30 µm.

Figure 5.5 Soil ciliate, living **(A)** and silver-impregnated specimens **(B, C)** of *Homalogastra setosa.* A and C from Plot 1 in Vicarage Farm; B from Plot 1 in Corfe Castle Farm. Ma: Macronuclei. Scale bars = 10 µm.



Figrue 5.6 Soil ciliates, silver-impregnated specimens. **A**. *Stylonychia vorax*, **B**. *Holosticha adami*, **C**. *Colpoda cucullus*, **D**. *Colpoda steinii*, **E**. *Uroleptus lepisma*. Scale bar = 40 µm in B; Scale bars = 30 µm in A, C, E; Scale bar = 10 µm in D. A, B and E from Plot 2 in the Fen; C, D from Plot 1 in the Fen.

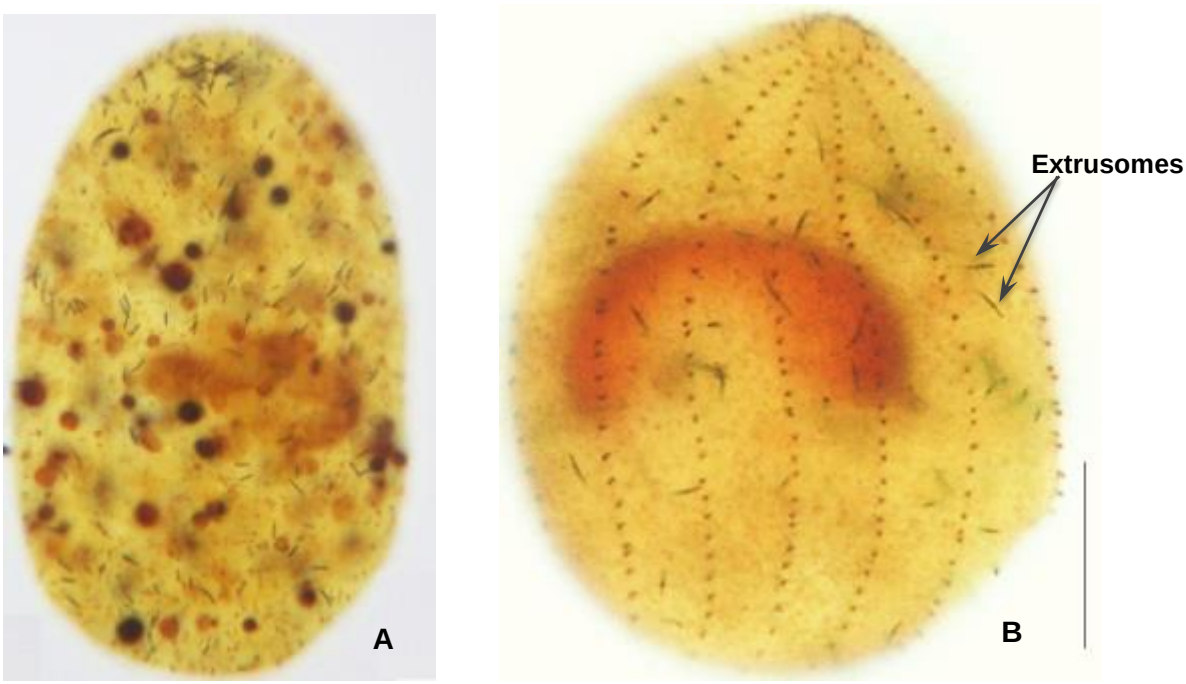


Figure 5.7 A-B. *Pseudoholophrya* sp. after silver impregnation from Plot 1 in Vicarage Farm. Scale bars = 10 µm.

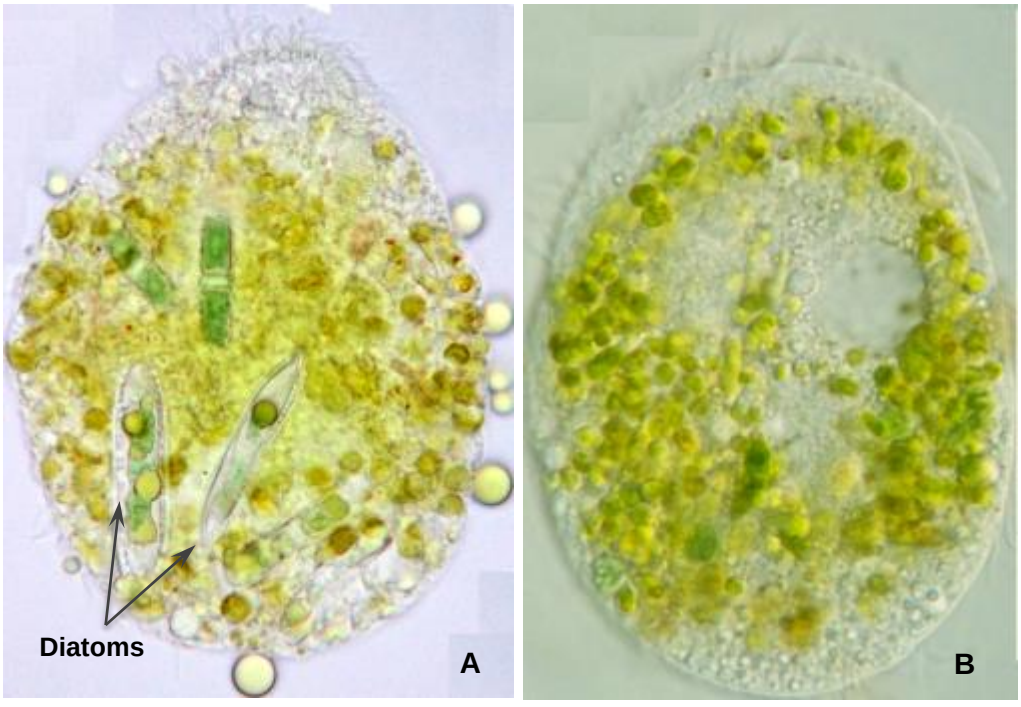


Figure 5.8 A-B. Soil ciliates in life. **A.** *Oxytricha* sp. (~150x50 µm in size), feeding diatoms from Plot 2 in Vicarage Farm and **B.** *Oxytricha chlorelligera* (~110x30 µm) from Plot 1 in Corfe Castle Farm.

5.3.2 Morphology and phylogeny of a new soil hypotrich ciliate (Alveolata, Ciliophora) from Dorset, the United Kingdom: *Psilotricha silvicola* nov. sp.

Occurance and growth. *Psilotricha silvicola* nov. sp. was isolated from soil samples collected at East Stoke Fen Nature Reserve in Southern England (Wareham, UK). Soils were examined during 19 months as part of a larger project; *P. silvicola* nov. sp. was recorded in all sampling occasions, and was detected after rewetting air-dried soils for ten days (see Methods). The ciliate grows well in rewetted soils at room temperature if some boiled wheat grains are added to encourage bacterial growth. *P. silvicola* nov. sp. thrives in this enriched cultures for approximately ten days.

Ethymology. The species named *silvicola*, referring to the woodland site where the species was discovered.

Description of *Psilotricha silvicola* nov. sp.

Table 5.2 shows the morphometric characters of *Psilotricha silvicola* nov. sp. Its size (in vivo) is 55-80 µm long x 30-50 µm wide, usually about 65 x 40 µm. Cells are colourless or slightly greyish due to the presence of particles in the cytoplasm (Figure 5.9-A, B). The cirri are very long and sparse - a common morphological characteristic for the species of the genus *Psilotricha*. Living organisms of *P. silvicola* nov. sp. have three types of movement: swimming, crawling, and filter-feeding, as also observed in *P. acuminata* (Esteban et al. 2001a) (Figure 5.9-D).

Living cells are oblong or slightly rounded in outline, dorso-ventrally flattened (Figure 5.9-A, B), resembling ciliates of the genus *Euplotes*. Cells of older populations are more elongated than younger ones. The anterior and posterior ends are rounded but the latter can also be pointed (Figures 5.9-A and 5.10-A). The single contractile vacuole is placed in the middle of the cell, towards the left margin (Figure 5.9-E). The dorsal surface has two longitudinal ribs positioned to the left and right of the main longitudinal axis (Figure 5.10-A), with the one on the right being longer than the one on the left. Lateral view of some cells shows a caudal tail due to the twisted long dorsal rib (Figure 5.9-C, D). All cells investigated had two elliptical macronuclear nodules, about 9 x 3 µm, one in the anterior half of the cell, the other in the posterior half. There is one micronucleus, 3 µm across on average, located between the two macronuclei and close to the equator of the cell (Figure 5.9-A, Table 5.2). However, 36 % of the cells investigated presented two micronuclei, one between the two macronuclei, and the other one on top of the anterior-most macronuclear nodule.

The Adoral Zone of Membranelles (AZM) is well developed (occupies an average of 46 % of body length) and consists of 19-26 polykinetids (Figure 5.9 and 5.10, Table 5.2), each formed by three rows of kinetosomes, one of them is shorter than the rest. In protargol-stained specimens the paroral membrane optically intersects the endoral membrane (Figure 5.12-A), although in several specimens it follows a parallel pattern.

The somatic ciliature of interphase cells of *P. silvicola* nov. sp. is composed of cilia and cirri. The right marginal row of cirri (RMC) and the left marginal row of cirri (LMC) are non-confluent posteriorly. As a common characteristic of the genus *Psilotricha* and typical of other oxytrichids, the RMC starts close to the cell equator and runs along the line of two of the fronto-ventral cirri (Figures 5.11-A and 5.12-A).

The RMC consists invariably of three cirri (Figures 5.11-A and 5.12-A). This characteristic is consistent in all our observations. Meanwhile the LMC comprises four (sometimes three) cirri (Table 5.2; Figure 5.11-A and 5.12-A).

The infraciliature on the ventral surface also includes: three frontal cirri close to the edge of the anterior part of the cell, four fronto-ventral cirri, one post-oral ventral cirrus, one pre-transverse ventral cirrus, and three transverse cirri close to the posterior end of the cell, one to the right and two to the left of the cell axis (Figures 5.11-A and 5.12-A, Table 5.2). The pre-transverse ventral cirrus (PTVC) is smaller than the transverse cirri (TC) and placed very close to the right-most transverse cirrus (Figure 5.11-A).

The dorsal infraciliature is composed of five dorsal kineties (DK) including one dorso-marginal kinety (DMK) (Figures 5.11-B and 5.12-B). Only two out of the 30 cells investigated presented four dorsal kineties, i.e., they lacked DK4. However, we cannot exclude that we overlooked DK4 in these two cells, since it is very short and it could go unnoticed due to the poorer impregnation of the dorsal infraciliature of those two specimens. DK1 starts about the level of the proximal end of AZM, DK2 occupying only the middle third of the body, DK3 bipolar but slightly shortened anteriorly, DK4 extremely shortened anteriorly as it is located in the posterior quarter of the cell, and DK5/DMK extending from slightly above the level of anterior end of DK3 to the level of the first right marginal cirrus (Figures 5.11-B and 5.12-B, Table 5.2). There are three caudal cirri, each at the posterior end of dorsal kineties 1, 3 and 4 (Figures 5.11 and 5.12).

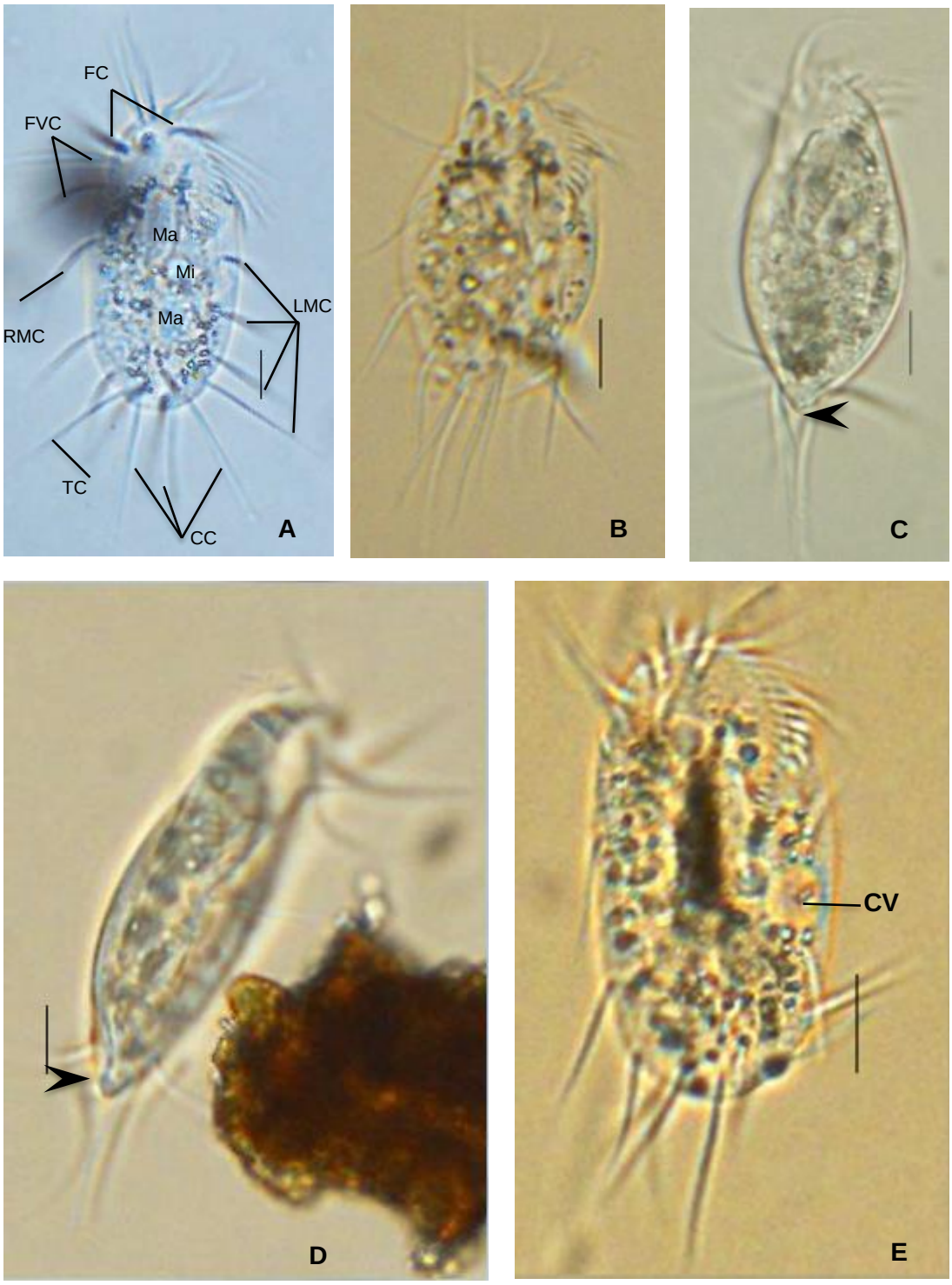


Figure 5.9 (A-E) *Psilotricha silvicola* nov. sp. from life. **A, B**. Ventral view of an old (A) and young (B) specimens. Note the elongated shape typical of older cells *vs.* rounded shape of younger cells; **C, D**. Lateral views of two specimens showing a 'caudal tail' (arrowhead); **E**. Ventral view showing the contractile vacuole. CC-caudal cirri, CV-contractile vacuole, FC-frontal cirri, FVC- fronto-ventral cirri, LMC-left marginal row of cirri, RMC- right marginal row of cirri, TC-transverse cirri. Scale bars = 10 µm.

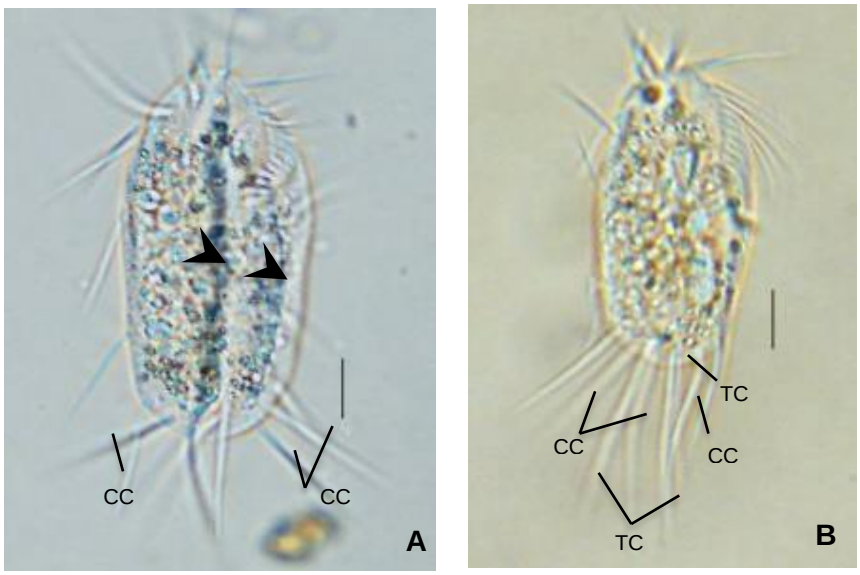


Figure 5.10 A-B. *Psilotricha silvicola* nov. sp. from life. **A**. Ventral side with slightly pointed posterior end. Arrowheads point to the two dorsal ribs, one is longer than the other. **B**. Ventral side showing the typical very long caudal and transverse cirri (up to 35 µm) CC-caudal cirri, TC-transverse cirri. Scale bars = 10 µm.

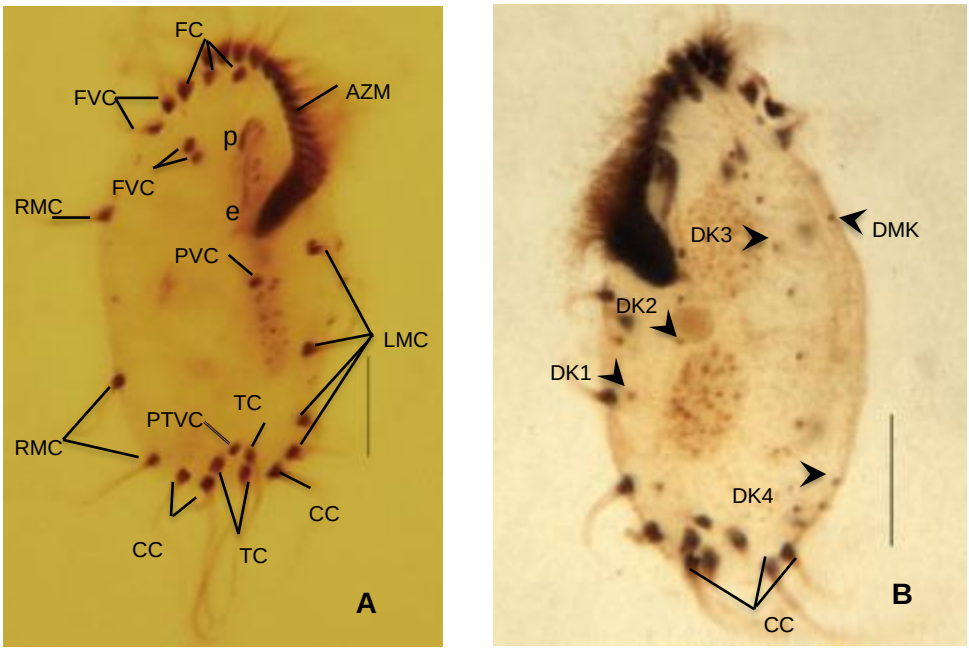


Figure 5.11. *Psilotricha silvicola* nov. sp. after protargol impregnation. **A**. Ventral view of a representative specimen showing the pattern of cirri, *inter alia*, two frontal cirri, three fronto-ventral cirri, one postoral ventral cirrus, one pre-transverse cirrus and three transverse cirri. **B**. Dorsal view of a specimen showing 5 dorsal kineties and three caudal cirri. CC-caudal cirri, DK1-4-dorsal kineties; DMK-dorso-marginal kinety, e-endoral membrane, FC-frontal cirri, FVC-fronto-ventral cirri, LMC-left marginal row of cirri, p-paroral membrane, PTVC-pre-transverse ventral cirrus, PVC-post-oral ventral cirrus, RMC-right marginal row of cirri, TC-transverse cirri. Scale bars = 10 µm.

Table 5.2 Morphometric data of *Psilotricha silvicola* nov. sp. from East Stoke Fen Nature Reserve (Wareham, UK).

Morphometric characters[a]	$\bar{x}$	M	SD	SE	Min	Max	n
Cell length in vivo (µm)	67.4	66.5	5.8	1.1	57	77	30
Cell width in vivo (µm)	37.8	37	4.5	0.8	29	47	30
Cell length (µm)	45	46	4.4	0.8	32	51	30
Cell width (µm)	21.2	21	3.1	0.6	17	29	30
Macronuclear nudules, number	2.0	2.0	0.0	0.0	2.0	2.0	30
Micronuclei, number	1.4	1.0	0.49	0.09	1.0	2.0	30
Macronucleus, length (µm)	9.3	9.1	2.05	0.37	6.15	13.4	30
Micronucleus, length (µm)	3.0	3.0	0.4	0.07	2.1	3.6	30
Adoral membranelles, number	22.9	23.0	1.98	0.36	18.0	26.0	30
Adoral zone, percentage of body length (%)	45.8	45.3	3.61	0.65	38.2	54.2	30
Cirri in right marginal row, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Cirri in left marginal row, number	3.9	4.0	0.3	0.05	3.0	4.0	30
Frontal cirri, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Fronto-ventral cirri, number	4.0	4.0	0.0	0.0	4.0	4.0	30
Post-oral cirri, number	1.0	1.0	0.0	0.0	1.0	1.0	30
Pretransverse ventral cirri, number	1.0	1.0	0.0	0.0	1.0	1.0	30
Transverse cirri, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Caudal cirri, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Dorsal kineties, number	4.9	5.0	0.3	0.06	4.0	5.0	30
Dorsal ribs, number	2.0	2.0	0.0	0.0	2.0	2.0	30

$\bar{x}$: arithmetic mean; M: median; SD: standard deviation; SE: standard error; Min: minimum; Max: maximum; n: number of individuals investigated. [a]Data based, if not mentioned otherwise, on mounted, protargol-impregnated, and randomly selected specimens.

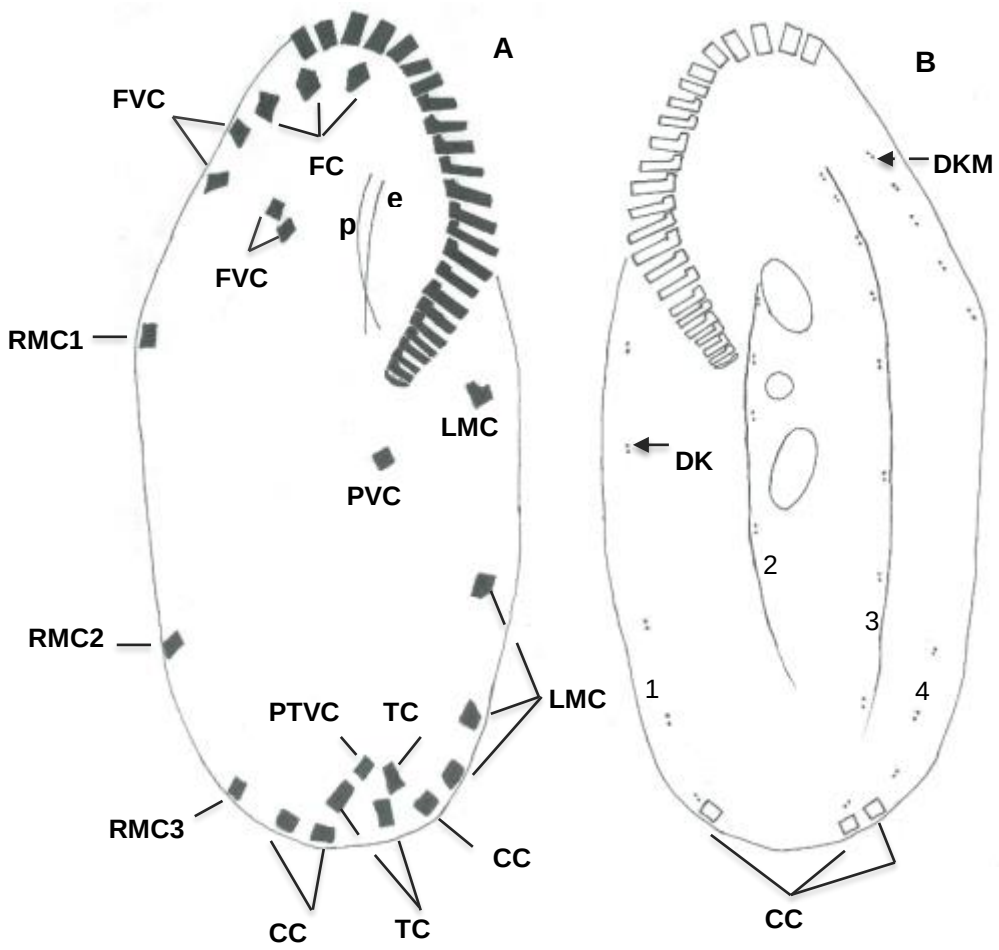


Figure 5.12 A-B. Line diagrams of *Psilotricha silvicola* nov. sp. after protargol impregnation. Ventral (A) and dorsal (B) views, CC-caudal cirri (note that they are located in the dorsal side but are easier to observe from the ventral side), DK1-4-dorsal kineties, DMK-dorso-marginal kinety, e-endoral membrane, FC-frontal cirri, FVC-fronto-ventral cirri, LMC-left marginal row of cirri, p-paroral membrane, PTVC-pre-transverse ventral cirrus, PVC-post-oral ventral cirrus, RMC-right marginal row of cirri, TC-transverse cirri.

Figure 5.13 *Hemiholosticha viridis,* population from East Stoke Fen (Wareham, UK); A. protargol impregnation; B. living specimen the endosymbiotic algae. Scale bars = 30 µm.

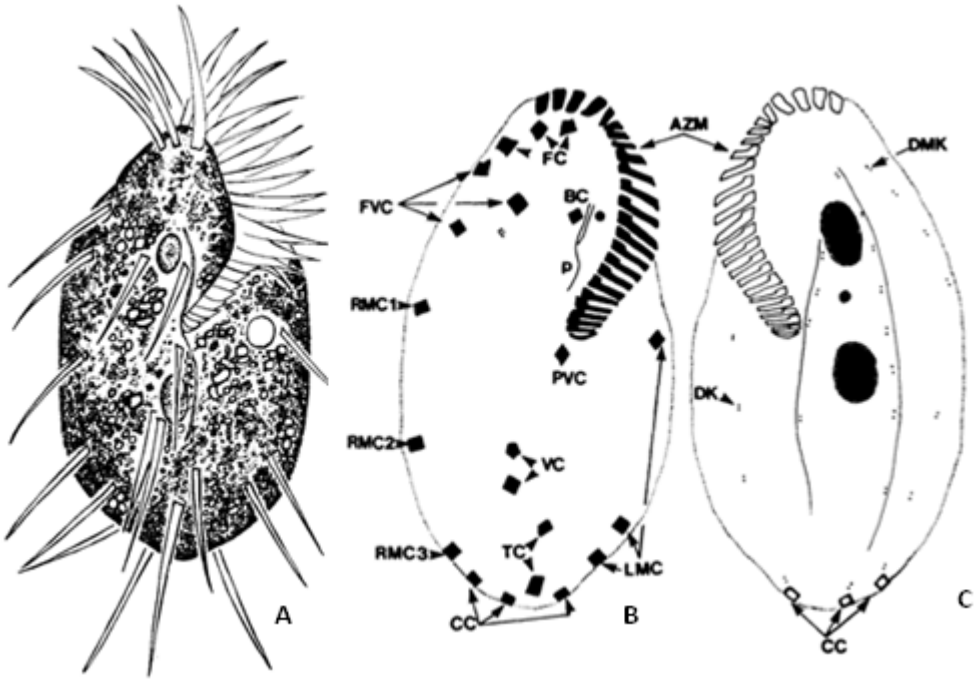


Figure 5.14 A-B-C. *Psilotricha acuminata* after Esteban et al*.* (2001a) from life (A) and after protargol impregnation (B-C). A. General morphology of a representative cell. B, C. Ventral (B) and dorsal (C) views. AZM-adoral zone of membranelles. BC-buccal cirrus, CC-caudal cirri, FVC-fronto-ventral cirri, FC-frontal cirri, LMC-left marginal row of cirri, PVC-post-oral ventral cirrus, p-paroral membrane, RMC-right marginal row of cirri TC-transverse cirri, VC-ventral cirri.

Table 5.3 Comparison between *Psilotricha silvicola* nov. sp. from East Stoke Fen (Wareham, UK), *Psilotricha acuminata* Stein, 1859 (from Scotland, UK; Esteban et al., 2001a), *Urospinula succisa* (Müller, 1789; Foissner, 1983), and *Psilotrichides hawaiiensis* (Heber et al., 2014).

Morphological character	*Psilotricha silvicola* nov. sp.	*Psilotricha acuminata*	*Urospinula succisa*	*Psilotrichides hawaiiensis*
Size in vivo (µm)	~ 65x40	~ 70x40	~ 120x70	~ 65x45
Macronuclear nudules, number	2	2-3	2	2
Micronucelei, number	1-2	1-2	1	1
Adoral membranelles, number	18-26	18-25	18-21	19-23
Buccal cirri, number	No	1	Not distinguishable	Not distinguishable
Ventral cirral rows[a], number	2	2	7	6
Cirri of right marginal row, number	3	3-4	6-10	4-10
Cirri of left marginal row, number	3-4	2-3	11-16	3
Post-oral ventral cirri, number	1	1	4-6[b]	1-3[b]
Frontal cirri, number	3	3	Not distinguishable	Not distinguishable
Fronto-ventral cirri, number	4	3+1unciliated cirrus	No	No
Ventral cirri, number	No	2	Form ventral rows	Form ventral rows
Pre-transverse ventral cirri, number	1	No	No	No
Transverse cirri, number	3	2	No	No
Dorsal kineties, number	4-5	4-5	3	3
Dorsal ribs, number	2	2	No	No
Caudal cirri, number	3	3	No	No

[a] include right and left marginal row of cirri and cirral ventral rows; [b] cirri form a cirral row.

Phylogenetic analysis

The investigated 18S rRNA sequence of *Psilotricha silvicola* nov. sp. was 1,766 base pairs long and had a 45.5 % GC content.

The maximum likelihood (ML) derived genealogy mostly matched the Bayesian inference (BI) topology, thus we present here the ML tree with node supports from both methods (Figure 5.15). *Psilotricha silvicola* nov. sp. nested within a clade of species mostly belonging to the family Oxytrichidae, i.e., *Paraurostyla weissei*, *Cyrtohymena citrina*, *Onychodromopsis flexilis*, *Rubrioxytricha ferruginea*, *Sterkiella histriomuscorum*, *Stylonychia mytilus*, *Gastrostyla steinii* and *Hemiurosomoida longa*), and one species of the family Plagiotomidae (*Plagiotoma lumbrici*), with moderate support from ML and full support from BI (i.e., 75.7/1.00).

The phylogenetic analysis showed that the family Oxytrichidae might be polyphyletic, which is congruent with previous publications (Schmidt et al. 2007; Kumar et al. 2014; Jung et al. 2015; Chen et al. 2017).

This phylogenetic analysis (Figure 5.15) also shows the position of *Urospinula* in relation to *Psilotricha*; the species *Urospinula succisa* formed a poorly supported clade by both ML and BI analyses (i.e., 38.4/0.57) with two species *Uroleptus lepisma* and *Uroleptus pisces*.

***Psilotricha viridis* sensu Kahl 1932**

The morphology and infraciliature of a population of *P. viridis* found in a freshwater pond, also at the East Stoke Fen Nature Reserve, was investigated (Figure 6.13), which could render some insight into the inclusion of this species within the genus *Psilotricha.* Unfortunately we could not obtain molecular data from *P. viridis*. However, the morphological study showed that this population of *P. viridis* does not match the diagnostic features of the genus *Psilotricha* but rather those of *Hemiholosticha viridis* after Gelei 1954 and after Dingfelder 1962 (as *Psilotricha viridis*) (Esteban et al. 2001a), *Psilotricha viridis* sensu Kahl, 1932 (Esteban et al. 2001a), and a German population of *Hemiholosticha* sp. showed in Heber et al. (2014).

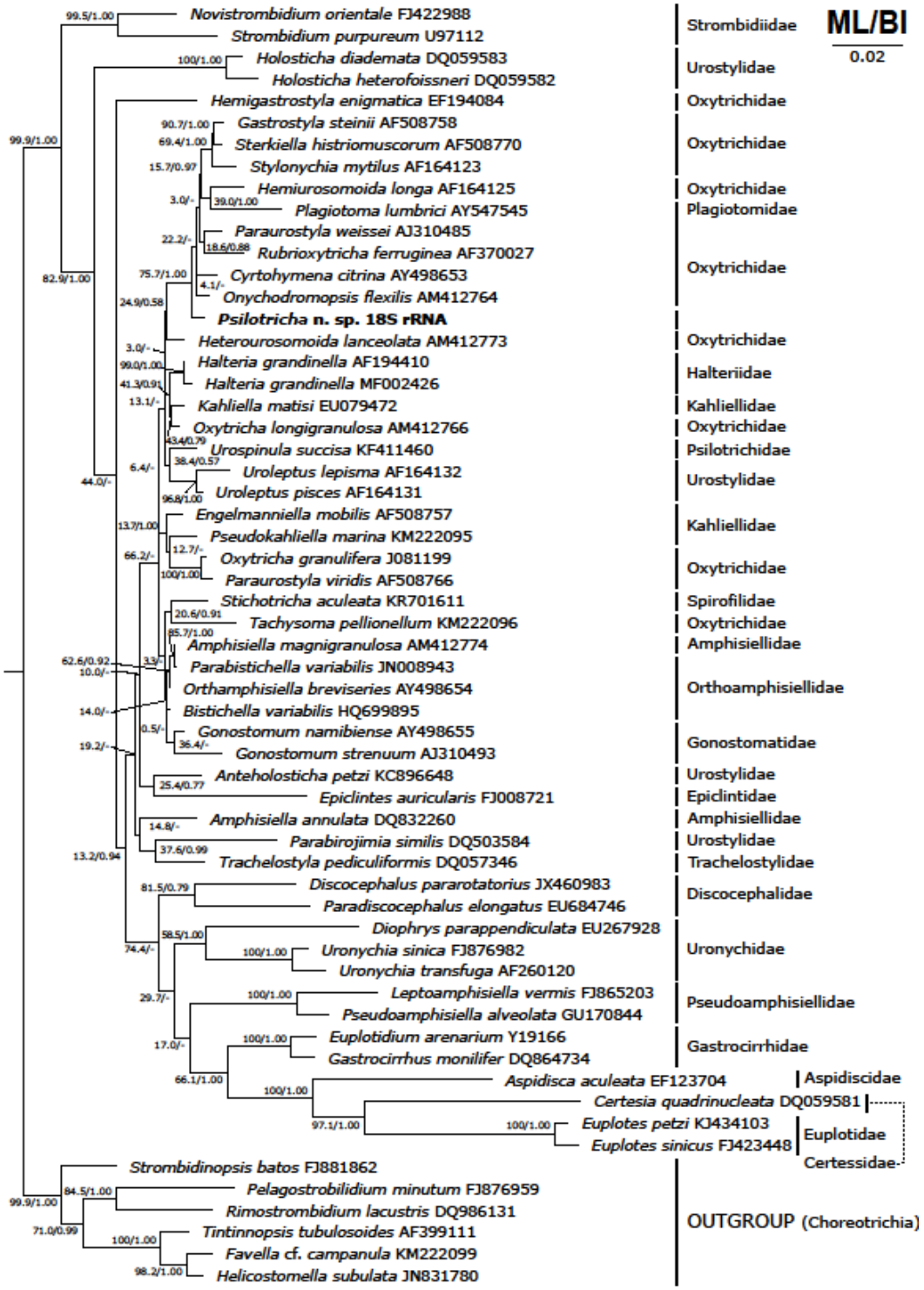


Figure 5.15 Maximum likelihood (ML) phylogenetic tree inferred from the 18S rRNA gene sequences, showing the position of *Psilotricha silvicola* nov. sp. Numerical support values are given at the respective nodes as: Maximum likelihood (ML) bootstrap out of 1000 replicates/Bayesian inference (BI) posterior probability. A hyphen (-) represents differences between ML and BI tree topologies. The GenBank accession numbers of these SSU rDNA sequences are provided after the species names. The scale bar corresponds to two substitutions per 100 nucleotide positions.

5.4. Discussion

5.4.1 General discussion

There are slight differences in soil ciliates species richness between the different type of studied soils, with total 82 ciliate species in East Stoke Fen compared to 88 an 84 species in Vicarage Farm and Corfe Castle Farm, respectively (Table 5.1). Foissner (1997c) indicated that there were slight differences in the soil ciliate species richness between natural ecosystems and agro-ecosystems, sometimes the number of ciliate species were higher in the latter than in the former. We found similar results in the present study; the number of ciliate species in agricultural soils was slightly higher than that in the natural habitat. Interestingly, 95 ciliate species were found in 150 soil samples at the Sourhope grassland in Scotland, UK (Finlay et al. 2001), whilst 82 different species were found in only 38 soil samples in natural soil in this project. Also, the Sourhope grassland was very open area, thus this might contribute to more soil ciliate species found compared with quite enclosed area which was covered by trees likes the Fen. Although the number of ciliate species differed, dominant species were similar in all the study sites in the present study, namely soil ciliate species of the Colpodid group. This is also in accordance with results from other terrestrial regions in the UK and the world (Foissner 1987, 1999c; Finlay et al. 2001; Foissner et al. 2005; Li et al. 2010a) that the ciliate species of Colpodid group were dominant and Colpodid had global distribution.

In the pesent study, nineteen species were common to all the studied sites: *Blepharisma hyalina*, *Chilodonella ucinata*, *Colpoda cucullus*, *Colpoda henneguyi, Colpoda inflata*, *Colpoda steinii*, *Cyclidium muscicola*, *Cyrtolophosis muscicola*, *Grossglockneria acuta*, *Gonostomum affine*, *Halteria grandinella*, *Hemisincirra gellerti*, *Hemisincirra interupta*, *Lepthopharyx costatus*, *Platyophrya spumacola, Pseudoholophrya* sp*., Pseudoplatyophrya nana, Pseudoplatyophrya terricola*, *Sathrophilus muscorum*. In particular, the common species found in the natural and agricultural soils of this study, *Colpoda* spp., *Cyclidium mucicola*, *Lepthopharyx costatus, Cyrtolophosis mucicloa* and *Sathrophilus muscorum* that had high frequency of occurrence (They were observed in almost all sampling months), also have high frequency at a global scale (Finlay et al. 2001; Foissner et al. 2002; Li et al. 2010b). Thereby, the findings of the present study confirm that of Finlay et al. (2001) that the rank in frequency of occurrence of local rare and common ciliate protozoan species were similar to rare and common recorded worldwide.

The potential new soil ciliate species

Oxytricha chlorelligera is about 115 µm in size and contain symbiotic algae (Kahl 1935; Berger 1999). This species was recorded at Corfe Castle Farm in several sampling occasions. In addition, similar species with endosymbiotic algae was also found at Vicarage Farm. However, this species was bigger (at least 150 µm in size) than *Oxytricha chlorelligera,* and seems to be feeding on diatoms (Figure 5.8-A). Apparent features suggest that it is a species of the genus *Oxytricha*. This species is potentially new. This species grew after 2 days after rewetting the soil samples, but active cells disappeared after 5 days of incubation. Furthermore, the number of individuals of this species was too low to carry out any silver impregnation techniques in order to carry out a full morphological description and to sequence its 18S rRNA gene.

Another potential new species is *Pseudoholophrya* sp. (Figure 5.7), which was found in all sampling occasions at all study sites. They bear the basic characteristics of *Pseudoholophrya terricola* (Berger et al. 1984), including the rows of monokinetids, extrusomes, one macronucleus; however we have not been able to see the nematodesmata, defined as characteristic of the genus (Berger 1984). Further research of this species is on-going.

5.4.*2 Psilotricha silvicola* nov. sp.

Comparison of *Psilotricha silvicola* nov. sp. with *Psilotricha acuminata* and other species

Table 5.3 shows the morphological differences between *Psilotricha silvicola* nov. sp. and related ciliates, including *Psilotricha acuminata* Stein, 1859 (Esteban et al. 2001a), *Urospinula succisa* Müller, 1786 as described by Foissner (1983), and *Psilotrichides hawaiiensis* Heber et al., 2014.

Psilotricha silvicola nov. sp. from the Fen (Dorset, UK) and *P. acuminata* Stein, 1859 from Sourhope (Scotland, UK; Esteban et al. 2001a) share common characteristics, such as the arrangement of the cilia and cirri on the dorsal and ventral surfaces, long and sparse cirri, types of motion, cell shape, having one right and left marginal ventral rows of cirri, presence and number of caudal cirri, deep and wide oral area with very long cilia in the anterior half of the membranelles, one post-oral ventral cirrus, variability of number of macronuclei and micronuclei and their position in the cell (Esteban et al. 2001b) (Figure 5.14). However, *P. silvicola* nov. sp. differs from *P. acuminata* (Esteban et al. 2001b) in some features: (i) absence of buccal cirrus *vs.* buccal cirrus present; (ii) three cirri on the right

marginal row and three/four cirri on the left marginal row (usually four cirri) *vs.* three/four cirri in the right marginal row and two/three in the left marginal row (usually three in both rows); (iii) absence of ventral cirri *vs.* two ventral cirri present; (iv) one pre-transverse ventral cirrus *vs.* none; (v) three transverse cirri of similar size *vs.* two transverse cirri one longer than the other; (vi) colourless or slight grey cytoplasm *vs.* dark cytoplasm (Table 5.3; Figures 5.12 and 5.14). Although unfortunately no molecular data of *P. acuminata* are available, based on the morphological similarities between these two species, they can be easily assigned to the genus *Psilotricha* Stein, 1859.

The case of "*Psilotricha viridis*"

P. silvicola nov. sp. differs from *P. viridis* sensu Kahl, 1932 in having caudal cirri, and lacking endosymbiotic algae and ventral cirral rows (Kahl 1935; Esteban et al. 2001b). *Hemiholosticha viridis* after Gelei 1954 and after Dingfelder 1962, and *Psilotricha viridis* sensu Kahl, 1932 are co-specific within the genus *Hemiholosticha* Gelei, 1954, as is *H. viridis* Heber, Stoeker and Foissner, 2014. Therefore, we consider the population of *P. viridis* (see Figure 5.13) found in the freshwater pond at the Fen (UK) to be *Hemiholosticha viridis* Geleii, 1954. The German population of *Hemiholosticha* sp. showed in Heber et al. (2014) is most probably *H. viridis* Geleii, 1954 based on the pattern of its infraciliature.

Taxonomy and phylogeny of *Psilotricha* species

The taxonomy of the genus has been unjustifiably convoluted despite the morphological evidence available in the literature. Stein (1859b) included the genus *Psilotricha* within the family Oxytrichidae; this was corroborated by Esteban et al. (2001b) who found and re-described the type species *P. acuminata* Stein, 1859. The inclusion of *Psilotricha* in the family Oxytrichidae has thus far been justified by morphology, i.e., having left and right marginal ventral cirri, reduced transverse cirri, and presence of caudal cirri. This arrangement of the infraciliature is also found in the new *Psilotricha* species here described, *P. silvicola* nov. sp. Furthermore, Esteban et al. (2001a) observed the formation of long primary primordia during the early stages of the ontogenesis of *P. acuminata* - a character typical of the family Oxitrichidae. Long primary primordia are considered a key morphogenetic character for classification of hypotrich species into Oxytrichidae (Eigner 1997; Esteban et al. 2001b).

Bütschli (1889) established the family Psilotrichidae for the genus *Psilotricha*. Lynn (2008) included *Psilotricha* Stein, 1859 and *Hemiholosticha* Gelei 1954 in the family

and Heber et al. (2014) added two more genera, *Urospinula* Corliss, 1960 and *Psilotrichides*, despite their lack of shared morphological features and without phylogenetic analysis because no molecular data were available for *Psilotrichides*. Nonetheless, Heber et al. (2014) remarked that *Psilotricha* actually looks like a typical *Oxytricha*, and the type species, *P. acuminata* (Stein, 1859b), re-described by Esteban et al. (2001a, b), and certainly presents the features of the family Oxytrichidae. With the discovery and characterization both at morphological and molecular (18S rRNA) levels of *P. silvicola* nov. sp., we contribute to increase the taxon sampling within the genus *Psilotricha*, although unfortunately from the remaining three genera included in the family Psilotrichidae, i.e., *Urospinula*, *Psilotrichides* and *Hemiholosticha*, only the 18S rRNA gene sequence of *Urospinula* is available in GenBank, which is insufficient to clearly establish phylogenetic relationships and resolve their family allocation. However, the fact that *Psilotricha silvicola* nov. sp. nested apart from *Urospinula succisa* in a clade mostly containing species in the family Oxytrichidae, is in agreement with the inclusion of *Psilotricha* within this family as previously proposed by Stein (1859b) and Esteban et al. (2001a, b).

Based on the results here presented, the genus *Psilotricha* family Oxytrichidae, includes *Psilotricha acuminata* Stein, 1859 (Esteban et al 2001a), and *Psilotricha silvicola* nov. sp., with the *incertae sedis Psilotricha dragescoi* Grolière, 1975 and *Psilotricha viridis* (Penard, 1922) Kahl, 1932.

The family Psilotrichidae would now consist of the genera *Hemiholosticha*, *Psilotrichoides* and *Urospinula*, subject to further molecular data to elucidate the phylogeny of the families Psilotrichidae and Oxytrichidae.

5.5 Conclusion

There was similar species richness of soil ciliates at the three study sites (natural and agricultural soils), although number of species was higher in the agricultural soils compared to the natural Fen soil. The most frequent soil ciliates in the three ecosystems were those of the Colpodid group.

In-depth analysis of the soils samples during 19 months rendered one new species within the genus *Psilotricha* and two other potential new species within the genera *Pseudoholophrya* and *Oxytricha*.

Chapter 6. IMPACTS OF COPPER AND PESTICIDES ON SOIL CILIATED PROTOZOA

Abstract

Agricultural soils are impacted by many potentially toxic substances, such as trace metals and pesticides. These can damage soil health and soil fertility. Ciliates may be sensitive to soil pollutants, and they could be bio-indicators of metal and pesticide impacts on the health of the soil. However, studies on the effect of trace metals like Cu and pesticides on soil ciliates are scant. Here we determine the effect of copper, herbicide glyphosate and insecticide cypermethrin on soil ciliates and on total microbial activity under pot trial conditions. A loam topsoil was used in the Cu and cypermethrin experiments, whilst soil collected from one of the field study sites was used in glyphosate experiment. All experiments were conducted under laboratory conditions for 15 days. In the glyphosate experiment, samples were collected at 1, 7, and 15 days after glyphosate application, whilst samples were collected after 15 days in the copper and cypermethrin experiments. Copper stimulated the abundance of soil ciliates at the highest concentration (960 mg kg^{-1}). The insecticide cypermethrin also increased the abundance of soil ciliates at concentrations ranging 80-640 mg kg^{-1}, but this increase was attributed to some dominant soil ciliate species. A positive correlation between the abundance of soil ciliates and soil total microbial activity when cypermethrin applied was shown. However, cypermethrin was toxic for *Homalogastra setosa* at concentration of 320 mg kg^{-1}, and the concentration of 640 mg kg^{-1} represents an LD_{100} for this species. Also, cypermethrin at the highest concentration affected the structure of the soil ciliate community by reducing species richness. Glyphosate herbicide had no effect on ciliate abundance, total ciliate species richness, and total microbial activity in samples examined 1 day and 7 days after glyphosate application, but had a toxic influence on ciliates abundance 15 days after glyphosate application. This could be due to a reduction in the abundance of *Homalogastra setosa* at the highest glyphosate concentrations of 16.82 and 33.6 mg kg^{-1}. It is suggested that *H. setosa* might be a potential bio-indicator of herbicide and insecticide-contaminated soils. In addition, there were no statistically significant effects of Cu, cypermethrin, and glyphosate, but changes in the structure of soil ciliate community were found at the highest concentrations of all pollutants at 15 days after application, when presence/absence of ciliate species is taken into consideration.

6.1. Introduction

Agricultural soils are subject to many potentially toxic substances, such as trace metals like Cu and pesticides. These can damage soil health and soil fertility (Vrba et al. 2006). How soil ciliates respond is little understood, but they could be important bio-indicators of metal and pesticide impacts on soil health. Although copper is typically found at trace levels, it is an essential and important element for all living organisms; it is a particularly important cofactor for a number of enzymes in cells (Krupanidhi et al. 2008). However, excess of copper can be toxic, for example, causing chronic liver diseases in mammals (Uauy et al. 2008). Copper was also shown to affect the micro-biota (Ekelund et al. 2003; Wyszkowska et al. 2006). It can impact activity of soil enzymes (Wyszkowska et al. 2006), reduce microbial and protozoa biomass, change the diversity of microbial communities and affect bacterial physiology (Aoyama 1993; Ekelund et al. 2003). Copper spiked soil experiments in laboratory conditions indicated that at high concentrations, Cu negatively affected mineralization by the soil microbial flora (Giller et al. 1998; Ekelund et al. 2003). Heavy metal contamination, including copper, of soil ecosystems can arise from using pesticides, sewage sludge, application of fertilizers, animals manures, industrial wastes, mining, smelting, etc. (Wuana and Okieimen 2011) and can therefore be considered to be widespread.

As previously mentioned in the literature review (Chapter 1), despite the important roles of soil protozoa in controlling biomass of smaller microorganisms and their potential as bio-indicators of soil quality, research on the impacts of trace metals in general, and copper in particular, on soil protozoa including soil ciliates is scant. Almost all studies focused on laboratory testing of trace metal toxicity and only a few ciliates and amoeba species have been investigated (Forge et al. 1993; Campbell et al. 1997; Pratt et al. 1997; Díaz et al. 2006; Martín-González et al. 2006; Bitencourt et al. 2016; Hao et al. 2016). Very few investigations have evaluated changes in abundance and diversity of protozoa in soil microcosm trials spiked with trace metals (Ekelund et al. 2003), which whilst providing conditions more closely resembling conditions in the field, still do not fully reflect the complex interactions found in the field. However, microcosm experiments allow complexity to be reduced, providing more controlled experimental conditions, which in turn can provide a clearer understanding of the toxic effects of metals.

Approaches using different methods and studying different soil protozoan species have showed big variations in the effects of trace metal toxicity due to metal resistance shown by certain distinct species (Giller et al. 1998; Díaz et al. 2006).

Thus, more studies regarding the effects of trace metals on soil protozoa at microcosm scale are necessary to evaluate thoroughly the vulnerability of protozoa under heavy metal contamination in order to assess potential of soil ciliates as bio-indicators for metal contaminated soils.

Besides an increasing concern regarding the effects of heavy metal contamination on microbial communities, intensive use of pesticides also has created many risks for the environment, especially non-target organisms. Thus, many types of pesticides, including herbicides, insecticides and fungicides have been evaluated for toxicity to microbial organisms, including protozoa (Petz and Foissner 1989; Todorov and Golemansky 1992; Ekelund et al. 1994; Ekelund 1999; Adebavo et al. 2007). Cypermethrin is a synthetic pyrethroid insecticide, used widely to control a wide range of insect pests in household and agricultural crops (Singh et al. 2012). Cypermethrin has a moderate persistence in soil; especially persisting in soil that has high organic matter content (Harris and Chapman 1981), but degradation in soil was raised in presence of microorganisms (Cole et al. 1981; Xie and Zhou 2008). However, there has been an increasing concern regarding toxicity of cypermethrin on soil microbial biomass and its activity in recent years (Goswami et al. 2012; Filimon et al. 2015). Studies indicated that cypermethrin causes a reduction in biomass and total enzyme activities of microorganisms in the short term, but after that may stimulate microbial growth (Goswami et al. 2012). However, research into impact of cypermethrin on protozoa, and in particular, on ciliates is very sparse. Up to now, there were studies only on the effect of the insecticide on freshwater ciliates (Friberg-Jensen et al. 2003; Dutta 2015). Thus, there is crucial need to evaluate the toxicity of this insecticide on protozoa in soil due to its widespread use.

Glyphosate is a type of foliar-applied broad-spectrum herbicide widely used since the 1970's to kill weeds in both agricultural and non-agricultural applications. It is the most commonly used herbicide globally, with use increasing sharply from 3.2 million kg in the year 1974 to 6133 million kg in the period of time 2005-2014 (Benbrook 2016). Because of its widespread use, there is a raising concern regarding its toxicity to the soil microbial biomass and general effects on ecosystem health (Benbrook 2016). However, study results were usually inconsistent; this can due to application of different concentrations, type of used soil or different parameters of soil used in tests (Parkinson and Wardle 1990; Haney et al. 2000; Partoazar et al. 2011; Nguyen et al. 2016). Furthermore, some studies investigating the impact of glyphosate containing herbicide on protozoa illustrated that some species of ciliated protozoa, i.e., *Colpoda cucullus* and *Colpoda steinii* suffered

mortality when exposed to low glyphosate concentrations. This raises serious concern, but also indicates that they have potential as bio-indicators of glyphosate-contaminated environments (Mbanaso et al. 2014). Other researches also showed that ciliate populations are sensitive to different glyphosate concentrations (Coupe et al. 2006; Bonnet et al. 2007). Due to the insufficiency of research evaluating the impacts of copper, glyphosate herbicide and cypermethrin insecticide on protozoan communities, especially on soil ciliates, further studies are needed in order to assess ciliates as bio-indicators of soil quality.

The aim of this Chapter is to determine the effect of copper (as copper chloride), glyphosate herbicide and cypermethrin insecticide on soil ciliated protozoa and on soil enzyme activity under pot trial conditions. The objectives of work presented in this Chapter are:

i) to determine the effect of Cu, glyphosate and cypermethrin on the abundance and species richness of soil ciliates in different treatments;
ii) to investigate the effect of Cu, glyphosate and cypermethrin on total microbial activity in different treatments;
iii) to determine the correlation of the abundance and species richness of soil ciliates with total microbial activity in different treatments.

6.2 Materials and methods

6.2.1 Determining soil water holding capacity

The optimum moisture content for the majority of soil micro-organisms is 60-80% of soil water-holding capacity (WHC). Soil micro-organisms could suffer from dryness if the moisture falls below 55-60 % of WHC and if it is over 80 %, they may suffer from a depletion of oxygen as soil pore fill with water (Kononova 2013). Consequently, moisture of the soil used in this experiment was adjusted to 60 % of soil water holding capacity (WHC) to ensure optimal conditions for growth of micro-organisms. Hence, determination of WHC of the soil was needed and was explained as below:

A 25 g sample of fresh soil was thoroughly soaked in distilled water. After 24 h, a filter paper was put in ceramic funnel, the weight of which was then recorded to 3 decimal places. The funnel was placed in a 250 mL conical flask and soaked soil was transferred into the funnel. The soil was left for 2 h to drain excess water from the soil or left until dripping has ceased. The soil was then at 100 % water holding capacity. The funnel containing the saturated soil was weighed and dried in an oven at 105 ^{o}C until constant weight (at least 24 h). The funnel containing oven-dried soil was weighed again. Each sample was replicated 3 times.

Formula to calculate the water holding capacity (WHC) of the soil by:

$$WHC\ (\%) = (W/F)\ x100$$

Where:

F - The weight of oven-dried soil, calculated by removing the weight of the funnel and filter paper.

W - The weight of water in the soil at saturation, calculated as: W = S - F where S is called the weight of soil at saturation. This was in turn calculated by taking away the weight of the funnel and soil from the weight of the funnel, filter paper soil at the point of saturated soil.

6.2.2 Effect of copper and cypermethrin insecticide on soil ciliates and soil total microbial activity

Neither copper nor cypermethrin have been applied to the study's field sites according to records. Consequently, the effect of these two important substances could not be studied in the field. This and the greater control of conditions, led to the choice of a pot trial to meet the objectives of this chapter.

Commercial loam topsoil was obtained from a commercial supplier (Wicks, Northampton, United Kingdom). This was used as it provided a homogenous soil that could be acquired in a large amount. In addition, this soil had a high organic matter content (OM), thus it can supply nutrients efficiently for microorganisms, and soils with high OM can increase persistence of Cu and cypermethrin in the soil (Harris and Chapman 1981). Thus, this represents a serious scenario where a soil expected to have a good microbial and ciliate community is affected over a relatively long period by contaminants.

Soils were treated with a 10 mg mL^{-1} Cu solution (as $CuCl_2$) to provide seven distinct treatment concentrations: 0 mg Cu kg^{-1} Soil (d.w.) as the control; 35 mg kg^{-1}; 70 mg kg^{-1}; 140 mg kg^{-1}; 240 mg kg^{-1}; 480 mg kg^{-1}; 720 mg kg^{-1}; 960 mg kg^{-1}. Similarly, cypermethrin was mixed well with soils at various concentrations, i.e., 0 mg kg^{-1} (control); 10 mg kg^{-1}; 20 mg kg^{-1}; 40 mg kg^{-1}; 80 mg kg^{-1}; 160 mg kg^{-1}; 320 mg kg^{-1}; 640 mg kg^{-1}. Py Bug Killer Concentrate (Vitax Ltd, UK) containing 10 mg mL^{-1} cypermethrin was utilized as the insecticide source. For both copper and cypermethrin, the appropriate volume of solution necessary to raise the concentration in the soil by the desired amount was added to 2 kg of soil. Distilled water was added to ensure that each treatment received the same volume of liquid.

Copper and cypermethrin were mixed well with the soil, and then divided into 4 replicate 1 L pots. The experiment was conducted under laboratory conditions for 15 days. Moisture of the soil was kept to 60 % of soil WHC by watering each pot to maintain a constant weight. Each treatment and control was replicated four times, and untreated soils with no contamination were used as control.

For both substances soil samples were collected from top soils (0-5 cm in deep) of all treated soil pots and controls after 15 days. Samples were taken 15 days after the Cu and cypermethrin amendments to ensure that the dynamics of important group of soil micro-organisms, i.e., ciliates, bacteria, and fungi could be suffered stresses from Cu and cypermethrin in a suitably long period to reveal their growth dynamics in toxic conditions. The abundance and species richness of soil ciliates after 4 days and 10 days of incubation was determined as explained in sections 2.3.1 and 2.3.2. The total species richness used in this experiment refers to the total number of ciliate species found at 4 days and 10 days of incubation together, not separately. Also, a sub-sample of fresh soil was kept 4 ^{o}C to analyse for total microbial activity as explained in section 2.6.3.

6.2.3 Effect of glyphosate herbicide on soil ciliates and on soil total microbial activity

Glyphosate was chosen due to its ubiquity and the fact that it was the only pesticide applied to both farms. In this experiment, the agricultural soil with pH 7.5 was collected from 0-5 cm topsoil of Corfe Castle Farm field that formed one of study sites in this project. This field was chosen due to its proximity to a road, allowing the large (*ca.* 100 kg) soil sample to be collected and returned to the laboratory. After collection, the soil was spread out on clean tables and dried sufficiently to allow sieving of out of stones or plant roots at room temperature in the laboratory for 6 days, then sieved through a 4 mm mesh sieve before storing in plastic bags until the experiment was carried out. Cultivated soil was utilized here because glyphosate herbicide was applied in both studied farms. As described above, soil pot trials were designed under laboratory conditions for testing the effects of glyphosate on soil ciliates and on soil microbial activity. Sampling in this experiment was carried out three times i.e., on days 1, 7, and 15 after application. This is because that glyphosate is non-persistent in soils (Al-Rajab and Hakami 2014); the half-life of glyphosate in soil can be a few days or several months depends on the type of soils (Al-Rajab and Hakami 2014; Myers et al. 2016). Furthermore, soil samples were taken at three points after the glyphosate applications to ensure that the dynamics of investigated groups in the present study could be monitored relatively intensively

to reveal their growth dynamics when the amount of glyphosate supposedly had reduced over period of time.

The glyphosate concentrations used in the experiment were based on the standard application of Roundup ProActive 360 (5 L ha^{-1}). Soils were treated with glyphosate at 0 (control), X1, X2, X5 and X10 of the standard rate. These treatments are equal to glyphosate concentrations: 0 mg kg^{-1} (control); 3.36 mg kg^{-1}; 6.73 mg kg^{-1}; 16.82 mg kg^{-1}; 33.6 mg kg^{-1}, respectively. The appropriate volume of solution necessary to raise the concentration in the soil by the desired amount was added to 7 kg of soil. Distilled water was added to ensure that each treatment received the same volume of liquid.

Glyphosate was mixed well with soil, and then divided into 12 replicate 1 L pots. The experiment was conducted under laboratory conditions for 1, 7, and 15 days as explained above. Moisture of soil was kept to 60 % of soil WHC by watering each pot to keep a constant weight. Each treatment and control was replicated four times for each sampling time, and untreated soils with no contamination were used as control.

Samples were collected after 1 day, 7 days and 15 days of application to investigate the effect of glyphosate on soil ciliates and total microbial activity after different periods of application time. After collecting the soil samples from the pots, they were handled as section 6.2.2 above to determine the abundance and species richness of soil ciliates and total microbial activity.

6.2.4. Statistical analysis

Statistical analysis was conducted with SPSS (version 20). Differences between dependent factors and treatments were compared by one-way ANOVA test. Data sets were analysed for homogeneity of variance with Levene's test. When assumptions were not met, data were log_{10} transformed or Welch's robust ANOVA used as appropriate. Effects of individual treatments were determined by Tukey's Honesty Significant Difference *post-hoc* test.

Bivariate correlations were conducted to test for relationship between variables. Spearman's rank order method was used for this.

6.3 Results

6.3.1 Impacts of copper and soil ciliates and total microbial activity

Impacts of copper on the abundance and species richness of soil ciliates

There were fluctuations in the abundance of soil ciliated protozoa after 4 days and 10 days of incubation (Figure 6.1). The highest values of ciliate abundance were observed in soil samples which had the highest Cu concentration (960 mg kg^{-1}) after both 4 days and 10 days at 306 and 49 cells g^{-1} soil (d.w.), respectively (Figure 6.1). The number of ciliates counted after 10 days were much lower than that after 4 days. The significance of the differences in soil ciliate abundance after 4 days of incubation between doses was determined by one-way ANOVA. This indicated that Cu treatment had a statistically positive significant impact on the abundance of soil ciliates ($F_{(7, 24)} = 7.836$, $p < 0.001$). To determine the significance of differences amongst specific treatment groups, Tukey's HDS *post-hoc* analysis was used. The test showed that there were no significant differences between the treatment concentrations of 35, 70, 140, 480, and 720 mg kg^{-1} and the control or amongst the treatment concentrations (ranged from 35-480 mg kg^{-1}), but the abundance of soil ciliates at the concentration of 960 mg kg^{-1} was significantly different to all other treatment concentrations and control. It also can be seen that there was a significant increase in the abundance of ciliates after 4 days in concentrations 720 mg kg^{-1} and 960 mg kg^{-1} compared to the remaining doses.

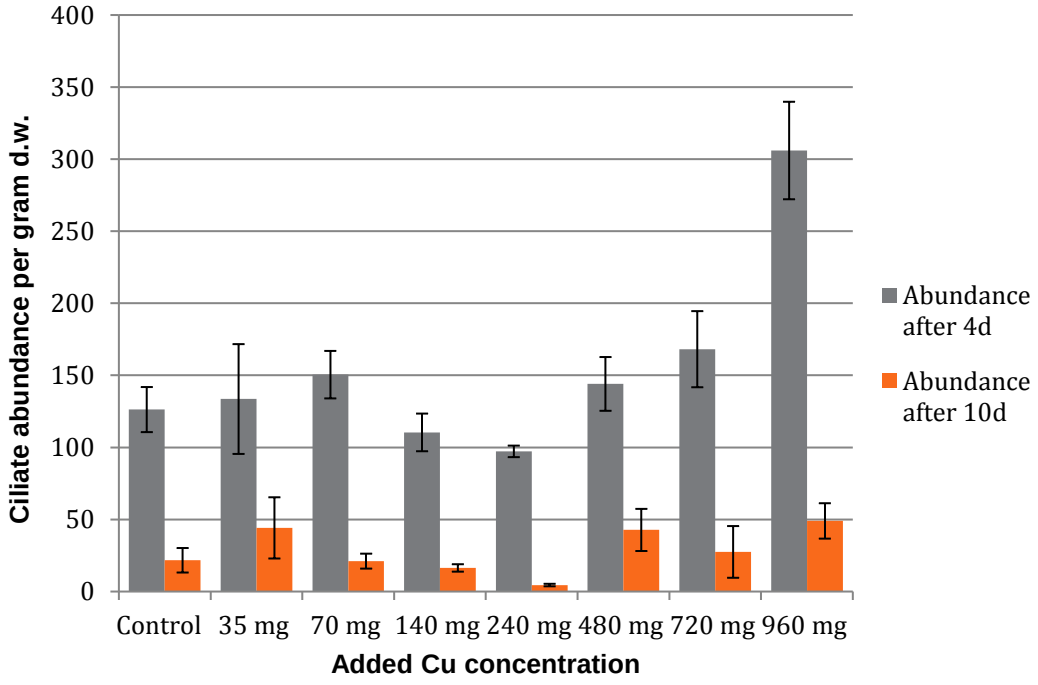


Figure 6.1 The abundance of soil ciliates at different Cu treatments (mg kg^{-1})-calculated mean abundance per gram dry weight after 4 days and 10 days of incubation (mean ± 1 SE).

A similar trend in ciliate abundance was also showed after 10 days of incubation (Figure 6.1). However, the ciliate abundances after 10 days were very low in all treatments and control when compared to after 4 days.

One-way ANOVA was conducted to investigate the impact of Cu on the number of ciliates after 10 days. The dependent variable (the abundance of soil ciliates) was inversely transformed to meet the assumption of homogeneity of variance. This showed that there was a significant difference in the number of ciliates among treatments after incubating for 10 days ($F_{(7, 24)} = 2.449$, $p = 0.048$). Tukey's HDS *post-hoc* test showed that there was only a significant difference between two Cu concentrations, 240 and 960 mg kg^{-1}. No significant difference between Cu treatments and control was indicated.

The total ciliate species richness recorded after 4 days and 10 days together had a relatively plateau between the concentration used, in which there was only a slight decrease at concentrations of 720 mg kg^{-1} and 960 mg kg^{-1} (Figure 6.2), with 9 and 8 different species respectively, in comparison with the control and lower treated concentrations. However, no significant differences were indicated by a robust one-way ANOVA test ($F_{(7, 24)} = 1.033$, $p = 0.435$). However, when presence/absence of ciliate species in all four soil samples together in each treatment and the control is taken into account, total 17 different species were found in the control, but just 14 species and 12 species were present at Cu doses of 720 and 960 mg kg^{-1}, respectively.

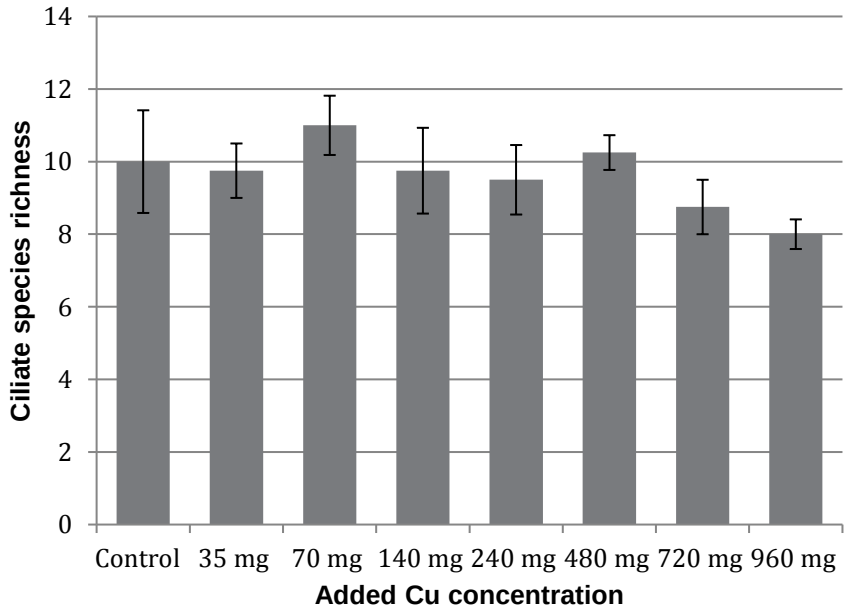


Figure 6.2 The total species richness of soil ciliates found under different Cu treatments (mg kg^{-1}) (mean ± 1 SE).

Interestingly, when the abundance of particular ciliate species is taken into account, the results showed that there was a significant increase in the population abundance of *Chilodonella uncinata* and *Homalogastra setosa* at dose of 960 mg kg^{-1} compared to the control soil (Table 6.1), though the number of total species richness decreased slightly. It was due to the rapid increase in the abundance of these two species that the highest number of soil ciliates was recorded at the highest Cu concentration (960 mg kg^{-1}).

Table 6.1 The abundance of two soil ciliate species in Cu treatments (mg kg^{-1})-calculated mean abundance per gram oven-dry weight after 4 days of incubation (mean ± 1 SE).

Cu treatment	*Homalogastra setosa*	*Chilodonella uncinate*
Control	50 ± 7.28	21.68 ± 6.96
960 mg kg^{-1}	100.57 ± 12.71	176.3 ± 28.89
$F_{(1, 6)}$	11.542	27.063
Sig.	p = 0.015	p = 0.002

Impacts of Cu on total microbial activity

Levels of soil total microbial activity at different Cu concentrations were determined and recorded (Figure 6.3). At concentrations from 35 to 140 mg kg^{-1}, Cu seemed to stimulate microbial activity; a highest peak was shown at 35 mg kg^{-1} concentration. However, the activity then decreased, and a rapid reduction can be seen at 960 mg kg^{-1} with fluorescein released at a rate of 10 µg g^{-1} compared with 17 µg g^{-1} in control.

Subsequently, differences in microbial activity between treatments were assessed. The results of this analysis indicated that there were significant differences among Cu treatments in soil total microbial activity ($F_{(7, 24)}$ = 5.787, p = 0.001). Tukey's HDS *post-hoc* test showed significant differences in soil total microbial activity between the highest Cu concentration (960 mg kg^{-1}) and control. Also, total microbial activity was significantly lower at Cu concentrations of 960 mg kg^{-1} compared at Cu concentrations of 35, 70 and 140 mg kg^{-1}. In contrast, insignificant differences in total microbial activity between Cu concentrations of 720 and 480 mg kg^{-1} and control and other treatments were shown.

No correlation between soil microbial activity and abundance or species richness were shown by Spearman's correlation in the Cu experiment.

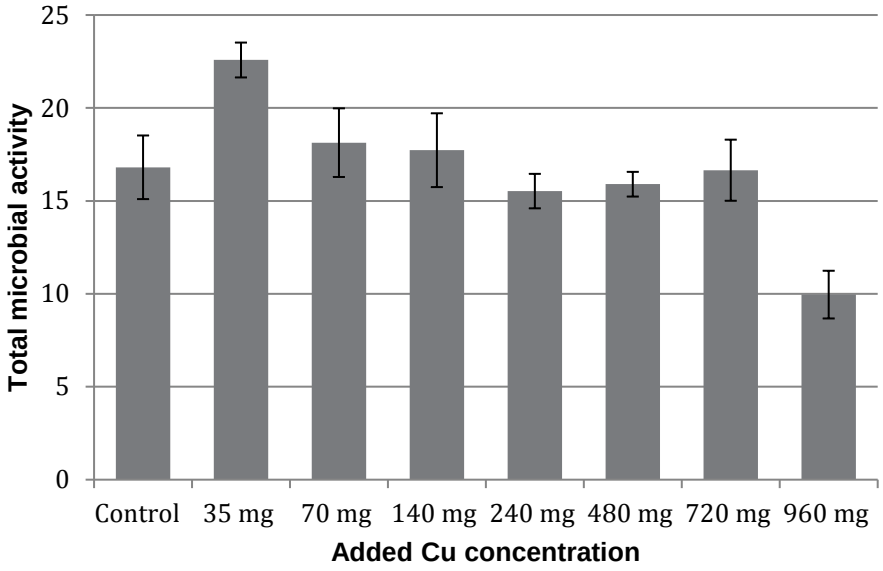


Figure 6.3 Soil total microbial activity (µg fluorescence released g^{-1} d.w. h^{-1}) in different Cu treatments (mg kg^{-1}) (mean ± 1 SE).

6.3.2 Impacts of cypermethrin insecticide on soil ciliates and total microbial activity

Impact of cypermethrin on the abundance and richness of soil ciliates

There were wide fluctuations in soil ciliate abundance after 4 days and 10 days of incubation (Figure 6.4). The abundance of ciliates after 4 days at cypermethrin concentrations of 10, 20, 40, and 640 mg kg^{-1} was lower than that in the control; but not in the 80-320 mg kg^{-1} range. After 10 days, a similar trend was found, except for the 640 mg kg^{-1} concentration where an increase in the number (up to 789 cells g^{-1} of dry soil) was recorded.

The highest ciliate abundance was recorded at a concentration of 160 mg kg^{-1} after 4 days and at 320 mg kg^{-1} after 10 days with 780 and 1008 cells g^{-1} of dry soil, respectively.

A statistical analysis by one-way ANOVA based on $\log_{10}$ transformation of the dependent variable showed that ciliate abundance in cypermethrin treatments was significantly different after 4 days and 10 days of incubation ($F_{(7, 24)}$ = 13.157, p < 0.001 and $F_{(7, 24)}$ = 19.073, p < 0.001, respectively).

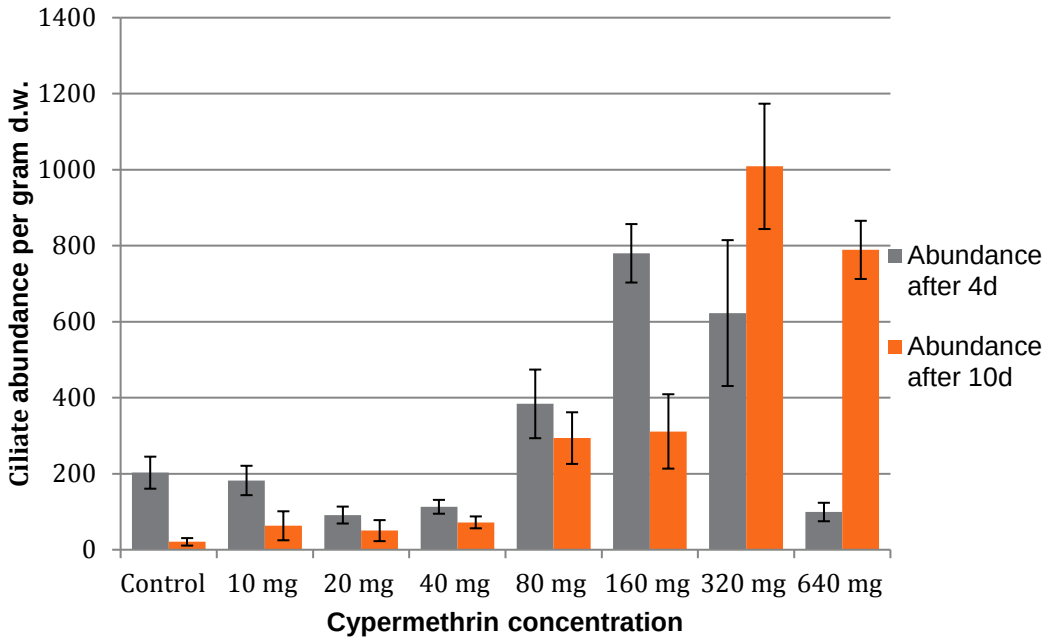


Figure 6.4 The abundance of soil ciliates at different cypermethrin treatments (mg kg^{-1})-calculated mean abundance per gram dry weight after 4 days and 10 days of incubation (mean ± 1 SE).

Tukey's HDS in *post-hoc* test showed that ciliate abundance after 4 days of incubation only differed significantly to the control at an applied dose of 160 mg kg^{-1}. The cypermethrin concentrations of 20, 40, 640 mg kg^{-1} were significantly different in the number of ciliate cells compared to treatments in the concentration range from 80 – 320 mg kg^{-1}. At 10 mg kg^{-1}, ciliate abundance differed significantly with that in levels of 160 and 320 mg kg^{-1} as well. However, only the increase of ciliate individuals at 160 mg kg^{-1} concentration compared to control was statistically indicated, i.e., no other treatment had a significant effect compared to the control.

Similarly, a *post-hoc* test showed that there was a significant increase in ciliate abundance after 10 days of incubation at the cypermethrin concentrations of 80, 160, 320, and 640 mg kg^{-1} compared to the control and the concentrations of 10 and 20 mg kg^{-1}. Also, there was significant difference in number of ciliates between concentrations of 40 mg kg^{-1} with 320 and 640 mg kg^{-1}. No significant difference was found between groups which had high cypermethrin level (from 80-640 mg kg^{-1}).

In term of total ciliate species richness, there was a slight increase at treatments of 10 to 160 mg kg^{-1} cypermethrin. However, at very high concentrations (320 and 640 mg kg^{-1}) the number of species decreased slightly (Figure 6.5)

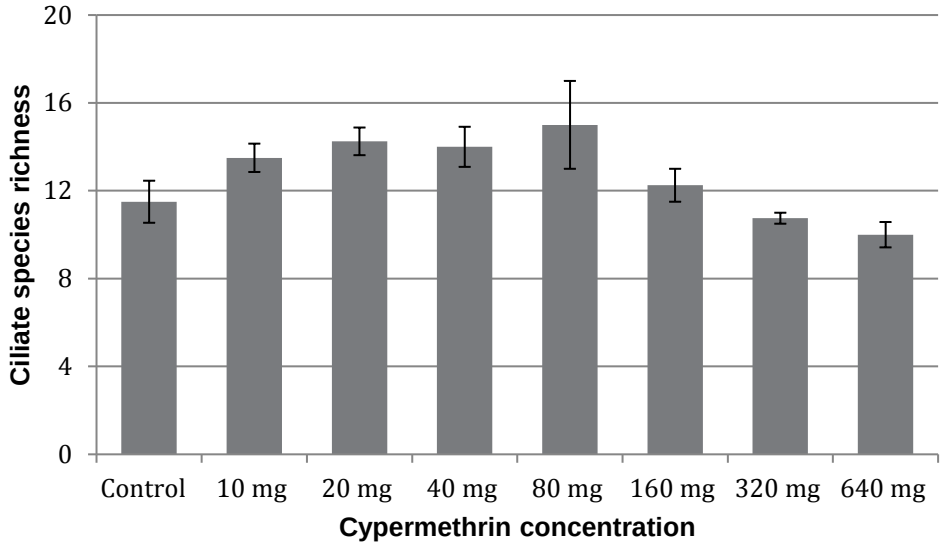


Figure 6.5 The species richness of soil ciliates at different cypermethrin treatments (mg kg^{-1}) (mean ± 1 SE).

One-way ANOVA was conducted to establish the significance of the difference between soil ciliate species richness (which also transformed into log_{10} to meet assumption of homogeneity) and applied cypermethrin concentration. This revealed that cypermethrin had a significant effect on soil ciliate species richness ($F_{(7, 24)}$ = 4.405, p = 0.003). Nevertheless, when difference amongst groups were taken into consideration, differences in species richness between control and all cypermethrin treatments were insignificant. The significant differences in ciliate species richness were only found between a concentration of 640 mg kg^{-1} and concentrations of 20, 40, and 80 mg kg^{-1}. However, when the presence of total ciliate species at all four soil samples collected from four replicates of the control and each treatment was taken into consideration, several ciliate species were completely absent at the highest concentration (640mg kg^{-1}), but still found in the control and lower cypermethrin concentrations such as *Halteria grandinella*, *Homalogastra setosa*, and *Arcuospathidium vermiforme*. In total, 21 ciliate species were found in the samples of the control, but only 12 ciliate species were recorded in the samples from the 640mg kg^{-1} treatment.

Similar to the impact of Cu on soil ciliates, detailed information about specific species after 4 days of incubation was considered. Statistical analysis by one-way ANOVA showed cypermethrin had positive significant impact on the ciliate species *Homalogastra setosa* at 80, and 160 mg kg^{-1-} (Table 6.2), but after that the abundance of this species decreased sharply (very few cells were recorded at 320 mg kg^{-1} in only 2 out of 12 petri dishes) and the species was totally absent at a

concentration of 640 mg kg^{-1}. The results of *post-hoc* test indicated that abundance of species *Homalogastra setosa* after 4 days differed at 160 mg kg^{-1} dose significantly with that the of control and dose of 80 mg kg^{-1}.

As mentioned above, although a significant difference in the abundance of soil ciliated protozoa between the control and 320 mg kg^{-1} concentration of cypermethrin was not reported by a robust statistical analysis, there was an increase in the abundance of two Colpodid species, i.e., *Colpoda steinii* and *Colpoda inflata* after 4 days of incubation, whilst no *Colpoda* cells were found in the control (presented in Table 6.2).

Table 6.2 The abundance of three soil ciliate species in cypermethrin treatments (mg kg^{-1}) - calculated mean abundance per gram dry weight after 4 days of incubation (mean ± 1 SE).

Cypermethrin treatment	*Homalogastra setosa*	*Colpoda steinii*	*Colpoda inflata*
Control	157.1±45.32	0	0
80 mg kg^{-1}	307.67±78.26	6.52±1.26	5.52±1.26
160 mg kg^{-1}	615.82±84.74	18.06±4.09	11.54±5.64
320 mg kg^{-1}	5.52±3.41	278.06±83.13	216.32±64.87
$F_{(2, 9)}$	10.68	-	-
Sig.	$p = 0.04$	-	-

Table 6.3 showed mean values of two different ciliate species in various cypermethrin treatments recorded after 10 days of incubation. No cells of *Colpoda inflata* were recorded in treatments which had cypermethrin concentrations under 320 mg kg^{-1}, including the control. Very few cells of *Homalogastra setosa* were observed in the control and low doses below 80 mg kg^{-1}. Similar to 4 days of incubation, at a dose of 320 mg kg^{-1} *Homalogastra setosa* was seen in two dishes only, and this species was not presented at the highest dose. Hence, cypermethrin stimulated the growth of *Homalogastra setosa* at doses of 80 and 160 mg kg^{-1}, but doses above 160mg kg^{-1} had a strong negative effect on this species and a concentration of 640 mg kg^{-1} was a lethal.

To test difference in abundance of soil species *Homalogastra setosa* after 10 days of incubation between control and two doses of 80 and 160 mg kg^{-1}, data was $\log_{10}$

transformed to meet the assumption in homogeneity of data. Subsequent statistical analysis showed that there were significant differences between control and treatments of 80 and 160 mg kg^{-1} in the abundance of this species (Table 6.3).

Table 6.3 The abundance of two soil ciliate species in cypermethrin treatments (mg kg^{-1}) - calculated mean abundance per gram dry weight after 10 days of incubation (mean ± 1 SE).

Cypermethrin treatment	***Homalogastra setosa***	***Colpoda inflata***
Control	5.02 ± 3.1	0
80 mg kg^{-1}	198.25 ± 57.48	0
160 mg kg^{-1}	268.26 ± 92.86	0
320 mg kg^{-1}	25.09±14.48	642.45 ± 190.59
640 mg kg^{-1}	0	509.94 ± 76.58
F (2,9)	26.938	-
Sig.	$p < 0.001$	-

Impacts of cypermethrin insecticide on total microbial activity

Soil total microbial activity was higher at all cypermethrin concentrations tested in comparison to the control (Figure 6.6). The highest concentration was found at 640 mg kg^{-1} with a fluorescence release of 30.61 µg g^{-1} h^{-1}, and the lowest is at control (with 16.81 µg g^{-1} h^{-1}).

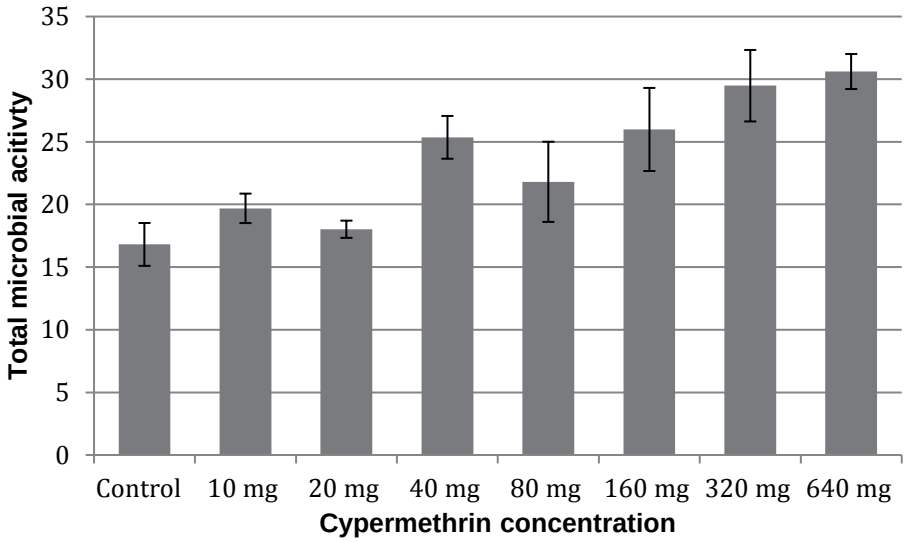


Figure 6.6 Soil total microbial activity (µg fluorescence released g^{-1} d.w. h^{-1}) in different cypermethrin treatments (mg kg^{-1}) (mean ± 1 SE).

Statistical analysis by one-way ANOVA showed that insecticide cypermethrin significantly affected soil total microbial activity ($F_{(7, 24)} = 5.510$, $p = 0.001$). Differences among groups was analysed by Tukey HDS in *post-hoc* test. It showed that there was a significant increase in soil total microbial activity at high concentrations of 320 and 640 mg kg^{-1} when compared to control and 20 mg kg^{-1}.

The relationship between the abundance and richness of soil ciliates and total microbial activity was indicated by non-parametric Spearman's rank correlation coefficient (Table 6.4). The results showed that soil ciliate abundance after 10 days of incubation had high positive correlation with microbial activity, but no significant correlation was found for the 4 day incubation period. In contrast, a significant negative correlation coefficient was recorded between the number of ciliate species and microbial activity.

Table 6.4 Correlation coefficient between the abundance after 4 days and 10 days of incubation, and species richness of soil ciliates and total microbial activity.

	Ciliate abundance_4 days	**Ciliate abundance_10 days**	**Ciliate species richness**
Microbial activity	0.242	0.624^{**}	-0.403^{*}

*: $p < 0.05$; **: $p < 0.01$

6.3.3 Impacts of glyphosate herbicide and soil ciliates and total microbial activity

Impacts of glyphosate on the abundance and species richness of soil ciliates

Effect of glyphosate on soil ciliate abundance and species richness 1 day after application

There was a slight increase in the abundance of soil ciliates after 4 days of incubation at doses of 3.36, 6.73 and 33.6 mg kg^{-1} in comparison with the control. Ciliate abundance after 10 days of incubation rose in 3.36 and 6.73 mg kg^{-1} concentrations, but decreased slightly at higher glyphosate doses (Figure 6.7).

However, there was no statistically significant difference in the abundance of soil ciliates in both after 4 days and 10 days of incubation between control and glyphosate treatments ($F_{(4, 15)} = 0.733$, $p = 0.584$ and $F_{(4, 15)} = 1.536$, $p = 0.242$, respectively).

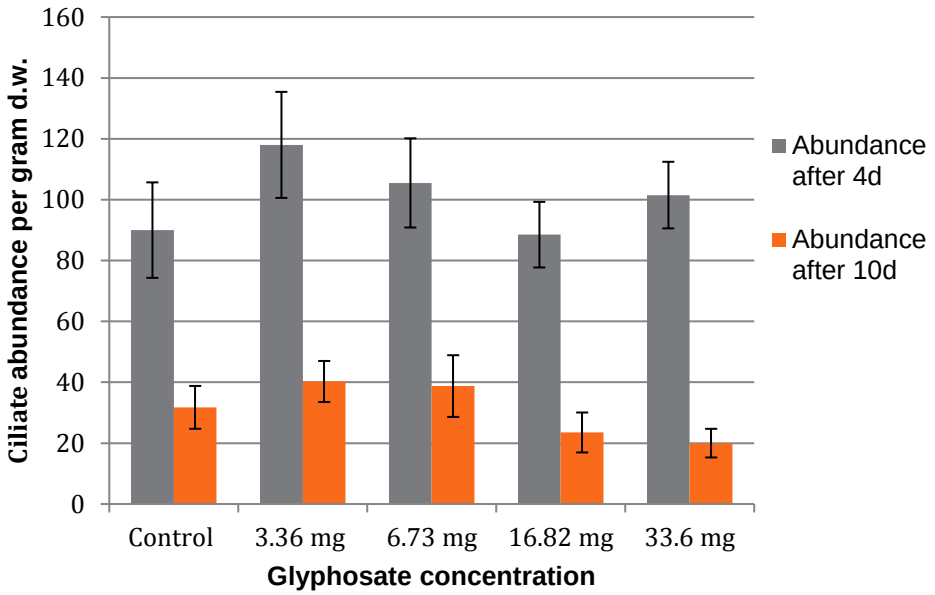


Figure 6.7 The abundance of soil ciliates in different treatments (mg kg^{-1}) 1 day after glyphosate application-calculated mean abundance per gram dry weight after 4 days and 10 days of incubation (mean ± 1 SE).

The species richness of soil ciliated protozoa was relatively similar between control and treatments (Figure 6.8). Consequently, no significant difference in species richness between glyphosate doses was showed by statistical analysis ($F_{(4, 15)}$ = 0.818, p = 0.534)

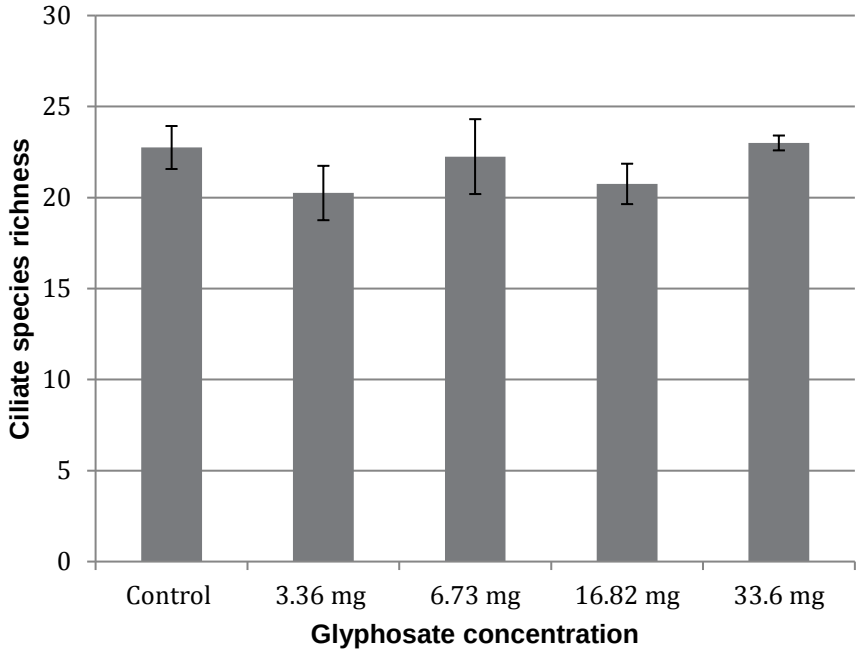


Figure 6.8 The species richness of soil ciliates in different treatments 1 day after glyphosate application (mg kg^{-1}) (mean ± 1 SE).

Effect of glyphosate on soil ciliate abundance and species richness 7 days after application

There was slight change of the abundance of soil ciliates after 4 days and 10 days of incubation from soil samples collected after applying glyphosate 7 days. The lower number of ciliates was observed in higher dose treatments (Figure 6.9). However, the results showed no significant differences in ciliate abundance after 4 days and 10 days between glyphosate concentrations ($F_{(4, 15)}$ = 0.482, p = 0.749 and $F_{(4, 15)}$ = 1.849, p = 0.172, respectively). Similarly, the ciliate species richness was observed with the highest value at control and lowest at a dose of 16.82 mg kg^{-1} (Figure 6.10). However, the species richness of soil ciliated protozoa between applied glyphosate herbicide concentrations did not differ ($F_{(4, 15)}$ = 0.801, p = 0.543).

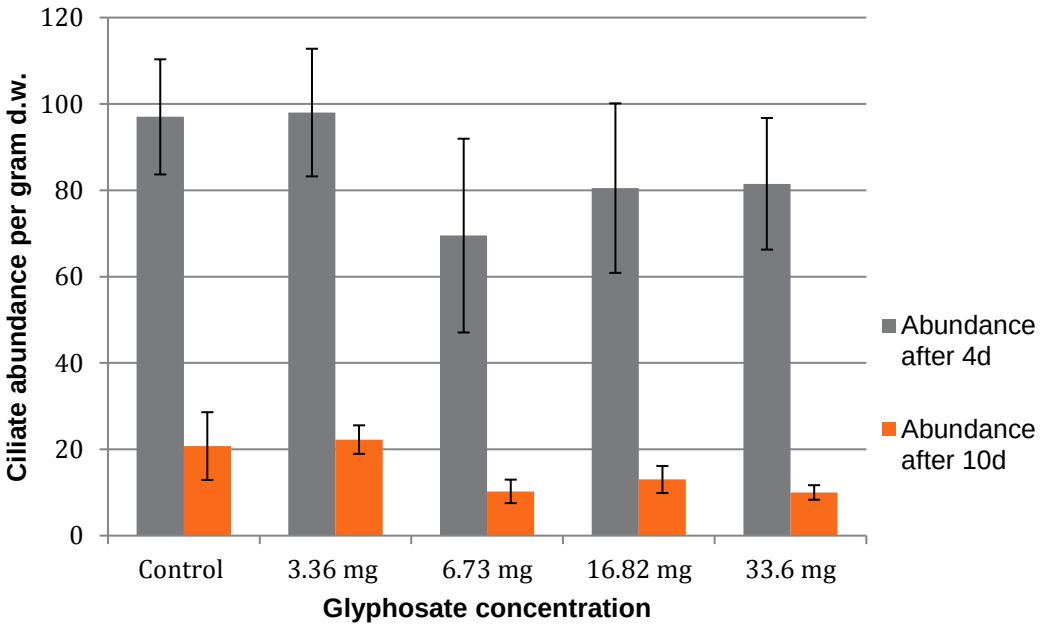


Figure 6.9 The abundance of soil ciliates in different treatments (mg kg^{-1}) 7 days after glyphosate application-calculated mean abundance per gram dry weight after 4 days and 10 days of incubation (mean ± 1 SE).

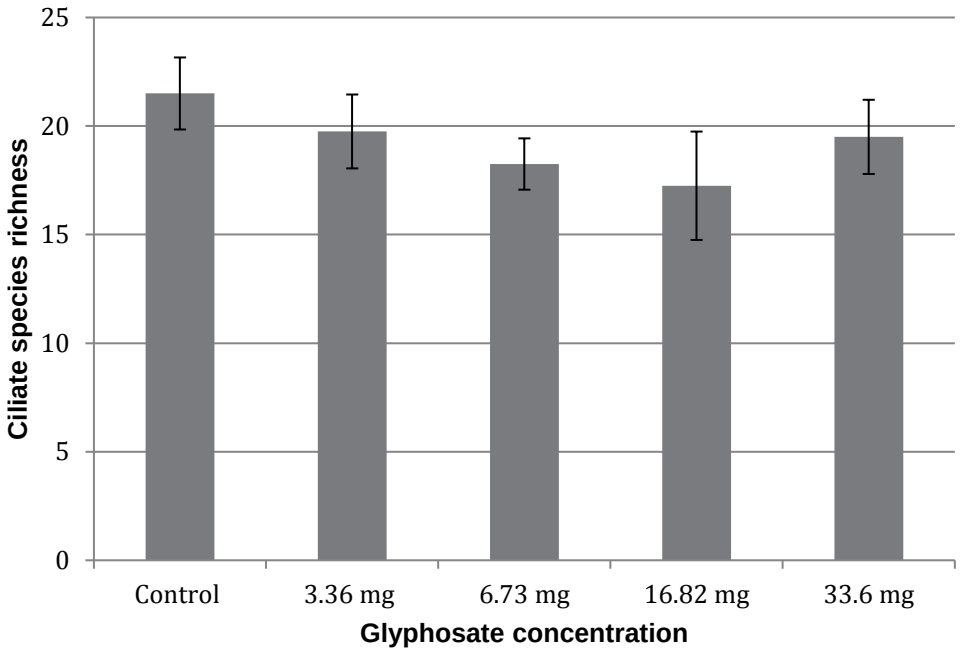


Figure 6.10 The species richness of soil ciliates in different treatments 7 days after glyphosate application (mg kg^{-1}) (mean ± 1 SE).

Effect of glyphosate on soil ciliate abundance and species richness 15 days after application

There was an increase of soil ciliate abundance after 4 days of incubation at a dose of 3.36 mg kg^{-1}, but then it started to reduce in higher concentrations (Figure 6.11). Statistical analysis by one-way ANOVA indicated that there was significant difference in the abundance of soil ciliates after 4 days between control and glyphosate dosages (Welch's $F_{(4,\ 7.1)} = 13.117$, $p = 0.002$). Differences among groups was analysed by Games-Howell in *post-hoc* test. It showed that there was a significant increase in soil ciliate abundance after 4 days of incubation at high concentrations of 16.82 and 33.6 mg kg^{-1} in comparison to the control.

In contrast to ciliate abundance after 4 days of incubation, a very low number of soil ciliates was counted after 10 days of incubation (Figure 6.11). Consequently, no significant impact of herbicide glyphosate on soil ciliate abundance after 10 days was indicated by one-way ANOVA analysis ($F_{(4, 15)} = 0.596$, $p = 0.672$).

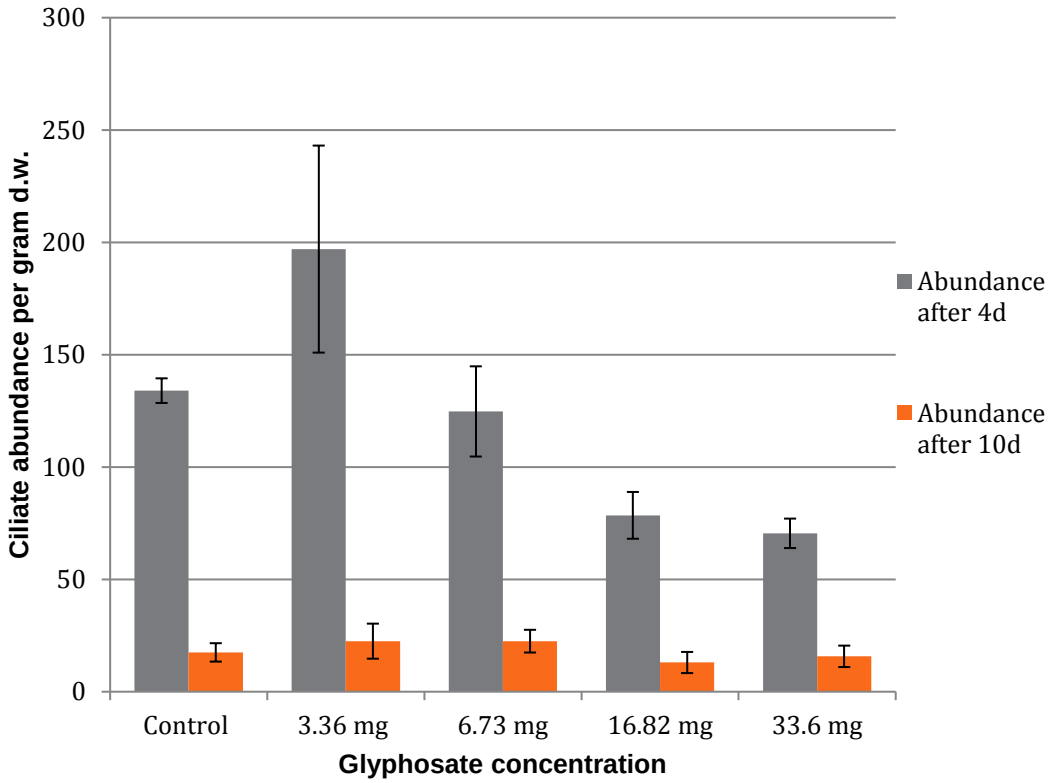


Figure 6.11 The abundance of soil ciliates in different treatments (mg kg^{-1}) 15 days after glyphosate application-calculated mean abundance per gram dry weight after 4 days and 10 days of incubation (mean ± 1 SE).

A consideration of the abundance of each ciliate species showed that *Homalogastra setosa* was always recorded after 4 days of incubation with the highest number in compare with other ciliate species. Table 6.5 shows mean of number of cells of *H. setosa* recorded 1 day, 7 days, and 15 days after glyphosate herbicide application.

Statistical analysis by one-way ANOVA indicated that the abundance of *H. setosa* was not significantly different in soil samples collected 1 day and 7 days after glyphosate application, but was significant in soils collected 15 days after glyphosate application (Table 6.5).

Table 6.5 The abundance of the ciliate species *Homalogastra setosa* 15 days after glyphosate application (mg kg^{-1})-calculated mean abundance per gram oven-dry weight after 4 days of incubation (mean ± 1 SE).

Treatment	After application 1 day	After application 7 days	After application 15 days
Control	50.96 ± 13.74	58.00 ± 12.36	96.66 ± 6.01
3.36 mg	76.92 ± 15.00	62.23 ± 18.11	157.95 ±44.00
6.73 mg	63.64 ± 16.76	57.6 ± 14.11	92.85 ± 16.34
16.82 mg	44.10 ± 8.85	47.33 ± 16.25	43.90 ± 6.87
33.6 mg	54.38 ± 12.31	47.53 ± 16.66	48.53 ± 5.42
F.	$F_{(4, 15)} = 0.873$	$F_{(4, 15)} = 0.168$	Welch's $F_{(4, 7.2)} = 9.827$
Sig.	$p = 0.503$	$p = 0.942$	$p = 0.005$

The species richness of soil ciliated protozoa in the soil 15 days after application is shown in Figure 6.12. The number of ciliate species was lower at glyphosate doses of 16.82 and 33.6 mg kg^{-1}, but increased slightly at a dose of 6.73 mg kg^{-1}. Consequently, one-way ANOVA was also conducted to analyse the significance of differences in soil ciliate species richness between glyphosate concentrations. The result showed that glyphosate had significant effect on ciliate species richness after 15 days of application ($F_{(4, 15)} = 3.825$, $p = 0.025$). However, no significant difference in species richness between the control and glyphosate doses was indicated by Tukey's HDS *post-hoc* test. The significant difference in species richness was only showed between glyphosate concentrations of 6.73 and 33.6 mg kg^{-1}.

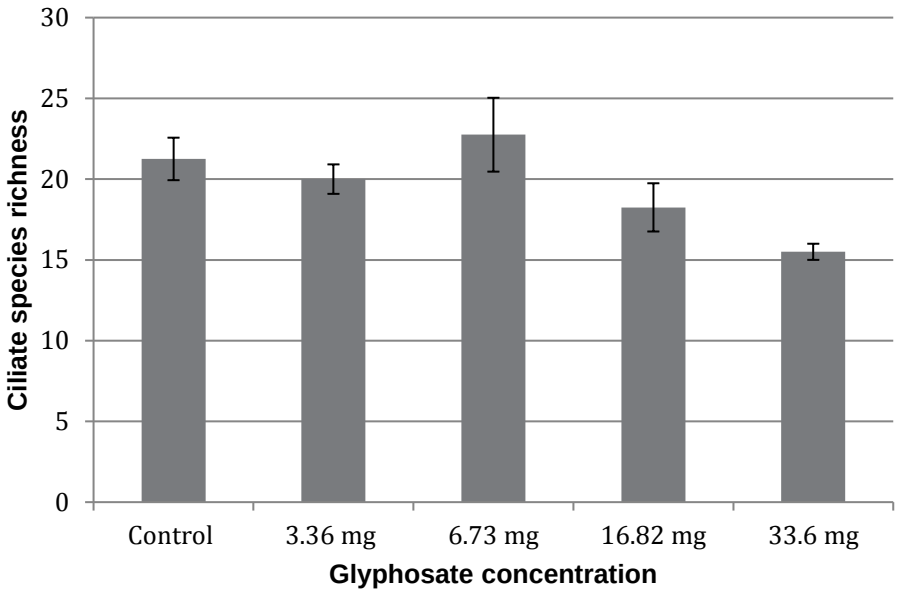


Figure 6.12 The species richness of soil ciliates in different treatments 15 days after glyphosate application (mg kg^{-1}) (mean ± 1 SE).

Interestingly, when considering the presence of all ciliate species in all samples combined together of the control and each glyphosate treatment, there was similarity in the total number of species between the control and treatments in samples collected at 1 day and 7 days after herbicide application, but there was a decrease in the number of ciliate species in the treatment of 36.6 mg kg^{-1} compared to the control, i.e., in total, 22 different ciliate species were found at a concentration of 36.6 mg kg^{-1} compared to 29 species presented in the control.

Impacts of glyphosate on total microbial activity of soil ciliates

Effect of glyphosate on soil total microbial activity 1 day after application

Total microbial activity increased with treatment until 6.73 mg kg^{-1} doses and then fell slightly (Figure 6.13). However, no significant impact of glyphosate on microbial activity was showed by one-way ANOVA (Welch's $F_{(4, 7.45)} = 1.279$, $p = 0.363$).

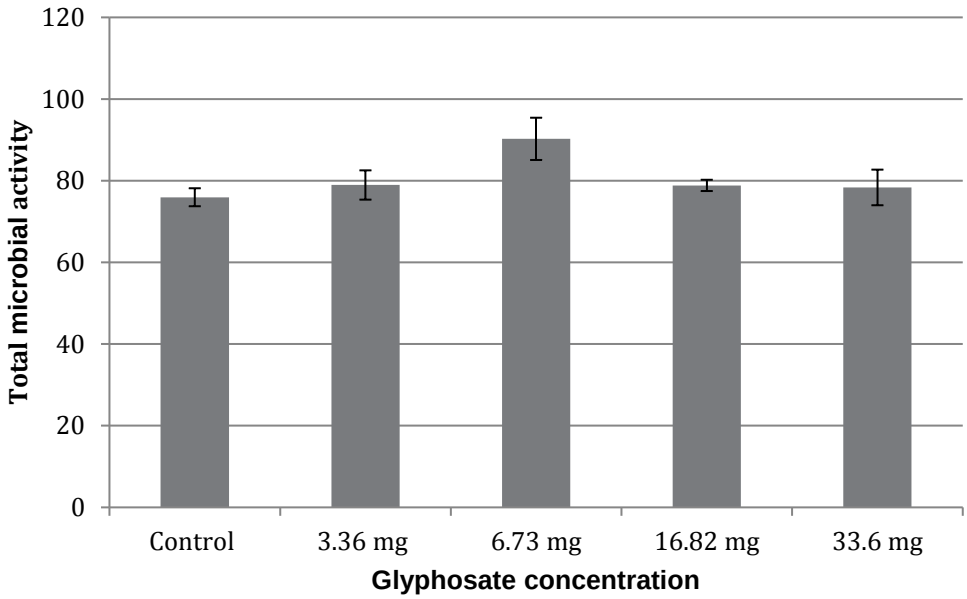


Figure 6.13 Soil total microbial activity (µg fluorescence released g^{-1} d.w. h^{-1}) in different treatments 1 day after glyphosate application (mg kg^{-1}) (mean ± 1 SE).

Effect of glyphosate on soil total microbial activity 7 days after application

The microbial activity is relatively similar between doses of glyphosate 7 days after application (Figure 6.14). Consequently, no significant difference in microbial activity was shown between treatments by one-way ANOVA (Welch's $F_{(4, 7.2)} = 2.229$, $p = 0.164$).

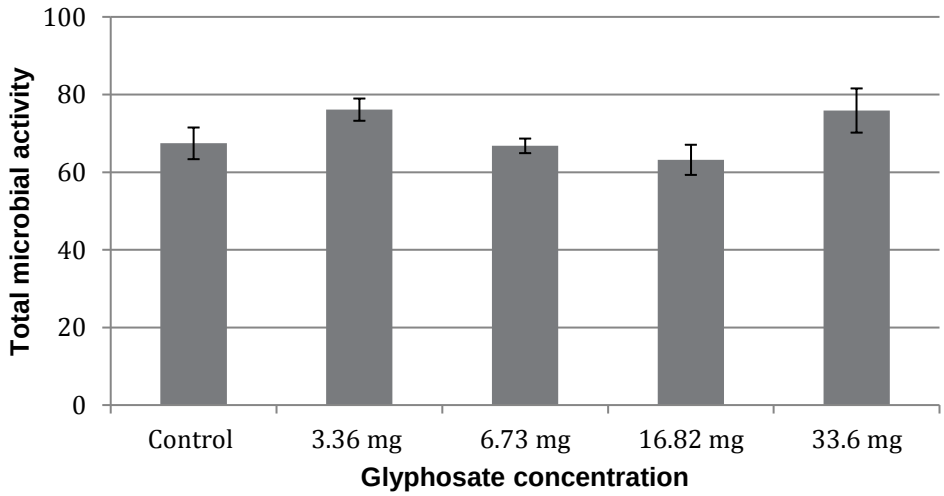


Figure 6.14 Soil total microbial activity (µg fluorescence released g^{-1} d.w. h^{-1}) in different treatments 7 days after glyphosate application (mg kg^{-1}) (mean ± 1 SE).

Effect of glyphosate on soil total microbial activity 15 days after application

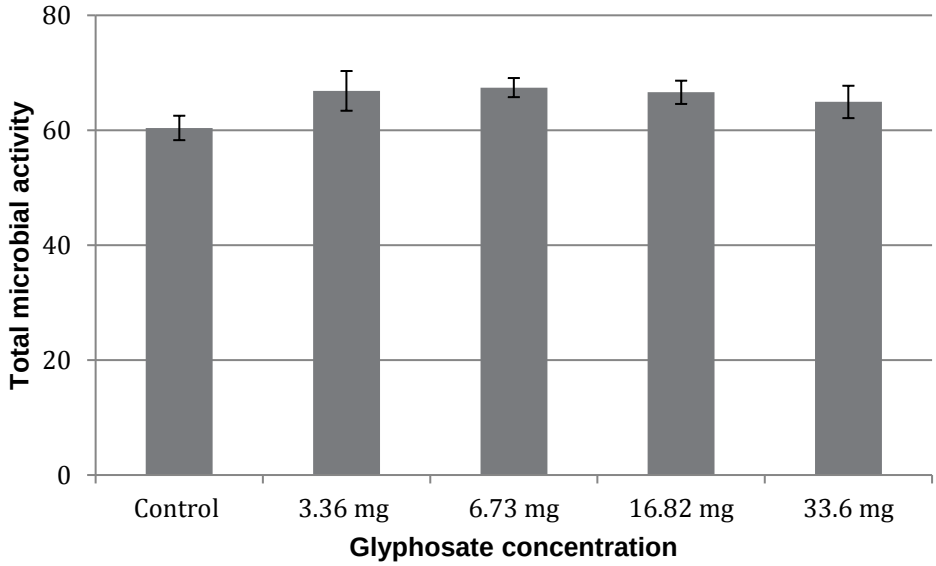


Figure 6.15 Soil total microbial enzyme activity (µg fluorescence released g^{-1} d.w. h^{-1}) in different treatments 15 days after glyphosate application (mg kg^{-1}) (mean ± 1 SE).

Total microbial activity after glyphosate treatment 15 days is shown in Figure 6.15. Although, concentration of microbial activity of treatments was higher than that of the control, the difference in microbial activity was not significant indicated by the analysis of one-way ANOVA ($F_{(4, 15)}$ = 1.31, p = 0.311).

Because no significant effect of glyphosate application on total microbial activity was found, subsequent analysis by Spearman's rank order correlation between total microbial activity and the abundance and species richness of ciliated protozoa was not carried out.

6.4 Discussion

Impact of Cu on the abundance and species richness of soil ciliates and total microbial activity

The Cu concentrations used in this present study, i.e., 35, 70, 140, 240, 480, 720, and 960 mg kg^{-1} were much higher than Cu available concentrations which were detected in the range of 0.001-0.3 mg kg^{-1} in the soils of the three present study sites including East Stoke Fen, Vicarage Farm, and Corfe Castle Farm.

Research into the toxicity of trace metals to soil ciliates is sparse. The work that is evident in the literature was carried out in solutions amended with trace metal or in solution extracted from metal contaminated soils and used common Colpodid species (Bowers et al. 1997; Campbell et al. 1997; Pratt et al. 1997; Díaz et al. 2006). These results showed that soil ciliate strains were sensitive to trace metals,

including Cu and the growth of the soil ciliate species in the tests was usually inhibited by present of Cu ions.

In the present study, the abundance of soil ciliates after 4 days of incubation was affected by a Cu concentration of 960 mg kg^{-1} at 15 days after application (Figure 6.1). However, no impact of Cu on soil ciliates abundance at the range of Cu concentration from 0 to 720 mg kg^{-1} was recorded. This corresponds with the finding of Ekelund et al. (2003), which showed that Cu with concentrations up to 1000 mg kg^{-1} did not affect the abundance of protozoa at 14 days and 70 days after application. Even Cu at a dose of 1000mg kg^{-1} at 70 days stimulated the abundance of soil protozoa. It is noted that significant negative effects of Cu on the abundance of soil protozoa in the experiment of Ekelund et al. (2003) were found at 2 and 7 days after application at the Cu concentrations of 1000 mg kg^{-1}. The authors explained that more Cu tolerant protozoa trains were favoured by the toxic effects in the experiment. In the present study, a significant increase in the abundance of soil ciliates was found in the highest concentration of Cu compared to the control. This was contributed to some opportunistic forms which favoured the toxic conditions, such as *Homalogastra setosa* and *Chilodonella ucinata*. This finding is attributed to an apparent inverse dose-respond of some soil ciliate species to Cu. Such a response was also shown in the findings of Ekelund et al. (2003), where an increase in the abundance of opportunistic protozoan forms at 70 days after Cu treatment of 1000 mg kg^{-1} was observed.

It is well-documented that microorganisms such as bacteria and fungi tolerate metal contamination (Lenart-Boroń and Boroń 2014). In addition, Ekelund et al. (2003) showed that respiration of micro-biota and microbial biomass decreased significantly at only the highest Cu concentration (1000 mg kg^{-1}) in their experiments. Tolerance of freshwater and wastewater ciliate species to heavy metal was also demonstrated in several tests under laboratory conditions using solutions spiked with trace metals (Shakoori et al. 2004; Rehman et al. 2008a, 2010). For example, Shakoori et al (2004) reported that *Vorticella microstoma* can tolerate 220 mg L^{-1} Cu. *Oxytricha fallax* and *Paramecium caudatum* isolated from industrial wastewater were tolerant to Cu at concentrations of 16 and 15 mg L^{-1}, respectively (Rehman et al. 2010). Meanwhile, *Euplotes mutabilis* from industrial wastewater showed resistance to 22 mg L^{-1} Cu (Rehman et al. 2008a). In contrast, some freshwater ciliates were found to be sensitive to Cu even at very low concentrations (Abraham et al. 2017a). Some Colpodid ciliates which are the most specific and common in terrestrial and semi-terrestrial habitats (Foissner 1993) were resistant to

Cu pollution up to 15 mg L^{-1} (Díaz et al. 2006). This may be due to autochthonous ciliate species that have adapted to biotopes which have persistent presence of trace metal contamination, including Cu, or might be related to the genetic structure of species (Díaz et al. 2006). Tolerant species and adaption may also explain the finding of a strongly significant increase in the number of ciliate cells after 4 days of incubation at dose of 960 mg kg^{-1} in comparison to the control, 303 compared with 126 cell g^{-1} dry soil, respectively. It was found that this increase was due to rise of two ciliate species *Homalogastra setosa* and *Chilodonella uncinata* (Table 6.1). Results in the present study seem to indicate that these two soil species can tolerate high Cu concentration. Interestingly, instead of inhibiting ciliate abundance as previous studies showed (Campbell et al. 1997; Pratt et al. 1997; Díaz et al. 2006), here Cu at the highest concentration stimulated the growth of these species. Metal tolerance exhibited by ciliates and microorganisms relates to their metal resistance mechanisms (Díaz et al. 2006; Martín-González et al. 2006). Copper is tolerated by cells due to the synthesis of metal binding proteins called metallomethioneins (Zahid et al. 2018) (Zahid et al. 2018). P_{1B}-ATPase pumps are also important in Cu tolerance as they efflux accumulated Cu from the cell (Díaz et al. 2006) to dissipate its toxicity. Both mechanisms could be contributing to Cu resistance observed in the two ciliates species. Furthermore, when presence/absence of ciliate species in all four soil samples together in each treatment and the control is taken into account, total 17 different species were found in the control, but just 14 species and 12 species were present at Cu doses of 720 and 960 mg kg^{-1}, respectively. This shows a tendency that some sensitive ciliate species to be lost from the community at highest Cu concentrations. Thus, tolerant ciliate species increased in abundance as they can exploit the resources not being used by the species that have been excluded by toxicity. This can partly explain why significant increases in the abundance of two species, *Homalogastra setosa* and *Chilodonella uncinata*, were seen at the highest concentration of 960 mg kg^{-1} to compare with the control.

Although the species richness of soil ciliates in this study was not significantly affected by elevated Cu in statistical terms, even at a very high concentration, there was a decrease in the number of soil ciliate species, i.e., a total of 17 species in the control compared to 14 and 12 in Cu concentrations of 720 and 960 mg kg^{-1}, respectively. Also, the same ciliate species were missed in Cu treatments of 720 and 960 mg kg^{-1}. This further demonstrates the general tolerance of many soil ciliates to Cu pollution. Abraham et al. (1997) also reported that there were many

ciliate species which were frequently present in activated-sludge sewage treatment plant because they were tolerant to trace metals at high concentrations. However, a reduction in the number of ciliate species at high concentration of Cu compared to control also suggests that some species are sensitive to Cu toxicity. Hence, the highest concentrations of Cu in the present study could affect the soil ciliate community. It suggests that the structure of soil ciliate community can be potential indicator to soil Cu contamination.

Compared to soil ciliates, assessments about impact of trace metal and Cu on microbial communities and microbial activity are much more frequently reported (Ekelund et al. 2003; Rajapaksha et al. 2004; Nwuche and Ugoji 2008). Much of this research focused on determining total microbial activity by measuring basal respiration (measuring CO_2 - evolution). However, fluorescein diacetate hydrolysis is also an effective method to measure total microbial activity and shows a linear relationship with microbial biomass (Schnürer and Rosswall 1982). The finding in the present study demonstrated that Cu had a negative effect on total microbial activity at the highest concentration. This confirms reports from other research that high Cu concentrations decrease microbial activity measured through CO_2 evolution (Ekelund et al. 2003; Nwuche and Ugoji 2008).

Correlation between ciliates and total microbial activity in Cu treatments

Through predation, soil ciliates control microbial populations (Finlay and Esteban 2013). Thus, a negative correlation between ciliate abundance and microbial activity might be expected. However, no such correlation was found in the present Cu investigations. However, at the highest Cu concentration, the abundance of ciliates increased significantly compared to the control (Figure 6.1), meanwhile, soil total microbial activity decreased significantly (Figure 6.3). The microbial population will have been exposed to the combined pressures of Cu stress and increased predation from the Cu tolerant ciliates, hence decreased in microbial activity. Ekelund et al. (2003) found that soil protozoa controlled the microbial growth at Cu concentrations that left soil protozoa unaffected, but at higher Cu concentrations when the abundance of protozoa was affected, the growth of microorganisms increased again because of diminished grazing pressure. This shows that ecological interactions are an important factor that needs to be taken into consideration when considering effects of metal pollutions in particular and other contaminants in general such as pesticides on microorganisms on real ecosystems. Otherwise, the toxicity of the pollutant may be incorrectly determined.

Impacts of cypermethrin insecticide on the abundance and species richness of soil ciliates and soil total microbial activity

The Pyrethroid insecticide group is widely used in domestic and agriculture situations (Singh et al. 2012). Impacts of cypermethrin on soil microbial biomass and its activity was investigated by previous studies (Zhang et al. 2008; Goswami et al. 2012). Toxicity of the insecticide was also tested on a limited number of freshwater protists (Friberg-Jensen et al. 2003; Dutta 2015; Hikal et al. 2015). Friberg-Jensen et al. (2003) reported that freshwater ciliate abundance increased after 2 days in the three highest concentrations spanning a range of 0.47-6.1 µg L^{-1} of cypremethrin. The study also showed a positive correlation between ciliates and cypermethrin concentration during the entire study period. However, assessing the effect of cypermethrin on soil ciliates has not been reported up to now.

In the present study, a significant increase in soil ciliate abundance after 4 days of incubation was also indicated at a cypermethrin dose of 160 mg kg^{-1} compared to the control. Although there was not a statistically significant difference between the control and exposure to 320 mg kg^{-1}, the mean number of individual ciliates was triple that in the control (Figure 6.4). Ater 10 days of incubation, soil ciliate abundance showed a similar tendency as after 4 days. Comparing with the control, the number of soil ciliates after 10 days increased significantly at the four highest concentrations (80-640 mg kg^{-1}). In fact, the high number of individual ciliates at doses of 80 and 160 mg kg^{-1} was attributed to the abundance of *Homalogastra setosa* and the ciliate abundance at doses of 320 and 640 mg kg^{-1} was contributed to the abundance of two species, *Colpoda stenii* and *Colpoda iflata* (Tables 6.2 and 6.3). This was also reported by Petz and Foissner (1989) when testing the toxicity of mancozeb (fungicide) and lindane (insecticide) on soil ciliates. Petz and Foissner (1989) showed that almost all active ciliate cells in plots 90 days after lindane was applied at high dose were from two species, *Colpoda steinii* and *Pseudoplatyophrya nana*. In general, microorganisms such as bacteria and fungi are known to be able to completely metabolize a pesticide to CO_2, H_2O, inorganic nitrogen and other inorganic elements via a mineralization process, thus they can tolerate pesticides (Hoagland et al. 2001; Van Eerd et al. 2003). It has also been reported that two ciliates isolated from industrial effluents, *Stylonychia mytilus* and *Paramacium caudatum*, have resistance to the insecticide endosulfan, and are able to utilize it as a carbon source (Rehman et al. 2008b). Therefore, the dominant ciliate species which had high abundance in different cypermenthrin concentrations (the range of 80-640 mg kg^{-1}) may have had the capacity to resist cypermethrin, and use

cypermethrin as a nutrient source for their growth. *Homalogatra setosa* was favoured in cypermethrin treatments at concentrations of 80 and 160 mg kg^{-1}. Increase microbial enzyme activity (although not significant) at the concentrations of 80 and 160 mg kg^{-1} compared to the control was also observed (*ca.* 21.8 and 26 compare to 16.8 µg g^{-1}, respectively; Figure 6.6), thus more food in this case may also be a factor to stimulate the abudance of this species. Thereafter, very few cells of *H. setosa* were seen at the higher concentration (320 mg kg^{-1}) and the species completely disappeared at the highest concentration (640 mg kg^{-1}). Instead, the abundance of two other species, *Colpoda stenii* and *Colpoda inflata*, increased in these higher concentrations. The results strongly suggest that cypermethrin was toxic to *H. setosa* at 320 mg kg^{-1} and 640 mg kg^{-1}, with the latter representing an LD_{100} (dose having 100 % probability of causing death). In addition, an inhibitory effect of cypermethrin at concentrations of 320 and 640 mg kg^{-1} on *H. setosa* would have reduced competition on food. This would facilitated an increase in abundance of more tolerant, but less competative species and thereby, at least partly, accounting for the increase in abundance of *C. stenii* and *C. iflata*. Such a competitive reduction also explained the increase in the abundance of some dominant species after 90 days insecticide lindane application (Petz and Foissner 1989).

Although no significant difference in the number of soil ciliate species was found between the control and all concentrations of cypermenthrin used, there was a decrease in the number of soil ciliates species when the presence of ciliate species in the control and different treatments was taken in consideration. Several ciliate species were completely absent at the highest concentration (640 mg kg^{-1}), but still found in the control and lower cypermethrin concentrations, such as *Halteria grandinella*, *Homalogastra setosa*, and *Arcuospathidium vermiforme*. In total, 21 ciliate species were found in the samples of the control, but only 12 ciliate species were recorded in the samples from the 640 mg kg^{-1} treatment. This indicated that the highest concentration of cypermethrin was toxic to some species in the ciliate community. There is no literature on the effect of cypermethrin on protozoa communities thus far, but evidence of cypermethrin induced change in the structure of microbial community was found (Zhang et al. 2008), demonstrating a wide effect on soil microbial communities. Hence, it suggests that cypermethrin at very high concentration may affect the structure of soil ciliate community. However, some species were favoured at high cypermethrin doses, as mentioned and explanined

above.This also contributes to view that many ciliate species are tolerant to this pesticide.

Concerning total microbial activity in the present study, there was a significant increase in microbial activity in treatments and a positive corelation between ciliate abundance after 10 days and total microbial activity (r = 0.624, p < 0.01). Increased microbial biomass in freshwater was shown by Friberg-Jensen et al (2003) and soil microbial biomass increased at recommended dosages of insecticide cypermethrin used in cucumber production (Zhang et al. 2008). In contrast, Goswami et al (2012) reported that soil total microbial activity in alluvial soils decreased up to 30 days after the use of cypermethrin at the dosage of 26.5 µg kg^{-1}. However, it is well-documented that soil protozoa play an important role in controlling microorganisms, and therefore stimulate their growth (Finlay and Esteban 2013). Thus, here, it can be hypothesized that ciliates had an indirect effect on microbial biomass by grazing them. The abundance of ciliate species specifically consuming bacteria was found to contrilbute the nutrients nitrogen and phosphorus to soil by their excretion (Griffiths 1994). This may stimulate the activity of microbial community. Furthermore, microorganisms have the capacity to metabolize pesticides (Hoagland et al. 2001; Van Eerd et al. 2003). Thus in this case, soil microbes may have used cypermethrin as carbon resourse for their growth. Consequently, the increased activity of the microbial community facilitated the growth of predators (ciliates), then ciliates stimulated the growth in the populations of other microorganims, as outlines above. This positive feedback explains why higher total microbial activity was found in treatments than in control and why a positvie correlation between ciliate abundance and total microbial activity was found.

A negative coefficient was shown for the relationship between soil ciliate richness and total microbial activity (r = - 0.403, p < 0.05). Although, there was no statistically significant difference in ciliate species richness between treatments and the control, cypermethrin at very high concentration had a negative effect on the structure of ciliate community, i.e., in total, 21 ciliate species were found in the samples of the control compared to 12 ciliate species recorded at the samples of 640 mg kg^{-1} treatment. It is possible that a reduction in grazing pressures on microorganisms arose due to the decrease in the number of bacteria feeding ciliate species, which may partly explain the weak negative correlation between soil ciliate richness and total microbial activity. However, in this case, it is difficult to establish a “cause-effect” relationship because the increase in total microbial activity may also have

been stimulated by the use of cypermethrin as a carbon source, or stimulated by ciliate grazers.

Impacts of glyphosate herbicide on the abundance and species richness of soil ciliates and total microbial activity

There was no significant difference between the abundance of ciliates after 4 days and 10 days of incubation and glyphosate concentrations 1 day and 7 days after application. However, there was a significant decrease in soil ciliate abundance after 4 days, but not for 10 days of incubation at 15 days after the application of glyphosate when compared with the control. Hence, a toxic effect on ciliate abundance was evident in the two highest dosages of 16.82 and 33.6 mg kg^{-1}. The reduction in number of individual ciliates was attributed mainly to one species, *Homalogastra setosa* (Table 6.4). This species seems to be more sensitive to glyphosate at high concentrations than other species. Similar sensitivity has been shown for other ciliate species, for example two freshwater species, *Tetrahymena pyriformis* and *Euplotes vannus,* had median lethal concentrations of glyphosate between of 23-30 mg L^{-1} (Tsui and Chu 2003). Furthermore, other research found that protists were inhibited at high concentrations (above 72 mg L^{-1}), but survived at concentration of 72 mg L^{-1}. Specifically, two soil species, *Colpoda cucullus* and *Colpoda steinii*, were very abundant at this concentration (Mbanaso et al. 2014). In the present study, *Homalogastra setosa* increased in the number at low concentrations 15 days after application, but then decreased at higher dosages. Thus this species has potential as a bio-indicator of herbicide-contaminated environments.

Mbanaso et al (2014) found that glyphosate impacted on the richness of soil protists at concentrations over three applied concentrations i.e., 72, 720, and 7200 mg L^{-1} (equivalent to concentrations of 14.88, 148.76, and 1487.6 mg m^{-2}). Although, the present study found no statically significant impact of glyphosate on soil ciliate specie richness up to 15 days following application, there was a reduction in the number of soil ciliate species at the highest concentration (33.6 mg kg^{-1} – equivalent to concentration of 1800 mg m^{-2}) compared to the control. In total 22 species were found in the treatment of 33.6 mg kg^{-1} compared to 29 species recorded in the control. This shows glyphosate at a dose of 33.6 mg kg^{-1} changed the structure of soil ciliate community. Therefore, the soil ciliate community may be a potential of a bio-indicator for soil glyphosate contamination.

Other research reported that glyphosate significantly stimulated soil microbial activity (Haney et al. 2000; Partoazar et al. 2011). Haney et al. (2000) showed that microbial activity determined through C and N mineralization increased significantly due to glyphosate concentrations in the range of 47-234 mg kg^{-1}. However, in the present study, glyphosate had no impact on total microbial activity at any of the concentrations used, probably because the glyphosate concentrations used were too low to have effect on microbial activity compared to the concentrations given by Haney et al. (2000).

6.5 Conclusion

Copper stimulated the abundance of soil ciliates at the highest concentration, which was attributed to the abundance of two species, *Homalogastra setosa* and *Chilodonella uncinata*. At the highest concentration, Cu had toxicity on the structure of soil ciliate community.

The insecticide cypermethrin also increased the abundance of soil ciliates at concentrations ranging over 80-640 mg kg^{-1}. This increase was also attributed to the increased abundance of dominant soil ciliate species. The high number of numbers of *H. setosa* was found at doses of 80 and 160 mg kg^{-1}, whereas high individuals of *Colpoda stenii* and *Colpoda inflata* were found at doses of 320 and 640 mg kg^{-1}. the abundance of *H. setosa* rapidly reduced at a cypermethrin concentration of 320 mg kg^{-1}, and by 640 mg kg^{-1} *H. setosa* was absent from samples, this concentration apparently toxic to this species. Furthermore, a toxic effect of cypermethrin on the structure of soil ciliate community was found at the highest concentration . A positive correlation between abundance of soil ciliates after 10 days of incubation and soil total microbial activity in the experiment of cypermethrin was recorded. This indicated an ecological interaction between soil ciliates and soil microorganisms. Soil microorganism possibly used cypermethrin as carbon source for their growth. Increased activity of the microbial community, thereafter, facilitated the growth of predators (ciliates). In turn, ciliates stimulated the growth of other soil microorganims again.

In general, herbicide glyphosate had no statistically significant effect on ciliate abundance after 4 days and 10 days of incubation, total ciliate species richness, and total microbial activity up to 15 days after application. However, a significant effect of glyphosate on the ciliate abundance after 4 days of incubation at 15 days after application only was shown. This decrease in the abundance of ciliates was due to a reduction in the abundance of *H. setosa* at high glyphosate concentrations

of 16.82 to 33.6 mg kg^{-1}. Therefore, this species is a potential bio-indicator of herbicide and insecticide contaminated soils. In addition, although there was no statically significant difference in ciliate species richness 15 days after glyphosate application, a change of structure in the soil ciliate community was found at a dose of 33.6 mg kg^{-1} compared to the control. To sum up, the soil ciliate community may also have a potential as a bio-indicator for contamination by Cu, cypermethrin, and glyphosate.

As a whole, the species *Homalogstra setosa* demonstrated an interesting pattern in response to pollutants in this series of experiments. The abundance of *H. setosa* increased at the highest concentration of Cu (960 mg kg^{-1}), whilst it reduced at high doses of glyphosate. In addition, *H. setosa* was abundant at lower cypermethrin concentrations (80 and 160 mg kg^{-1}), but the abundance of this species decreased at a dose of 320 mg kg^{-1} and a dose of 640 mg kg^{-1} was a LD_{100} for *H. setosa*. Therefore, it is possible that a greater investigation of underlying causes of change in the abundance of *H. setosa* is needed.

Chapter 7. OVERALL DISCUSSION AND CONCLUSION

7.1 Overall discussion of the present study

7.1.1 Summary of main findings and original contribution to knowledge

Ciliates are free-living single-celled organisms and are one of the functional groups of protozoa, with the other two being the flagellated and the amoeboid protozoa (Finlay and Esteban 2013). Ciliates are abundant in soil habitats and they seem to have a cosmopolitan distribution (Foissner 1998; Finlay et al. 2001). Soil ciliates have an important role in controlling the growth of other microorganisms and in recycling organic matter (Esteban et al. 2006), but they are also influenced by the soil characteristics (Foissner 1987; Foissner et al. 2005; Li et al. 2010a; Li et al. 2010b).

The aim of this Chapter 7 is to discuss how the overall aim and objectives of the PhD research presented here have been met. The limitations of the work are also discussed.

The overall aim of this study was to investigate the abundance and species richness of ciliated protozoa in natural and agricultural soils, and to determine the relationship between physio-chemical soil properties and the occurrence of the species and abundance of ciliates in order to determine the usefulness of soil ciliates as bio-indicator of soil quality.

The objectives of the current study were:

1. To determine the soil properties at three study sites, i.e., one natural ecosystem and two agricultural ecosystems (Chapter 3);
2. To investigate the abundance and richness of soil ciliated protozoa in the three study sites and to determine correlations between soil ciliates and soil properties in order to determine if soil ciliates can be used as bio-indicators of soil quality (Chapter 4);
3. To assess the impact of specific contamination factors, including coper metal, the herbicide glyphosate and the insecticide cypermethrin on soil ciliate abundance and species richness (Chapter 6);
4. To examine the taxonomy of soil ciliate species in the three study sites and to identify rare and new species (Chapter 5).

Chapter 3 of this research determined a wide range of soil properties in the natural and agricultural ecosystems, including soil texture; pH; moisture; soil and air temperatures; levels of major the nutrient elements K, P, S and Ca; soil organic

matter; nitrate-N and ammonium; selected metals; activities of β-glycosidase and acid phosphomonoestarase and total microbial activity. Differences were found in soil texture, pH, and content of soil organic matter in the three studied ecosystems, which are key factors causing effects on other soil characteristics. In particular, soil organic matter (SOM) content increased water holding capacity (Yang et al. 2014; Sujatha et al. 2016) and nutrients (Craswell and Lefroy 2001). SOM is a major property that differed between the natural Fen and the agricultural soils. It would be expected SOM would be low in intensively farmed soils. In the present study, the very high content of SOM in the natural Fen soil created significant increases in moisture and concentrations of nutrients such as available P, total P, and N compared to the agricultural soils of Vicarage Farm and Corfe Castle Farm. Soil organic matter also stimulated activity of enzymes (Schnürer et al. 1985; Wang and Lu 2006), thus significantly increased total microbial activity and β-glycosidase was found in the natural Fen soil. The increase in nutrient elements and enzyme activities related to the existence of high SOM content significantly increased the abundance of soil ciliates in the Fen in comparison with the agricultural soils. In addition, soil pH has a crucial importance in governing the bioavailability of nutrients (Ch'ng et al. 2014) and trace metals (Rieuwerts 2007; Takac et al. 2009; Moir and Moot 2010). Although almost all total concentrations of selected trace metals were the highest in the Vicarage Farm soil, available concentrations of the metals in this farm were very low because of neutral/alkaline pH, which limits the availability of undesirable metals. In contrast to the farm, the available concentrations of the metals were highest in the Fen due to the acidic pH of the soil. Even within the Fen, the effect of low soil pH was shown clearly, where Plot 2 had a lower pH than Plot 1 (pH~4 compared to pH~5), thus lower available concentration of P and greater available concentrations of all selected metals were recorded in Plot 2. The more acidic pH and higher availablity may account for the lower ciliate species richness found for Plot 2 than for Plot 1. Soil pH also affected soil nitrification, at acidic pH nitrification is slowed down (Sahrawat 2008). Thus, accumulation of ammonium increased significantly in the natural Fen soil compared to the agricultural soils. Fined-textured soil with greater content of clay and silt fractions in Vicarage also contributed to increase capacity to keep soil moisture and soil nutrient elements was shown. In general, the study sites which offered a wide range of soil physical, chemical, and biological properties were selected in order to assess interactions between soil properties and soil ciliates.

Chapter 4 investigated and compared the biodiversity of soil ciliates in the natural and agricultural ecosystems. The well-established non-flooded Petri dish method was used to investigate the potential abundance and species richness of soil ciliate communities in the present study (Finlay et al. 2000). Correlations between the abundance and species richness of soil ciliates were analysed. The results showed significantly greater abundance of soil ciliates in the natural Fen soil than in the agricultural soils, but no significant difference between Vicarage Farm and Corfe Castle Farm. This was due to a significantly greater level of nutrient contents, including available P; soil organic matter; and accumulation of ammonium; enzyme activities of total microbial activity and β-glucosidase; and soil moisture in the natural Fen soil than in the agricultural soils. Insignificant difference in ciliate abundance between the two farm soils showed that either insufficiently large differences in soil properties between two farms or the effects of farming override the influence of soil properties accounted for. In general, the highest abundance was recorded after 10 days of incubation in almost all investigated months in the natural ecosystem and this confirmed the similar finding of Finlay et al. (2000). In the agricultural soils, the highest number of ciliates was always recorded after 4 days of incubation in all months investigated. This may relate to either poorer nutrient conditions such as soil organic matter, phosphorus and enzyme activity in the agricultural soils than in the natural Fen soil or difference in ciliate species between the natural and agricultural soils. Nevertheless, there was no significant difference in the total species richness of soil ciliates amongst the three ecosystems; a total 82 species were found in the Fen compared with 88 species and 84 species found in the Vicarage and Corfe Castle Farms, respectively (see Table 5.1 of Chapter 5). This finding confirmed the finding Foissner (1997c) that the number of ciliate species does not differ between natural and agro ecosystems. Indeed, sometimes the species richness of soil ciliates was higher in agro-ecosystems.

In the present study, Spearman's rank order correlation showed that soil ciliates were strongly correlated with soil properties. This is in agreement with previous studies (Foissner 1987, 1997c; Foissner et al. 2005; Li et al. 2010a; Li et al. 2010b)., The present study progressed this understanding by also investigating correlations between ciliate abundance and richness and soil biological properties, specifically, soil enzyme and microbial activity. Ciliate abundance after 4 days and 10 days of incubation and total species richness of soil ciliates at all three ecosystems had positive correlations with soil moisture, soil organic matter,

available concentrations of phosphorus, ammonium, the available concentrations of Cu, Ni and Zn, total microbial activity and β-glycosidase, but a negative correlation with soil pH was found. No correlations between the abundance of soil ciliates after 4 days and 10 days and the available concentrations of K and S and activity of acid-phosphomonoesterase were found in the three ecosystems together. However, with the exception of weak positive correlations with available P and total microbial activity, the total richness of soil ciliates had no correlation with the measured soil properties in the three habitats together. Due to the big difference in soil characteristics between Plot 1 and Plot 2 of the natural Fen such as pH and nutrient and metal bioavailability the relationship between soil ciliates and soil properties was taken into consideration here in order to determine interactions between soil ciliates and soil properties under natural conditions without human disturbances like farming. The abundance of soil ciliates after 4 days and 10 day positively correlated with ammonium, whilst the available concentrations of Ni and Pb had negative correlations with ciliate abundance after 4 days of incubation in the Fen site only. Nevertheless, more impacts of soil properties on total species richness of soil ciliates were found in the natural Fen soil, in which pH, total P, available P, total Ca, and nitrate-N positively correlated with ciliate species richness, but the available concentrations of almost all measured trace metals, except for Cr, showed strong negative correlations. In addition, seasonal variation in soil ciliate abundance was also seen clearly in the natural Fen habitat (Figures 4.2 and 4.4).

A group of common bacterivorous and fungivorous ciliate species, including *Cyclidium mucicola*, *Grosglockneria acuta*, *Lepthopharyx coastatus*, *Colpoda* spp., *Nivaliella plana*, *Pseudoplatyophrya nana* was very abundant in both Plots of the natural Fen habitat. Additionally, two species, *Sathrophilus muscorum* and *Drepasomonas revoluta*, were abundant in Plot 1 of the Fen in almost all sampling months, but were hardly recorded in Plot 2. In contrast, more species of the Hypotrichia group, i.e., *Gonostomum affina*, *Tachysoma pellionellum*, *Psilotricha silvicola* nov. sp. were found in the Plot 2. By contrast, *Homalogastra setosa* was observed only in the agricultural soils, where it was the main contributor to the abundance of soil ciliates in both the farms studied. *Drepasomonas sphagni* was only found in coarse textured soil, i.e., Corfe Castle Farm.

The list of soil ciliates species in the three study ecosystems is presented in Chapter 5 (Table 5.1), in which several of the species found in this research are first records for the UK, including *Colpoda minima* (Figure 5.2), *Holosticha adami* (Figure 5.6-B), *Nassulla terricola*-Complex (Figure 5.3), and *Stylonychia vorax*

(Figure 5.6-A). One new ciliate species *Psilotricha silvicola* nov. sp., found in Plot 2 of the natural Fen site, was described using morphological and molecular methods. Another two potentially new ciliate species belonging to the genera *Pseudoholophrya* and *Oxytricha* were also found.

Chapter 6 of the present study reported experiments utilising a trace metal, a herbicide and an insecticide, which were carried out to assess the effect of these common agricultural substances on the abundance and species richness of soil ciliates and soil enzyme activity. In general, in this experiment, there were no evident negative effects on the abundance and species richness of ciliates due to Cu toxicity. Total microbial activity was unaltered except at the highest Cu concentration (960 mg kg^{-1}), which significantly decreased total microbial activity. Concurrently, the highest copper treatment significantly increased the abundance of soil ciliates after 4 days of incubation. This was attributed to an increase in abundance of two species, *Homalogastra setosa* and *Chilodonella uncinata*. However, when considering presence/absence of ciliate species in the control and each Cu treatment, a total of 17 ciliate species were found in the control, and only 12 species were present in a Cu treatment of 960 mg kg^{-1}. Hence, Cu had a negative effect on the structure of the soil ciliate community at the highest concentration. Similarly to copper, the insecticide cypermethrin, a synthetic pyrethroid insecticide, used widely to control a wide range of insect pests in household and agricultural crops (Singh et al. 2012), significantly increased the abundance of soil ciliates after 4 days and 10 days of incubation in the treatment range of 80-640mg kg^{-1}. This was attributed to the abundance of some dominant soil ciliate species, such as *Colpoda steinii*, *Colpoda inflata*, and *Homalogastra setosa* (Tables 6.2 and 6.3). However, the abundance of *Homalogastra setosa* reduced sharply at cypermethrin concentration of 320 mg kg^{-1}, and it was completely absent at 640 mg kg^{-1} (Tables 6.2 and 6.3). At the highest concentration of the insectide the structure of the soil ciliate community also changed. When considering presence/absence of ciliate species in the control and each treatment, only 12 ciliate species were found in cypermethrin dose of 640mg kg^{-1}, compared to 21 species presented in the control. In general, glyphosate, a type of foliar-applied and non-target herbicides used to kill broadleaf weeds, had no statically significant effects on ciliate abundance after 4 days and 10 days of incubation, ciliate species richness and total microbial activity up to 15 days after application, except for a reduction in soil ciliates abundance after 4 days of incubation at 15 days after application. *Homalogastra setosa* was inhibited by glyphosate at highest

concentrations of 16.82 and 33.6 mg kg^{-1}. In addition, glyphosate also had a negative effect on the structure of the ciliate community; only 22 species were present in the glyphosate treatment of 33.6 mg kg^{-1} 15 days after application, whilst 29 species were found in the control.

7.1.2 Soil ciliates as indicators of soil quality

Systems with a high diversity of living micro-organisms are able to respond quickly to changes in environmental conditions (Foissner 1987; Bamforth 1995; Esteban and Finlay 2010). Thus, the biodiversity of soil ciliates is also able to help them to exhibit resilience to changes in their environment. It is well-documented that soil ciliates are sensitive to changes in environmental conditions and contaminants such as trace metals and pesticides because ciliates do not have a cell wall (Petz and Foissner 1989; Foissner 1994, 1997c; Díaz et al. 2006). Soil ciliates have been already shown as bio-indicators of soil conditions in several previous studies, for parameters such as soil CO_2 (Gabilondo and Bécares 2014); herbicides, fungicides and insecticides (Petz and Foissner 1989; Mbanaso et al. 2014); trace metals (Forge et al. 1993; Bowers et al. 1997; Campbell et al. 1997; Pratt et al. 1997; Díaz et al. 2006); fertilizers (Aescht and Foissner 1992); soil compaction; soil O_2 deficit (Foissner 1999b).

In the present study (Chapter 4), correlations of soil ciliates and a wide range of soil factors were taken into account in order to assess soil ciliates as bio-indicators of soil quality. The abundance of soil ciliates in the natural ecosystem with high soil organic matter (SOM) was significantly higher than at the agricultural soils, which had much lower content of SOM. The findings of the present study show that soil ciliate abundance had strong positive correlation with organic matter after 4 days and 10 days of incubation in the three ecosystems. Furthermore, the abundance of ciliates was due to some dominant bacterivorous and fungivorous species like *Cyclidium mucicola*, *Grosglockneria acuta*, *Lepthopharyx coastatus*, *Colpoda* spp., *Nivaliella plana*, *Pseudoplatyophrya nana*, *Sathrophilus muscorum* and *Drepasomonas revoluta*, and Hypotrichia group of *Gonostomum affina*, *Tachysoma pellionellum*, *Psilotricha silvicola* nov. sp. Verhoeven (2001) also showed that the abundance of soil ciliates increased significantly with increase SOM and two bacterivorous ciliate species, *Colpoda aspera* and *Cyrtolophosis elongata* contributed to the increase in abundance. Moreover, available P strongly and positively correlated to the abundance of soil ciliates after 4 days and 10 days in three ecosystems (r = 0.615 and 0.665, respectively). Thereby, the abundance of

soil ciliates may be bio-indicators for the nutrient status of soil habitats, i.e., content of SOM, and available concentrations of P.

Soil enzymes, including total microbial activity and β-glycosidase can indicate quantitative changes in organic matter and its turnover (Schnürer and Rosswall 1982; Stege et al. 2010b). Soil enzyme activities, themselves in turn are bio-indicators of soil quality because of their sensitivity to environmental changes (Nielsen et al. 2002). Esteban et al. (2006) also showed that soil ciliates stimulate the growth of microbial community by feeding on them. In the present study, total microbial activity and β-glycosidase strongly and positively correlated to the abundance of soil ciliates after 4 days and 10 days in three ecosystems (r = 0.551 and 0.617 for total microbial activity after 4 days and 10 days, respectively; and r = 0.57 and 0.579 for β-glycosidase). This suggests that the abundance of soil ciliates may be a bio-indicator for total microbial activity and activity of enzyme β-glycosidase. Furthermore, total microbial activity and enzyme β-glycosidase themselves are bioindicators of soil quality (De La Rosa and Sobral 2008). Higher enzyme activity are related to higher nutrients such as soil organic matter and higher nutrient availability such as nitrogen and phosphorus (Craswell and Lefroy 2001; Rita et al. 2013; Błońska et al. 2017). Hence, soil enzyme activities such as total microbial activity and enzyme β-glycosidase could be considered good indicators for soil quality which subject to productivity and sustainability of terrestrial ecosystems. To sum up, the abundance of soil ciliates shows potential as a good indicator of soil quality and compares favourably to other methods such as soil enzyme activity.

Homalogastra setosa was only found in the agricultural ecosystems in the present study. Foissner (1992) also found *Homalogastra setosa* abundant in fertilized soils. Consequently, the abundance of this species can be used as a bio-indicator of agricultural disturbance, particularly the application of fertilizers. However, as discussed in Chapter 4 of this thesis, the presence and abundance of *Homalogastra setosa* seem to be limited by pH factors. Thus, this species may also potentially be used as a bio-indicator of soil pH. Nevertheless, more research on how pH impacts on growth of this species is needed because its presence has been recorded in a wide range of soil habitats with pH oscillating from very acidic to alkaline pH by previous studies (Foissner 1987; Esteban et al. 2006). Therefore, factors other than pH, for example available Al, need to be investigated and ruled out.

The results of the laboratory experiment on the impact of the insecticide cypermethrin on soil ciliates (Chapter 6) showed that a cypermethrin concentration

of 320 mg kg^{-1} was detrimental for the growth of *H. setosa*, and at concentration of 640 mg kg^{-1} this species did not grow. Moreover, in the glyphosate experiment, *H. setosa* decreased significantly at doses of 16.82 and 33.6 mg kg^{-1} 15 days after glyphosate application. Thus, *H. setosa* could potentially to be used as a bio-indicator of pesticide contamination.

On the whole, it can be seen that *H. setosa* is sensitive to soil factors such as pH, herbicide and insecticide contamination, and fertilized conditions. An indicator is chosen to assess soil quality when analytic methods are developed fully (Nortcliff 2002) in order to describe or determine what that indicator signifies. *H. setosa* is easy to recognize *in vivo* because of its typical shape and jumping movement behaviour. Also, its infraciliature can be quickly revealed using silver-carbonate impregnation to identify the species (Figure 6.5). Thus, the presence or abundance of *H. setosa* has high potential as bio-indicator to assess soil environmental conditions such as pH, herbicides, insecticides, and in agro-ecosystems with fertilizer uses. Therefore, it suggests that the presence or abundance of *H. setosa* may have potential as an indicator for soil health.

Although there were no significant differences in species richness of soil ciliates in the three ecosystems (Chapter 4), the number of soil ciliate species correlated strongly and negatively with the available concentration of measured metals in the natural Fen soil, including Al, Cd, Cu, Ni, Pb, and Zn (Figure 4.7). In addition, two species, *Sathrophilus muscorum* and *Drepasomonas revoluta* were always present and quite abundant in Plot 1 of the Fen in all sampling occasions, but they were seldom recorded or completely absent in Plot 2. As discussed in Chapter 4 of this thesis, many ciliate species cannot survive in soils with strongly acidic pH (Foissner et al. 2005; Geisen et al. 2018). In addition, low pH increased availability of trace metals in soils (Rieuwerts et al. 1998; Takac et al. 2009; Moir and Moot 2010). Plot 2 of the Fen had a lower pH than Plot 1 (~4.0 *vs.* ~5.0, respectively), this led to increase availability of measured metals in Plot 2. Hence, metal availability with a strong acidic pH in Plot 2 of the Fen accounted for a reduction in ciliate species richness here. Therefore, it is possible that the soil ciliate community can provide an indication of the status of available concentrations of trace metals in soil and low soil pH. In addition, the presence and abundance of two species *Sathrophilus muscorum* and *Drepasomonas revoluta* can be limited to soil pH lower than *ca.* 4.

7.2 Conclusion

This thesis highlighted significant differences in soil physical, chemical and biological properties between three ecosystems. In general, the Fen is typical for a natural ecosystem with high content of nutrients, high moisture and high enzyme activities, while Vicarage Farm and Corfe Castle Farm have soil characteristics typical of cultivated land.

The work presented in this thesis has also shown significantly higher abundance of soil ciliates in natural soil than in agricultural soils, and the relationships of soil ciliates with a wide range of soil properties, for which positive correlations between soil ciliates and soils enzymes and total microbial activity have been demonstrated for the first time. In particular, soil moisture, soil organic matter, available P concentration ammonium, the available concentrations of Cu, Ni and Zn had strong positive correlations with the abundance of soil ciliates, but soil pH correlated negatively with ciliate abundance in three ecosystems combined together. However, there was no significant difference in the species richness of soil ciliates between the natural and agricultural soils in the three ecosystems together. Interestingly, strong negative correlations between the species richness available concentrations of Al, Cd, Cu, Ni, Pb and Zn were found in the natural habitat when considered separately.

This thesis also highlighted the other findings. In particular, a group of common ciliate species *Cyclidium mucicola*, *Grosglockneria acuta*, *Lepthopharyx coastatus*, *Drepasomonas revoluta*, *Colpoda spp.*, *Sathrophilus muscorum*, *Nivaliella plana*, *Pseudoplatyophrya nana*, and Hypotrichia group was very abundant in the natural Fen habitat. This group can potentially indicate soil habitats which have rich content of organic matter. The presence/abundance of the species *Homalogastra setosa* may be potential indicator for agro-ecosystems applied fertilizers and soil pH. Also, *Drepasomonas sphagni* was only found in coarse textured soil with low organic matter. Potentially, this type of soil texture is the favoured habitat of this species.

In the series of the experiments conducted under laboratory conditions with Cu, an insecticide cypermethrin and a herbicide glyphosate, copper at the highest concentration of 960 mg kg^{-1} significantly decreased total microbial activity, but stimulated the abundance of soil ciliates after 4 days of incubation and this was attributed to the abundance of *Homalogastra setosa* and *Chilodonella uncinata*. Cypermethrin insecticide also significantly stimulated the abundance of soil ciliates. However, *Homalogastra setosa* was inhibited at the cypermethrin concentration of 320 mg kg^{-1} and was absent at the highest concentration of 640 mg kg^{-1}. In addition, this species was inhibited by glyphosate at the concentrations of 16.82 and above.

Interestingly, the structure of soil ciliate communities were affected at high concentrations of Cu, cypermethrin and glyphosate at 15 days after application when presence/absence of all ciliate species is taken into consideration. To sum up, this thesis highlighted the potential of soil ciliates as bio-indicators of several soil factors such as SOM content, P nutrient status, enzyme activities, soil pH, soil texture, herbicide, insecticide and trace metals in order to assess soil quality.

This thesis has also improved our understanding of biodiversity of soil ciliates. In particular, the morphology and phylogeny of a new species, *Psilotricha silvicola* nov. sp. has been described; two other potential new species of *Pseudoholophrya* sp. and *Oxytricha* sp. have been found; some species were first records for the United Kingdom.

7.3 Limitations

This present study targeted one natural and two agricultural sites to investigate soil ciliate biodiversity in order to find correlations between soil ciliates and soil properties; the use of a field-scale approach allowed for complex and uncontrolled experimental conditions to be tested, and how they may have an impact on soil microbial communities (ciliates in this case). The advantages of the method used in this study are that a wide range of soil physical, chemical and biological properties can be investigated to assess interactions between ciliates and soil properties, and the ecological interactions between ciliates, microbial activity and soil enzymes that take place in the real world. However, there are specific limitations associated with such a study. These limitations are explained below and some of them are discussed under separate subheadings.

Firstly, it is difficult to clarify the main causes which account for significant increase in the biodiversity of soil ciliates amongst the three soil ecosystems because of a wide range of soil characteristics found to have significant correlations to the abundance and species richness of soil ciliates (as shown in Chapter 4). In addition, the soil of the farm ecosystems has suffered interactions of potentially toxic substances, such as undesirable metals and pesticides. Thus, to determine which factor(s) had toxic effect on soil ciliates is impossible under field conditions.

Appropriateness of the natural Fen site

Given the limitations of funding, time, and the labour-intensive work involved in analysing the soil ciliate communities within a time frame that would allow robust and trustworthy results, one natural study site was selected. The natural habitat would act as a ‘control’ site that is not influenced by human activities, thus the Fen

site was chosen in this present study. The Fen is a woodland area and part of a nature reserve, with typical soil characteristics of natural woodland habitats, such as no human disturbance, high organic matter, high moisture, high nutrients, and high enzyme activity. In contrast, we did select more than one farm site due to the variability of the soil type, soil structure and other properties; above all, the main driver for the selection of the agricultural sites was the influence of, and level of human intervention. An another reason for choosing the Fen site is that it is difficult to find undisturbed ecosystems in the UK. This is particularly true of the southern UK. Most soils suitable for agriculture have been turned over to this use and the remaining, mainly semi-natural, systems are normally extreme and hence also unsuitable for agricultural production. For example, heathlands persist largely because of the difficulty growing crops on the nutrient poor, very acidic soils that characterise heathland. Thus, the Fen woodland represents the least disturbed ecosystem available in the study area. The alternative systems, such as grassland, would not only have been disturbed, but also the nature of the disturbance is different to that of an arable field. Thus, the Fen site provided the best option to compare farming to a natural system. However, either more natural soil ecosystems particularly sites with alkaline soil pH or more agricultural ecosystems (e.g. organic farm or field crop conversion to shrub) should be taken into account in the future.

Besides, only two plots within each habitat (natural and agricultural) habitat were selected for the study since a greater number of plots would not allow efficient analysis within the time frames. A longer sampling time period and more plots at each study site could potentially render a more accurate picture of the changes (if any) in the soil ciliate communities. However, the findings in this present study were consistent; thus, expanding sampling time and/or number of plots at each study site may not make any significant contribution to the overall outcome of the findings.

"Top-down" and "bottom-up" processes in soil food web

Both "top-down" and "bottom-up" processes in the food web need to discussed in relation to the study. "Top-down" process refers to the effect of top level consumers (predators) on the population of lower trophic levels on which they prey; the "bottom-up" process focuses on the interaction between food sources and higher trophic forms (Estes 1996). Several studies have shown that predatory nematodes and rotifers can feed on ciliates in laboratory tests and in soil (Arndt 1993; Ekelund et al. 2002). Hence, either predators or food sources may have effects on soil ciliates. In the present study, higher level predators such as these may have played a role in controlling the soil ciliate populations ("top-down"); whilst smaller micro-

organisms such as bacteria and fungi may be prey for soil ciliates (Esteban et al. 2006) and changes in the populations of these organisms may have affected ciliates ("bottom-up"). Especially, interaction between "top-down" and "bottom-up" can been partly seen in cypermethrin experiment, but it is difficult to know which process is dominant. In this experiment, a positive correlation between soil ciliate abundance after 10d and total microbial activity was shown (Table 6.4). This increase in bacterial numbers is likely due to the use of cypermethrin as carbon source for their growth, which facilitated the increase in soil ciliate abundance (as shown in Figure 6.4). Nevertheless, in this study the only interaction between higher trophic forms (ciliates) and food sources (bacteria/fungi/carbon source, the bottom-up process of the food web) was examined. It may be the case that cypermethrin had negative effects on top level predators of soil ciliates (nematodes and rotifers), thus reduce in predator numbers might partly facilitate the increase of ciliate abundance. However, up to now, there are no studies on impacts of cypermethrin on soil rotifers or nematodes to support for the above supposition of negative impact on ciliate predators. Furthermore, Friberg-Jensen et al. (2003) showed that freshwater rotifers significant increased with cypermethrin concentrations. Also, nematodes are quite tolerant to pesticides, although each species have different sensitivities with each different type of pesticides (Radova 2011).

In addition, soil nematodes feed on variety of foods such as bacteria, fungi, algae, and ciliates. The majority of nematode genera feeds on bacterial and fungi, only some genera are predatory (Yeats et al. 1993). Similar to feeding habit of nematodes, soil rotifers are the most dominant in species which feed on bacterial, fungi, and algae (Devetter 2009; Devetter et al. 2017). Hence, this suggests that there may have been a possible exclusion of or reduction in the abundance of ciliate competitors. Furthermore, in the cypermethrin experiment of this present study, presence/significant abundance of rotifers and nematodes was not observed in samples, this it is difficult to have further discussions. Importantly, the main reason behind the decision to examine "bottom-up" process was one of the aims of this research and the investigation of ciliate species as potential bio-indicators of soil quality – a task that involved in-depth analysis of ciliate species richness, abundance, and measurement of soil characteristics. Analysis of further trophic interactions, albeit important, was beyond the scope of this research. Nevertheless, it is interesting to consider how higher trophic predators (metazoans) may interact and/or impact soil ciliates.

7.4 Recommendations for further work

This thesis has highlighted issues in respect of interactions between soil ciliates and a wide range of soil properties in the natural and agricultural soils and effect of pollutants such as Cu, insecticide cypermethrin and herbicide glyphosate on soil ciliates. However, because of limitations as mentioned in section 7.3 of this Chapter, broadening the type of ecosystems and extending sampling time regarding assessing changes in the biodiversity of soil ciliates are needed. In addition, the controlled soil experiments under the field conditions to assess impact of other metals, pesticides and fertilisers on soil ciliates should be carried out in the future.

Furthermore, *Homalogstra setosa* has shown an interesting pattern in response to pollutants in the series of experiments of Chapter 6. The abundance of *H. setosa* increased at the highest concentration of Cu (960 mg kg^{-1}), but they reduced significantly at high doses of herbicide glyphosate. However, *H. setosa* was abundant at lower cypermethrin concentration (80 and 160 mg kg^{-1}), and then its abundance decreased at a dose of 320 mg kg^{-1} and a dose of 640 mg kg^{-1} was a LD_{100} for *H. setosa*. Therefore, it is possible that a greater investigation of underlying causes of change in the abundance of the species *H. setosa* is needed.

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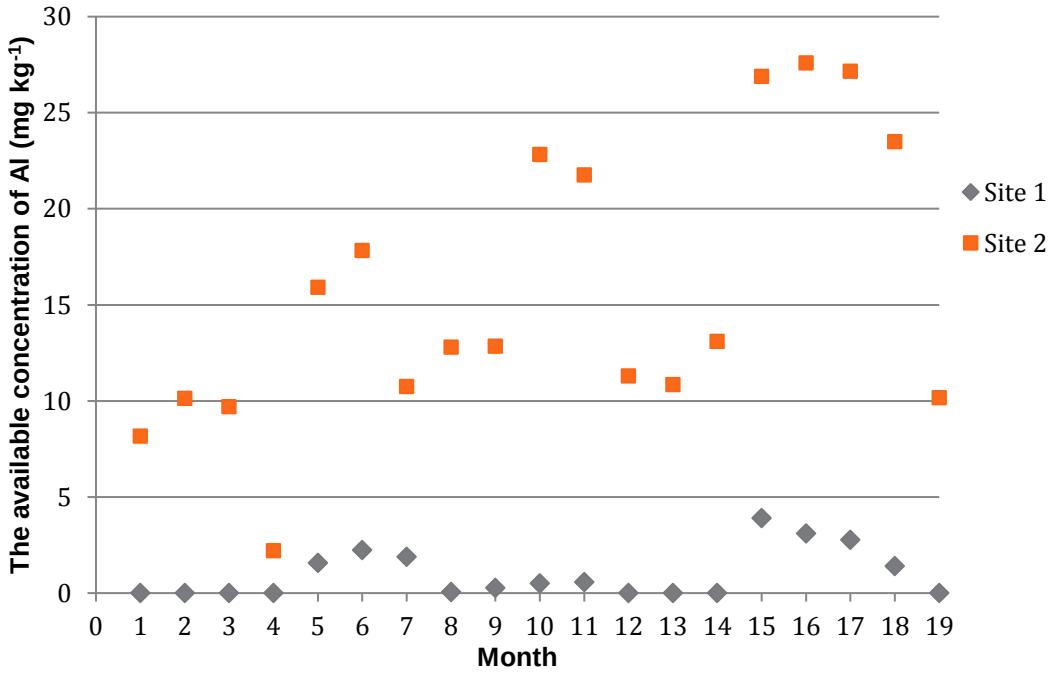
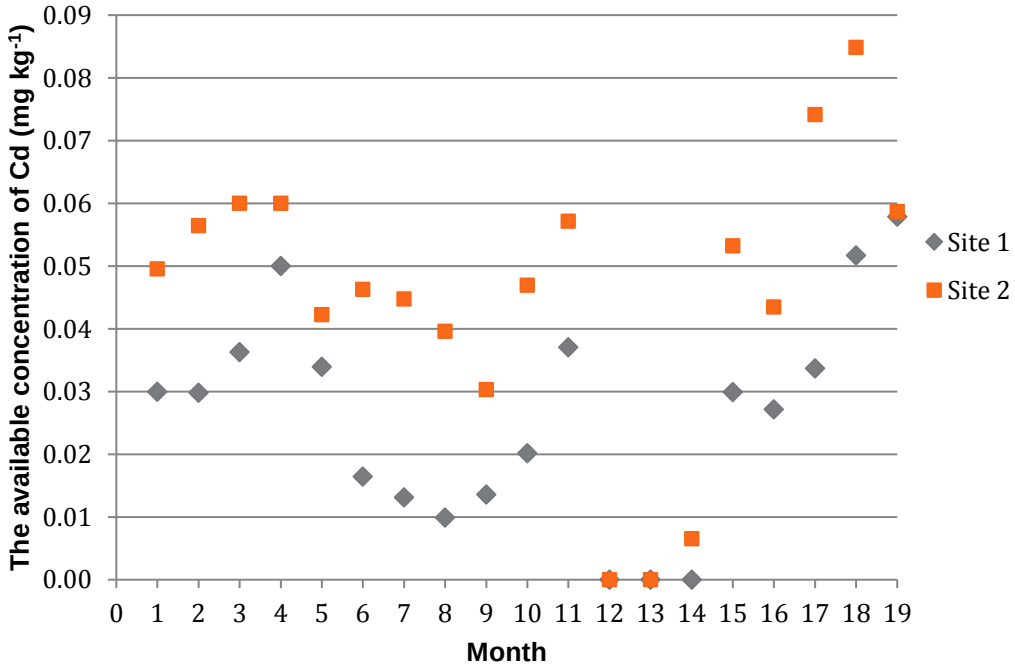
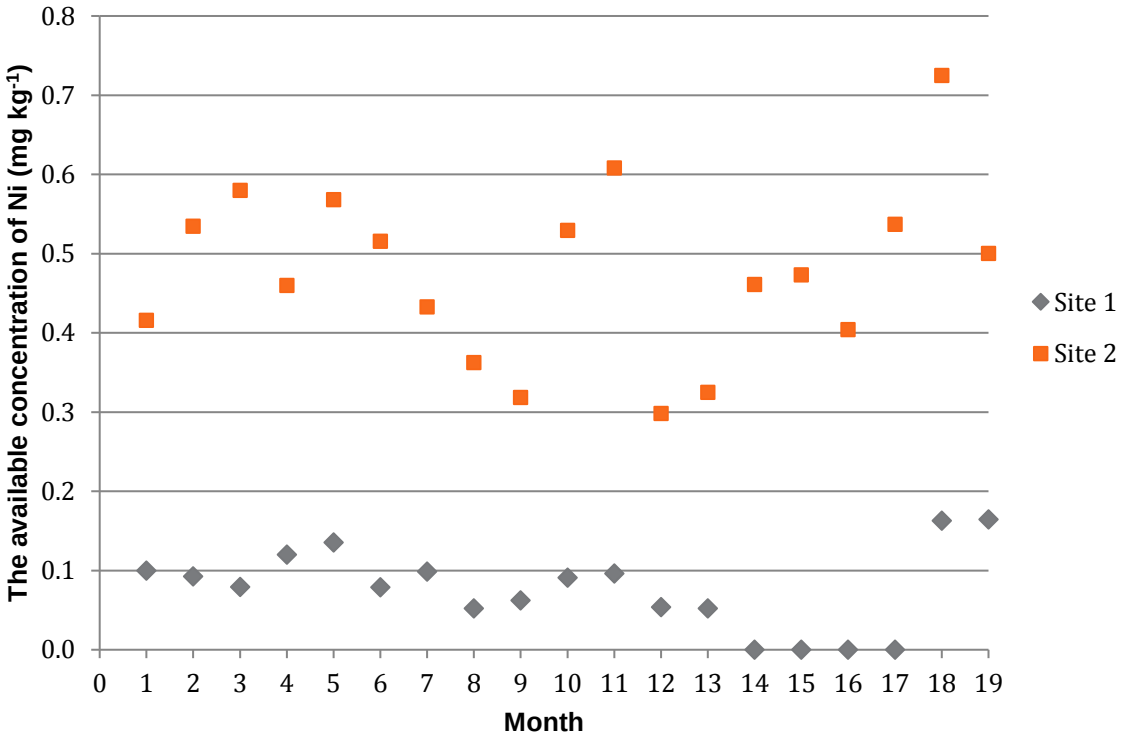
Appendix A

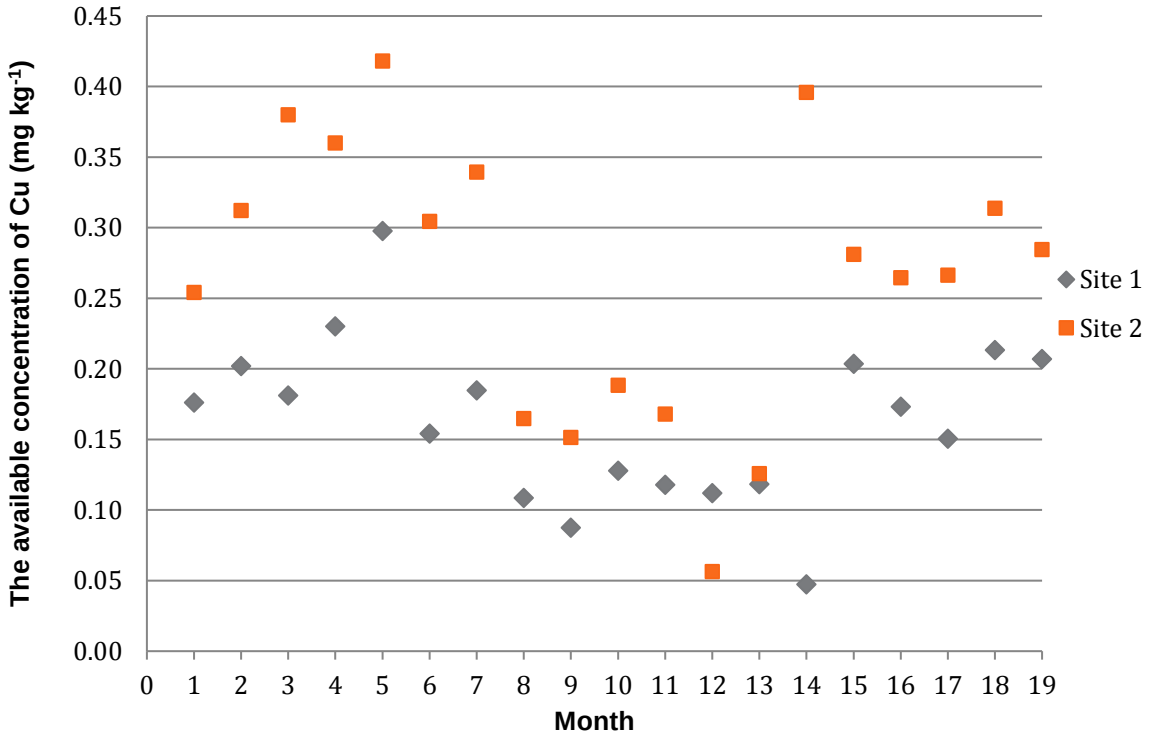
Changes in the available concentrations of trace metals in the natural Fen ecosystem during the time investigated.

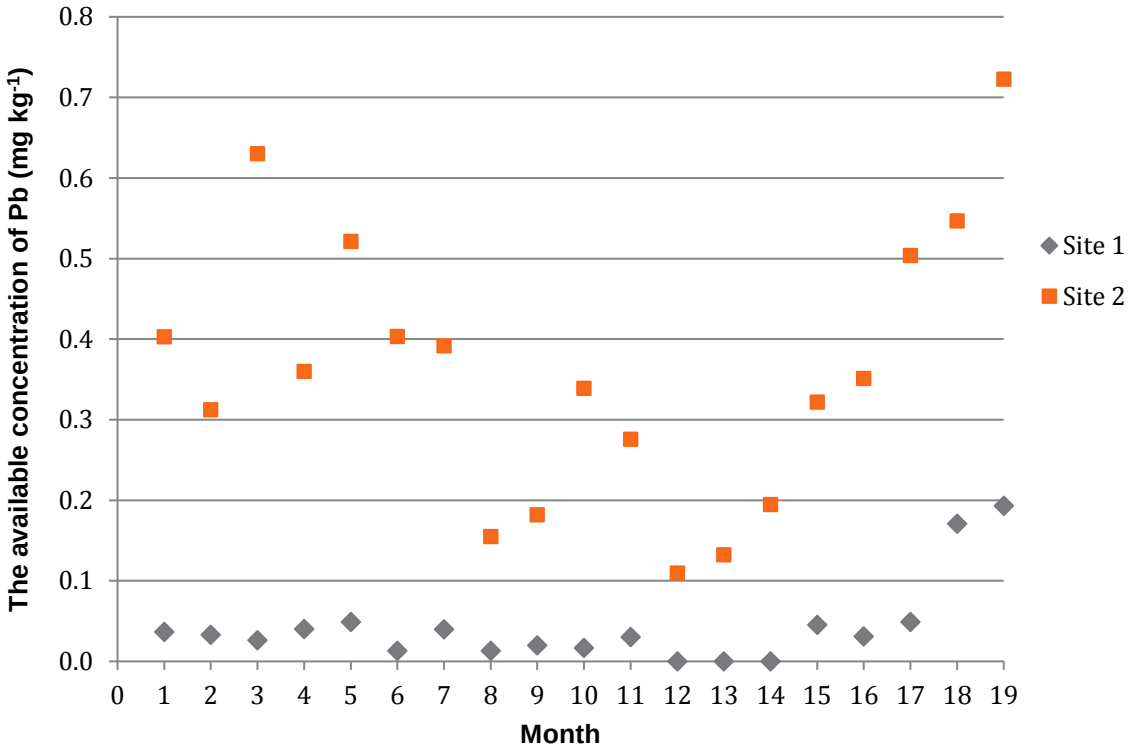
Numbering of 1-19 in horizontal axis represents for 19 sampling months from January 2016 to December 2017.

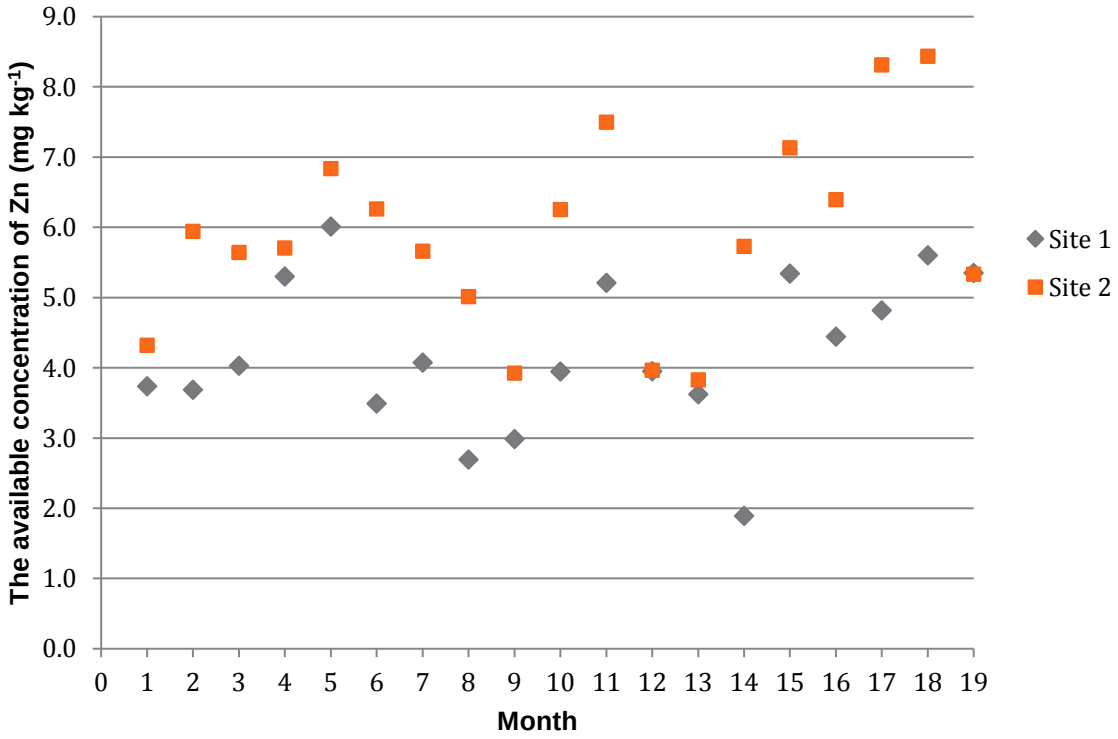





The available concentration of Ni (mg kg-1)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Month
Site 1
Site 2


The available concentration of Cu (mg kg-1)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Month
Site 1
Site 2

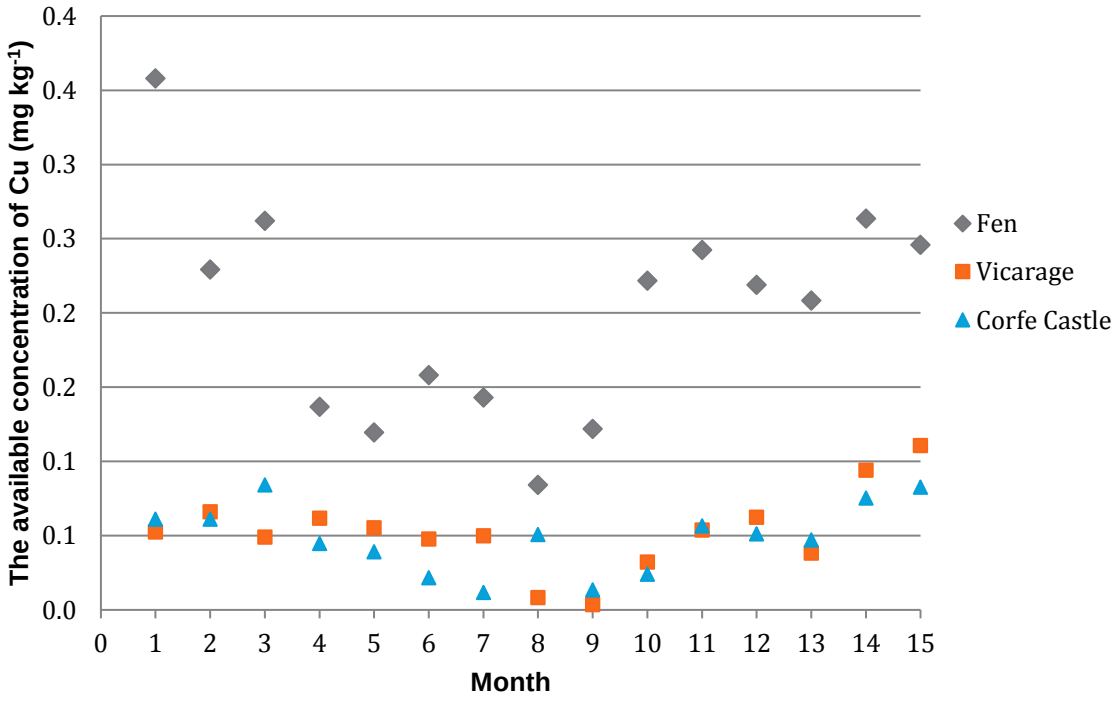

The available concentration of Pb (mg kg-1)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Month
Site 1
Site 2

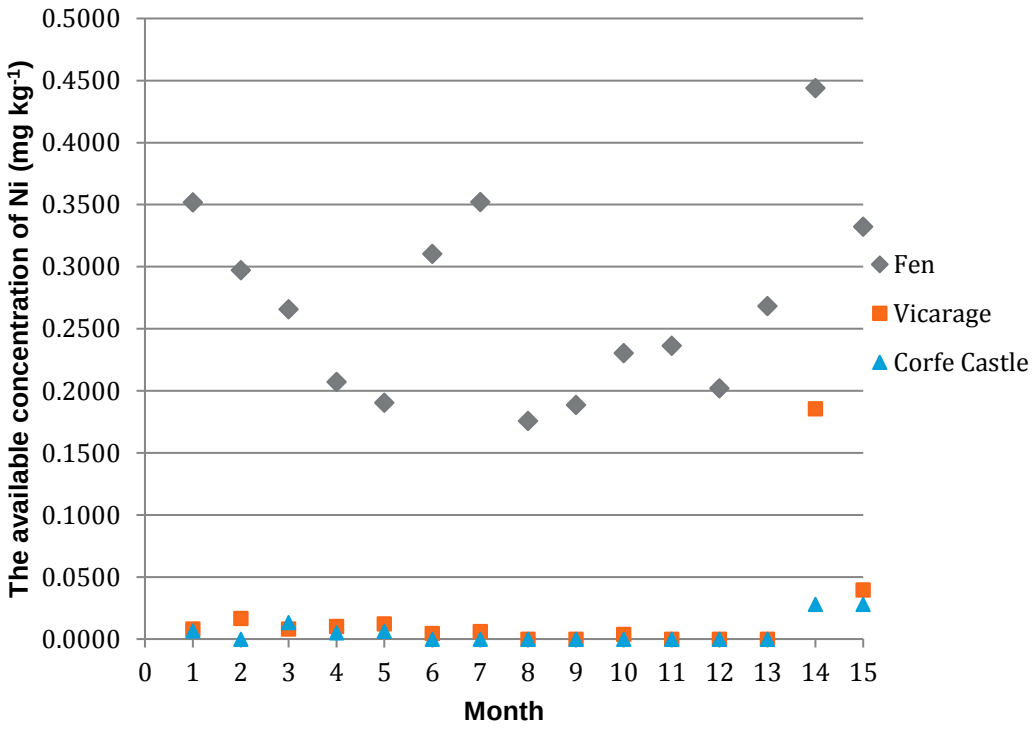

The available concentration of Zn (mg kg-1)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Month
Site 1
Site 2

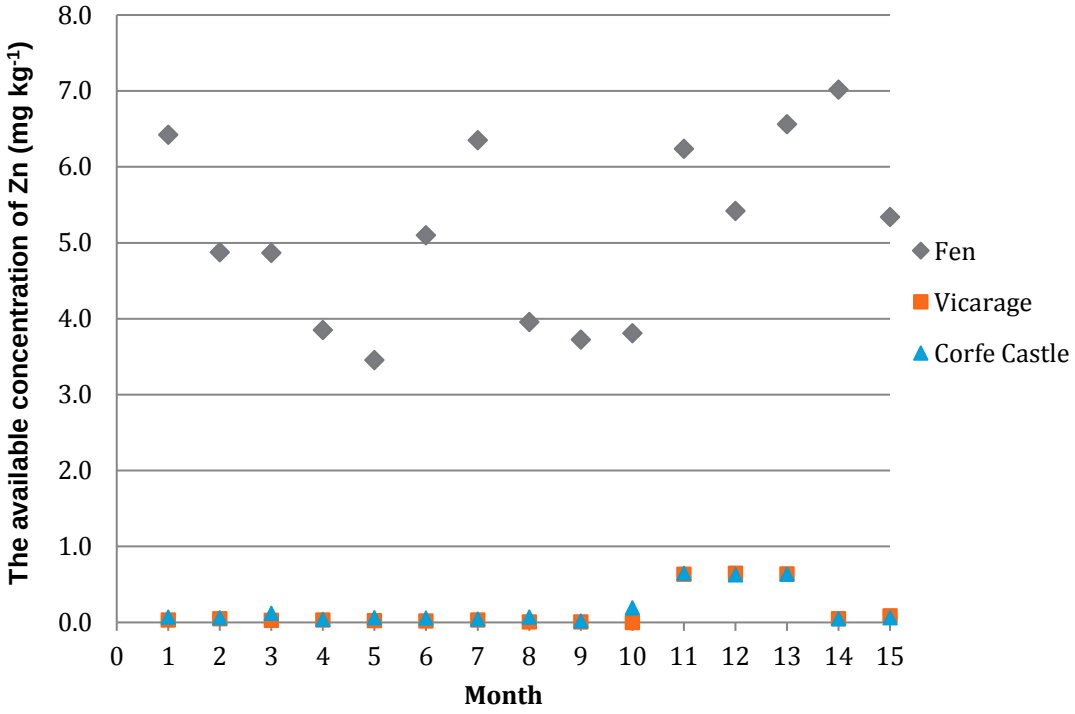
Appendix B

Changes in the available concentrations of trace metals in three ecosystems during the time investigated.

Numbering of 1-15 in horizontal axis represents for 15 sampling months from May 2016 to December 2017.






8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
The available concentration of Zn (mg kg-1)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Month
Fen
Vicarage
Corfe Castle