The Soil Life Cycle:

Food webs and ecosystem services during soil transformations

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Abstract

Soil is one of the most important natural resource for life on Earth and provides important ecosystem services, such as food production, carbon sequestration, water regulation and contaminant attenuation. Soil quality, defined as the soil's ability to provide these services, is drastically reduced in many locations and regions worldwide due to human activities. This loss in soil quality ultimately leads to soil degradation, erosion and desertification, imposing a severe and increasing risk for the growing human population. It is therefore essential that we are aware of the importance of protecting soil, and at the same time understand processes that build up and regenerate soil. The key objective of the present study was to obtain a better understanding of soil food web structure and functioning, and how these develop in stages along the soil life cycle. Using field surveys, I investigated the soil food web structure and functioning in different sites along the soil life cycle, including soils developing in glacial chronosequences, productive soils under different land use and management, and soils under risk of degradation.

The soil food web was expected to build up in biomass and structure, be highest in the intermediate soils, and decrease in soils at or nearby degradation. This was indeed the case when comparing developing soils in the chronosequences, and comparing productive soils with degrading soils. But also land use type turned out to be very important for the structure of the soil food web. Biological measures such as biomass, activity and diversity of soil organisms, especially that of soil microarthropods, were found to be indicative for soil quality in all sites.

I also investigated the possible role of soil organisms in the soil ecosystem functioning, in terms of soil structure formation and C and N mineralisation. Although soil organisms are known to have an important role on soil structure formation, no clear indications of such a role were found for that function in the studied sites. However, soil microbial biomass and activity, and the biomass of other trophic groups, did play a crucial role in soil ecosystem process rates, especially the C and N mineralisation rates.

In conclusion, I have found that soil food webs assemble in a directive manner: organism biomass and activity increase with soil productivity. In productive soils, land use type and land management are the main drivers affecting soil food web structure and functioning, although this effect is limited to the topsoil. Under harsh conditions, soil organisms reach a relatively low biomass and are sensitive to aspects of intensive agricultural land use.

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Chapter 1

General introduction: The soil life cycle

Jeroen P. van Leeuwen

1.1 Introduction

Soil is one of the most important natural resources for life on Earth and provides important ecosystem services, such as the production of food, feed and fibre, climate regulation through sequestration of carbon, purification and regulation of water and attenuation of contaminants (Costanza et al., 1997). Soil forms very slowly while it erodes up to a hundred times faster on the majority of farmland and in urban areas, making soil a finite resource (Banwart et al., 2011). Soil quality, here defined as the soils' ability to deliver ecosystem services, is being drastically reduced in many locations worldwide, mostly due to human activities such as intensive agriculture, commercial forestry and urban development. The decrease in soil quality ultimately leads to soil degradation, erosion, and desertification (Milgroom et al., 2007) and imposes a severe and increasing risk for human populations. It is therefore essential that we are aware of the importance of protecting soil, and at the same time understand processes that build up and regenerate soil.

1.2 SoilTrEC: understanding soil processes

In order to come up with effective strategies to protect and enhance soil quality, the Critical Zone Observatory (CZO) network was established across the USA and Europe (Anderson et al., 2008). The CZO network is an internationally coordinated, interdisciplinary research effort aiming for a better understanding of the chemical, physical and biological processes that shape the Earth's surface and support terrestrial life.

As part of the CZO research effort, the European Commission has provided funding for a large multi-disciplinary research project: Soil Transformations in European Catchments (SoilTrEC). This project aims to describe and quantify the physical, chemical, and biological processes that are critical to soil ecosystem functions (Banwart et al., 2011; Menon et al., 2014). The European CZO network studied in the SoilTrEC project consists of sites along the soil life cycle gradient (Figure 1.1). Included are natural sites along soil formation gradients (Switzerland, Iceland), productive agricultural and forested sites differing in soil management (Austria, Iceland, Czech Republic), and agricultural sites under risk of degradation (Greece) (Banwart et al., 2011; Menon et al., 2014).

These sites were selected involving different environmental factors. Each research site targets its own research aim. The glacial forefields in Switzerland and Iceland allow the study of incipient soil ecosystem development at sites where the retreating glaciers expose the underlying bedrock to weathering. Studying the productive soils in Austria, Iceland and Czech Republic enables assessment of the effects of land use and management on soil processes and soil quality in agricultural and forested

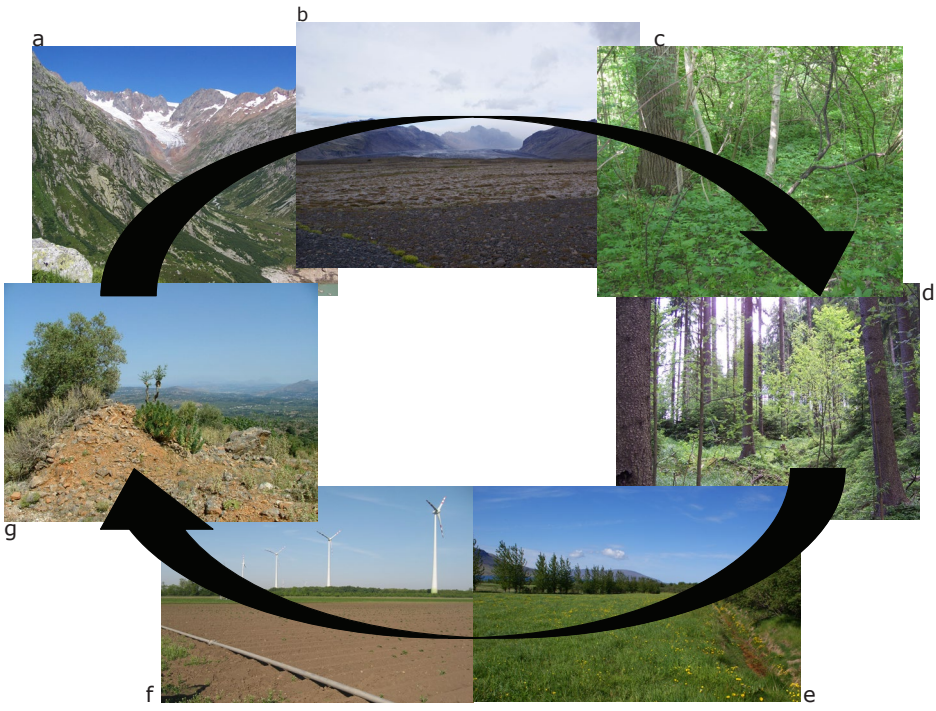

Figure 1.1 The soil life cycle, from newly formed soils in glacial forefield in Switzerland (a) and Iceland (b), via productive soils under forestry in Austria (c) and Czech Republic (d), and under agriculture in Iceland (e) and Austria (f), to soils under risk of degradation in semi-arid Crete (g).

soils. The sites in Crete allow the study of processes in degraded soils that are under threat of desertification due to millennia of intensive land use. This thesis is based on the soil biological measurements performed in these European CZOs.

1.3 Soil organic matter and soil structure formation

Soil organic matter (SOM) is an essential component of soil quality, governing processes like carbon sequestration, nutrient cycling, water retention, and soil aggregate turnover. For the global carbon (C) balance, the soil is highly significant: Currently more than two thirds of organic C in terrestrial ecosystems is stored in SOM, while yearly 60 Pg C (in the form of CO_2) is respired from the soil (Six et al., 2006). SOM dynamics are driven by land use through root-turnover, deposition of plant residues, and decomposition by the soil microbial populations. Soil organisms are known to play an important role in SOM dynamics (Brussaard et al., 2007; de Ruiter et al., 1994; Lavelle et al., 2006; Wardle et al., 2004). The decomposition of SOM by microbes mineralises C and nutrients like nitrogen (N), making these available for plant uptake. To understand the role of soil organisms in decomposition processes, SOM has been

defined in terms of fractions based on decomposability (Golchin et al., 1994). The idea behind this fractioning is that the labile fractions, such as dissolved and particulate organic matter, are better available for biological decomposition, contribute more to soil structure formation, and are more sensitive to soil management than more stable fractions such as lignin (Beare et al., 1994; Tisdall and Oades, 1982).

SOM dynamics are strongly linked to soil aggregate turnover. Incorporation of SOM into the soil aggregates "protects" it from microbial decomposition, thereby stabilizing SOM content and sequestering carbon in the soil (Golchin et al., 1994). When aggregates are disturbed (e.g. due to tillage), C and N mineralisation rates often increase as a result of increased bioavailability of SOM (Six et al., 2006). Furthermore, soil aggregates are important for infiltration of water and avoiding soil erosion (Caesar-TonThat et al., 2010). Finally, soil aggregates and the pores between the aggregates provide space, water, and oxygen, thereby creating habitats for a large diversity in soil organisms (Anderson, 1978; Sulkava and Huhta, 1998).

1.4 Importance of soil organisms

In soil ecosystem development and functioning, soil microbial populations play an important twofold role. Firstly, soil microbes produce exudates that enhance aggregation of soil particles (De Gryze et al., 2005; Tisdall and Oades, 1982; Wright et al., 2007). Arbuscular mycorrhizal fungi produce glomalin, a glycoprotein, which promotes the abundance and stability of water-stable aggregates (Abiven et al., 2007; Six et al., 2006; Wright et al., 2007). Also saprotrophic fungi and bacteria produce extracellular polymeric substances that can bind soil particles into stable aggregates (Caesar-TonThat et al., 2011; Vogel et al., 2014). Besides the production of adhesive polysaccharides, fungal hyphae also physically enmesh soil particles (Bossuyt et al., 2001). Although the biological mechanisms behind soil formation are well-recognized, the relation between microbial biomass and activity, microbially derived C and aggregate formation and stability has not yet been quantified (Helfrich et al., 2008).

Secondly, microbial organisms determine the dynamics of soil organic C and N, and hereby link belowground processes and aboveground vegetation growth. While microbial mineralisation provides the nutrients for vegetation development (Barea et al., 2005), vegetation promotes microbial biomass in return through the supply of plant residues, root exudates and root turn-over (Ohtonen et al., 1999).

In addition to soil microbes, soil fauna has been increasingly recognized for its important role in soil ecosystem functioning and the provision of soil ecosystem services (Brussaard et al., 1997; Brussaard et al., 2007; Lavelle et al., 2006). The soil fauna plays a role in creating a stable soil

pore structure through moving in the soil and the formation of faecal pellets (Jastrow and Miller, 1991; Lavelle et al., 2006; Lee and Foster, 1991; Oades, 1993). Soil fauna also facilitates the decomposition of organic matter by microbes through shredding of larger particles, which increases the availability and reactive surface of SOM. In addition, grazing by microbivores such as protozoans and nematodes may stimulate microbial activity (Brussaard et al., 1997; Buchan et al., 2013). Apart from influencing microbial activity and hence decomposition of soil organic matter, soil fauna can also contribute directly to soil ecosystem functioning through trophic activities in the soil food web (de Ruiter et al., 1993; Verhoef and Brussaard, 1990).

From a different perspective, the soil as habitat for species rich communities has received increased attention emphasizing both the intrinsic and the functional value of soil biodiversity. High levels of biodiversity are thought to enhance stability of soil functions such as nutrient cycling and decomposition of SOM against perturbations and disturbances, and aid in the suppression of soil-borne pests and diseases (Altieri, 1999; Barrios, 2007; Griffiths et al., 2000). Soil biodiversity is also recognised as a sensitive biological indicator for effects of environmental change and disturbance (Pattison et al., 2008; Ponge et al., 2006; Ritz et al., 2009; Wardle, 1995). One of the key indicator groups is the group of soil microarthropods, because microarthropods are abundant, functionally diverse, and respond to a variety of ecological and environmental factors (Gardi and Parisi, 2002; Parisi et al., 2005). Also microbial biomass and activity are regularly used as indicators for soil ecosystem functioning (Brussaard et al., 2007; van Eekeren et al., 2009).

1.5 Soil food web characteristics

Many studies have approached ecosystem services such as nutrient cycling by analysing the structure and functioning of soil food webs (de Ruiter et al., 1994; Holtkamp et al., 2008; Hunt et al., 1987). These food web studies quantify the material flows among trophic groups in the soil food web (Figure 1.2). In this way, estimates are obtained of the contribution of a particular group on the functioning of other groups and the whole soil food web (de Ruiter et al., 1994; Sackett et al., 2010). Given the large biological diversity in soils, soil food web models are constructed in terms of 'functional' or 'trophic groups'. These groups are defined on the basis of taxonomy, diet and life-history traits, following the method of Moore et al. (1988).

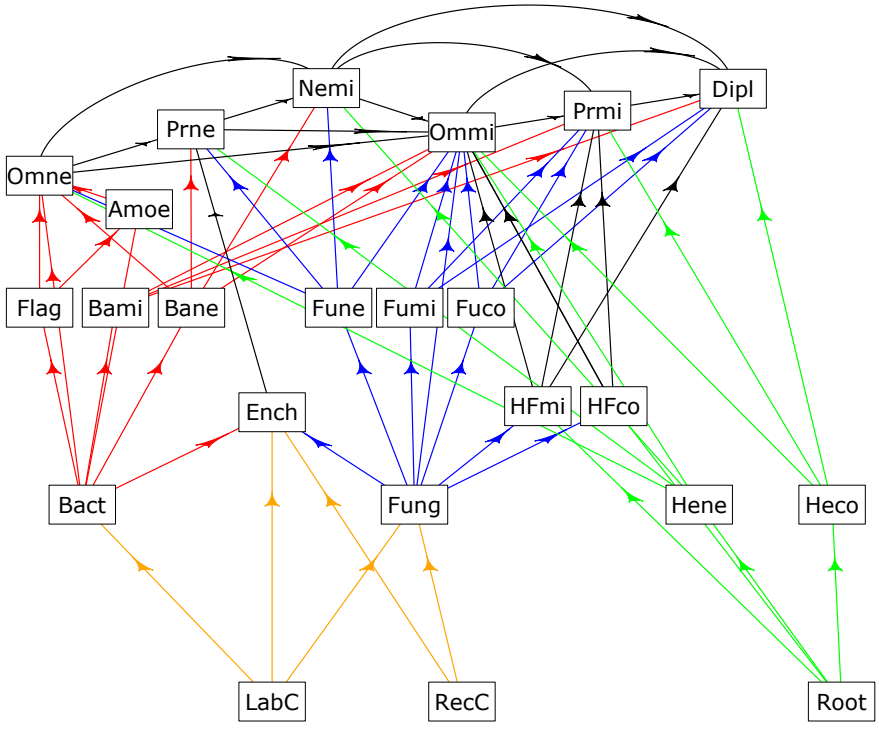

Figure 1.2 Soil food web diagram representative for the farms in chapter 3. Boxes represent the presence of trophic groups in the soil food web. Arrows represent feeding interactions, colour represents feeding source (yellow: detritivorous, green: herbivorous, red: bacterivorous, blue: fungivorous, black: omnivorous). Included soil organisms: Diplura (Dipl), Predaceous Mites (Prmi), Omnivore Mites (Ommi), Nematovore Mites (Nemi), Predaceous Nematodes (Prne), Omnivore Nematodes (Omne), Amoebae (Amoe), Flagellates (Flag), Bacterivore Mites (Bami), Bacterivore Nematodes (Bane), Fungivore Nematodes (Fune), Fungivore Mites (Fumi), Fungivore Collembola (Fuco), Enchytraeids (Ench), Herbofungivore Mites (HFmi), Herbofungivore Collembola (HFCo), Herbivore Nematodes (Hene), Herbivore Collembola (Heco), Bacteria (Bact), Fungi (Fung), Labile C (LabC), Recalcitrant C (RecC) and Plant Roots (Root).

Changes in soil food webs, e.g. due to soil management or environmental change, have been shown to alter C and nutrient cycling rates, with important implications for plant growth, nutrient use efficiency and C sequestration (Andrén et al., 1990; Brussaard et al., 1997; Hendrix et al., 1986; Holtkamp et al., 2011; Kardol and Wardle, 2010). However, relations between soil food webs and soil ecosystem functioning are highly context dependent (Bardgett, 2005; Bezemer et al., 2010). The present study has used the food web approach in the context of soil age and land use, and has linked food web structure and functioning to abiotic variables, such as C and N content, pH and soil aggregate structure.

1.6 Objectives and approach

The key objective of the present study is to obtain a better understanding of soil food web structure and functioning, and how soil food web structure and functioning will develop during soil formation and degradation under various land use types. These processes have been extensively approached from an abiotic perspective, with emphasis on soil structure, water erosion and nutrient cycling (Celik, 2005; Kosmas et al., 1997; Solomon et al., 2000). Soil organisms received less attention, and the studies that have been done often focused on single groups of organisms, such as microbes (Bending et al., 2004), nematodes (Ferris et al., 2001), or microarthropods (Hodkinson et al., 2003; Kaufmann, 2001), instead of on the level of food web structure. Although the importance of biology in soil quality and fertility is more and more acknowledged (Brussaard et al., 2007; Cole et al., 2006; De Deyn et al., 2003; Lavelle et al., 2006), soil organisms have mostly been studied in productive agricultural fields (Birkhofer et al., 2008; Carpenter-Boggs et al., 2000; Doran and Zeiss, 2000) or in well-developed natural ecosystems (De Deyn et al., 2003; Schröter et al., 2003), but not during initial soil formation stages or in degraded soils. Therefore, the main research question of my thesis is: "How do soil food webs, and the ecosystem services they provide, respond to changes in land use at various stages of the soil life cycle?" To answer this question, I have formulated some more detailed objectives, each focusing on a particular stage of the soil life cycle:

1. To investigate soil food web assembly during early soil formation and vegetation succession.
2. To investigate soil food web structure and functioning under different agricultural practices in productive soils.
3. To investigate the soil microbial community structure and activity in different land uses and at different soil depths.
4. To investigate soil food web structure and functioning in soils that are considered to be at risk of degradation and desertification.

In order to meet these objectives, I have carried out field surveys in the CZO network in the context of the EU-FP7 SoilTrEC project. The CZOs encompass different land use types and gradients in soil formation and degradation. In the field surveys the complete soil food webs are sampled, based on measurements of presence and abundance of soil microbes (bacteria and fungi) and soil fauna (protozoans, nematodes, enchytraeids and microarthropods), representing the main taxonomic groups and trophic levels in the soil food web. In addition, in most sites the taxonomic richness and diversity within the group of microarthropods are measured.

Soil physical and chemical measurements include soil aggregate size fractions, soil organic matter contents and distribution (based on different organic matter fractions), nutrient contents and soil pH.

1.7 Outline

In this thesis soils and soil food webs are followed through the soil life cycle, starting from freshly formed soils in glacial forefields, via productive soils under agricultural land use, to soils under threat of degradation.

In chapter 2, I analyse soil food web development during soil formation along retreating glaciers in Iceland and Switzerland at the start of the soil life cycle. Glaciers are retreating due to the temperature rise of the last decades and thereby provide natural chronosequences in soil formation and weathering. In these chronosequences, I investigate soil food web assembly in terms of biomass, complexity and stability. To relate soil food web assembly to soil ecosystem development and functioning, I also measure soil physicochemical parameters, as well as vegetation cover and plant species richness.

When soils are fully developed and productive, land use and management play an important role in the soil's ability to provide ecosystem services. Especially intensive agricultural production can be an important driver for the loss of soil quality. In Chapter 3 I present a soil quality assessment for agricultural sites under different farming managements in Iceland and Austria, comparing the soil food webs in conventionally and organically farmed fields. Biological, physical, and chemical soil quality parameters are investigated, focusing on soil structure formation, soil organic matter dynamics and nutrient cycling, and the soil as a habitat for biotic communities.

Even in highly productive soils, the effects of land use on the role of soil biota in the soil's processes, including soil formation, are not well understood. Different land use types not only have an effect on the topsoil, but effects can continue in the deeper layers of the soil profile. Also the microbes in the soil subsurface potentially play an important role in soil biochemical processes. Therefore, I assess the effects of different land use types on soil microbiological biomass, activity and community structure at different soil depth layers in chapter 4.

When intensive agricultural practices are continued for centuries or even millennia in an unsustainable way, especially under harsh conditions, soils can become seriously threatened by soil degradation, leading to the loss of soil quality, or even the complete erosion of soils. I investigate biological soil properties in agricultural soils under risk of degradation

in semi-arid conditions in Crete (Greece) in chapter 5. Soil food web structure and functioning in relation to soil structure, soil organic matter, nutrient availability, and soil as a habitat for species-rich communities are analysed.

This thesis concludes with a general discussion in chapter 6. In this final chapter, I synthesize the findings and place them in the context of scientific literature. I discuss the characteristics of soil food webs and their role in ecosystem functioning during the soil life cycle, taking together the results of chapters 2-5. Finally, I also evaluate the use of biological parameters for the assessment of soil quality over the range of sites studied.

Chapter 2

Soil food web assembly in glacial chronosequences in Iceland and Switzerland

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J.P. van Leeuwen, G.J. Lair, G. Gísladóttir, S.M. Bernasconi , T.M. Lehtinen, J. Bloem, L. Hemerik, P.C. de Ruiter. Soil food web assembly in glacial chronosequences in Iceland and Switzerland

Abstract

Due to human activities, soil quality in terms of the soil's ability to deliver ecosystem services is under threat worldwide. It is therefore essential that we are aware of the importance of protecting soil, and at the same time understand processes that build up and regenerate soil. Studying natural soil ecosystem development is the first step in understanding these processes, and at the same time fundamental for developing ecological theory. The present paper investigates how the community of soil organisms, i.e. the soil food web, develops along soil formation in chronosequences in two glacier forefields in Iceland and Switzerland. We hypothesised that along with increases in nutrient content, vegetation cover and plant species richness, soil food webs in glacial forefields show increases in biomass, diversity, complexity and stability with successional age. We indeed found that soil organisms increased in biomass and diversity with age, although some of the groups of soil organisms peaked at an intermediate successional stage. In contrast to our expectations, some of the calculated food web metrics such as the number of trophic groups, trophic chain length and complexity, did not increase linearly, but showed an intermediate peak or even decreased, whereas food web stability showed no consistent trend with successional age. In line with our expectations all measured carbon and nitrogen pools, vegetation cover and plant species richness increased with age in the two glacial forefields. Summarising, our study revealed that all components of terrestrial ecosystems (soil formation, vegetation development and soil food web assembly) occur in concert, and along stable pathways of ecosystem development.

2.1 Introduction

Soil is the most important natural resource for life on Earth after water, and provides important ecosystem services, such as food and fibre production, carbon sequestration and nutrient cycling. Soil forms very slowly, at the rate of several millimetres per 100 years, but erodes much faster on the majority of farmland and in urban areas. Additionally, soil quality in terms of the soil's ability to deliver ecosystem services is under threat worldwide due to human activities. It is therefore essential that we are aware of the importance of protecting soils and soil quality, and at the same time understand processes that build up and regenerate soil. One way to do this is by studying the processes underlying soil formation in soils developing without human interferences.

The present study focuses on soil ecosystem development in natural chronosequences, with a special attention to the development of the soil biological community. In soil ecosystem development, soil microbial populations play an important twofold role. Firstly, they influence soil formation chemically by producing exudates (polysaccharides) and physically by enmeshing soil through fungal hyphae, both enhancing aggregation of soil particles (De Gryze et al., 2005; Wright et al., 2007). Secondly, microbial organisms decompose soil organic carbon and mineralise nutrients, and hereby link belowground processes and aboveground vegetation growth.

In addition to soil microbes, soil fauna has been increasingly recognized for their important role in soil ecosystem functioning and the provision of soil ecosystem services (Brussaard et al., 1997; Lavelle et al., 2006). Much less is known about how soil food webs, i.e. the communities of soil organisms, assemble and develop during soil formation. Soil food web assembly has been studied in chronosequences of primary succession (Neutel et al., 2007; Wardle et al., 1995) and secondary succession (Holtkamp et al., 2008; Korthals et al., 2001), but these studies either did not relate soil food webs with soil ecosystem formation or were hampered with a legacy from the previous agricultural land use, creating a time-lag between above- and belowground succession.

In the present study we analysed soil food web development during soil formation along retreating glaciers. Glaciers are retreating due to the temperature rise of the last decades and provide natural chronosequences in soil formation and weathering (Egli et al., 2001; Milner et al., 2009; Schmalenberger and Noll, 2010; Stevens and Walker, 1970). Chronosequences are considered to be sequences of soils, developed on similar parent materials and relief, under the influence of constant, or ineffectively varying, climate and biotic factors. Differences between these soils can thus be ascribed to the laps of differing increments of

time since the initiation of soil formation (Huggett, 1998). This reasoning makes glacier forefields model systems for studying soil formation and the concomitant colonization and succession of above- and belowground organisms (Hämmerli et al., 2007; Ingimarsdóttir et al., 2012; Noll and Wellinger, 2008).

Soil organisms have been studied in glacier forefield chronosequences, but a complete overview of soil ecosystem functioning and food web development is still lacking. Some studies focused on microbes, showing that microbial populations increase in biomass and growth efficiency with soil age (Hämmerli et al., 2007; Insam and Haselwandter, 1989; Lazzaro et al., 2009; Ohtonen et al., 1999; Rime et al., 2015; Sigler and Zeyer, 2002). Studies that have looked at other organisms than microbes in glacier forefields focused mostly on single clades of organisms, especially microarthropods (Hågvar et al., 2009; Hodkinson et al., 2003; Kaufmann, 2001).

Understanding soil food web development during the process of soil formation is fundamental for both soil conservation and ecological theory (Neutel et al., 2007). Following the hypotheses posed by Odum (1969) and the observations on soil food webs done in primary chronosequences in sand dunes by Neutel et al. (2007), and on microbial organisms in glacial forefields by Ohtonen et al. (1999), we expected that soil food webs will increase in number of trophic groups, biomass, diversity, complexity and stability, together with increases in soil nutrient contents, vegetation cover and plant species richness.

To test these hypotheses, we investigated soil food webs in terms of the presence and abundance of microbes (bacteria, fungi) and soil fauna (protozoa, nematodes and microarthropods), representing the main taxonomic groups and trophic levels in soil food webs. In addition, we measured the taxonomic richness and diversity within the group of soil microarthropods. From these measurements, various food web metrics were calculated, i.e. trophic chain length, connectance, link density, complexity and stability. To evaluate soil food web assembly in relation to soil ecosystem development and functioning, soils were characterized in terms of soil pH, C and N pools, mineralisation rates, and vegetation cover and plant species richness were measured. The study was carried out in two glacier forefields in contrasting landscapes (due to climate and parent material) in Iceland and Switzerland.

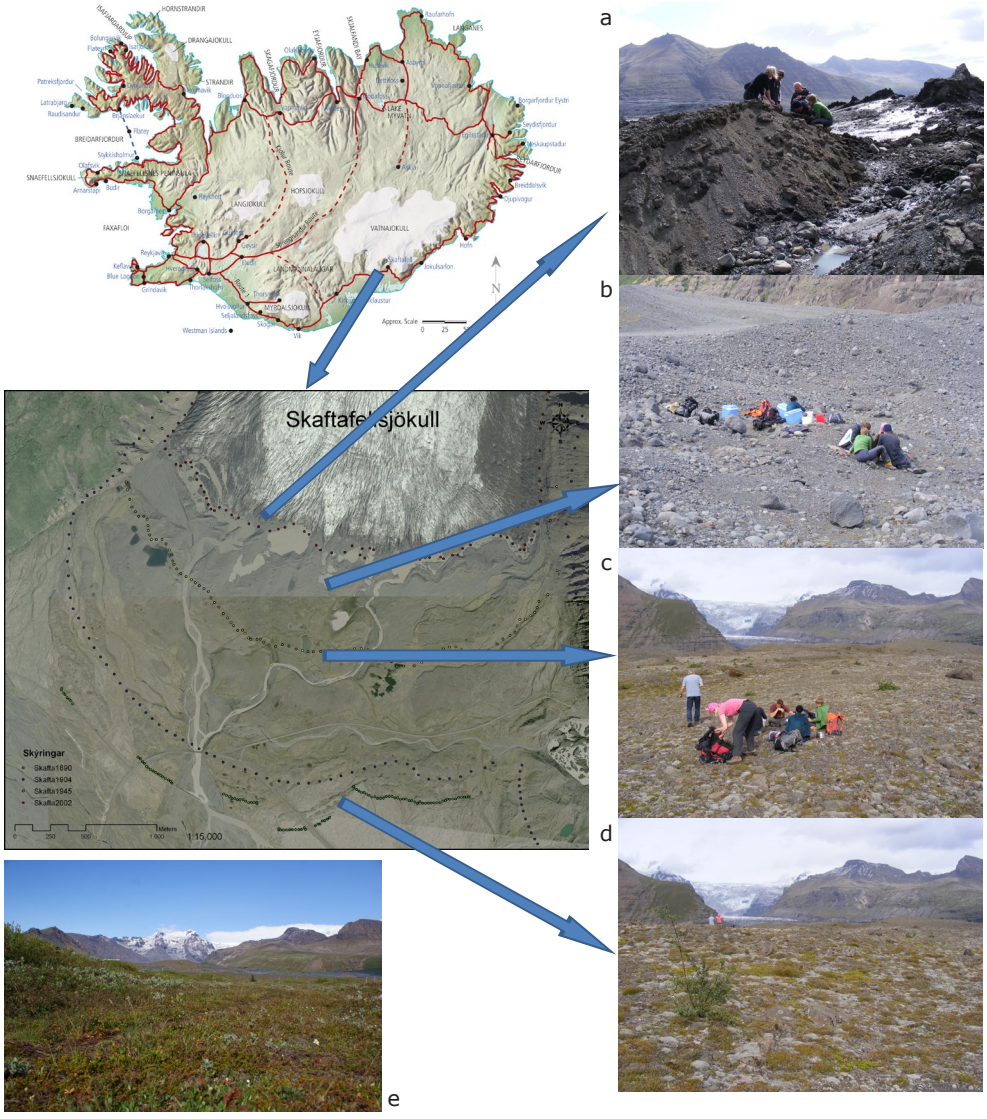

Figure 2.1 Proglacial area of the Skaftafelljökull in southeast Iceland. Samples were taken of soils of 1(a), 30(b), 65(c) and 120(d) years old, and from a reference site of about 500 years old (e).

2.2 Methods

2.2.1 Site description

The proglacial area of Skaftafellsjökull (abb. S, Iceland, Figure 2.1) is an outlet glacier extending from the Vatnajökull ice cap down south to the lowlands, with an elevation of 90-120 m asl. The climate is cold-temperate oceanic with an average annual temperature of 5°C, and a yearly precipitation of 1800 mm (Vilmundardóttir et al., 2014). Since 1890, the outlet glaciers south of Vatnajökull have retreated up to 5 km, exposing poorly sorted sediments, while leaving behind moraines resulting from short glacier advances in 1890 (S4), 1945 (S3), 1980 (S2) and 2010 (S1, on the edge of the glacier) (Hannesdóttir et al., 2015; Vilmundardóttir et al., 2014). Samples were taken on top of these moraines, to prevent influences of erosion and sedimentation fluxes by glacial creeks, and for certainty of the age of the soil material. In addition, we sampled a reference site on a Leptosol further from the glacier hosting a natural climax vegetation (S5). The glacial retreat has escalated rapidly over the past decade (averaging about 100 m yr^{-1}). Parent material consists mainly of basaltic lava and hyaloclastite. Also tephra, deposited by volcanic eruptions, is a substantial component of the soils (Vilmundardóttir et al., 2014). The proglacial area has been traditionally grazed before the establishment of Skaftafell National Park in 1967.

The Damma Glacier Critical Zone Observatory (abb. D, Switzerland, Figure 2.2) (Bernasconi, 2014), located in the central Alps, is a 9.9 km^2 catchment with an elevation range between 1940 and 3630 m asl, 50% of which is glaciated. The climate is cold-temperate continental, with an average annual temperature of 1°C and a yearly precipitation of 2400 mm (Bernasconi, 2014). Since 1850, the retreat of the glacier has formed a soil chronosequence on a relatively flat area of about 1 km length at an altitude between 1950 and 2050 m asl (Bernasconi et al., 2011). The youngest sites include soils from 2 to 15 years old (D1), the intermediate group comprises locations freed from the ice between 1930 and 1950 (D2) and the third group soils started to form between 1870 and 1897 (D3). A grassland site on a more than 3000 year old Cambisol is included as reference site (D4). The bedrock in the glacial forefield is composed of relatively homogeneous metamorphic granite. Vegetation evolves from scattered pioneer plants in the young soils to a well-developed vegetation cover with grasses and shrubs (Bernasconi et al., 2011). Some minor sheep grazing occurs in an otherwise pristine environment.

2.2.2 Soil and vegetation sampling

Samples from the topsoil (0-5 cm) were taken in June 2011 in Skaftafelljökull and June 2010 in Damma Glacier. At each site (age)

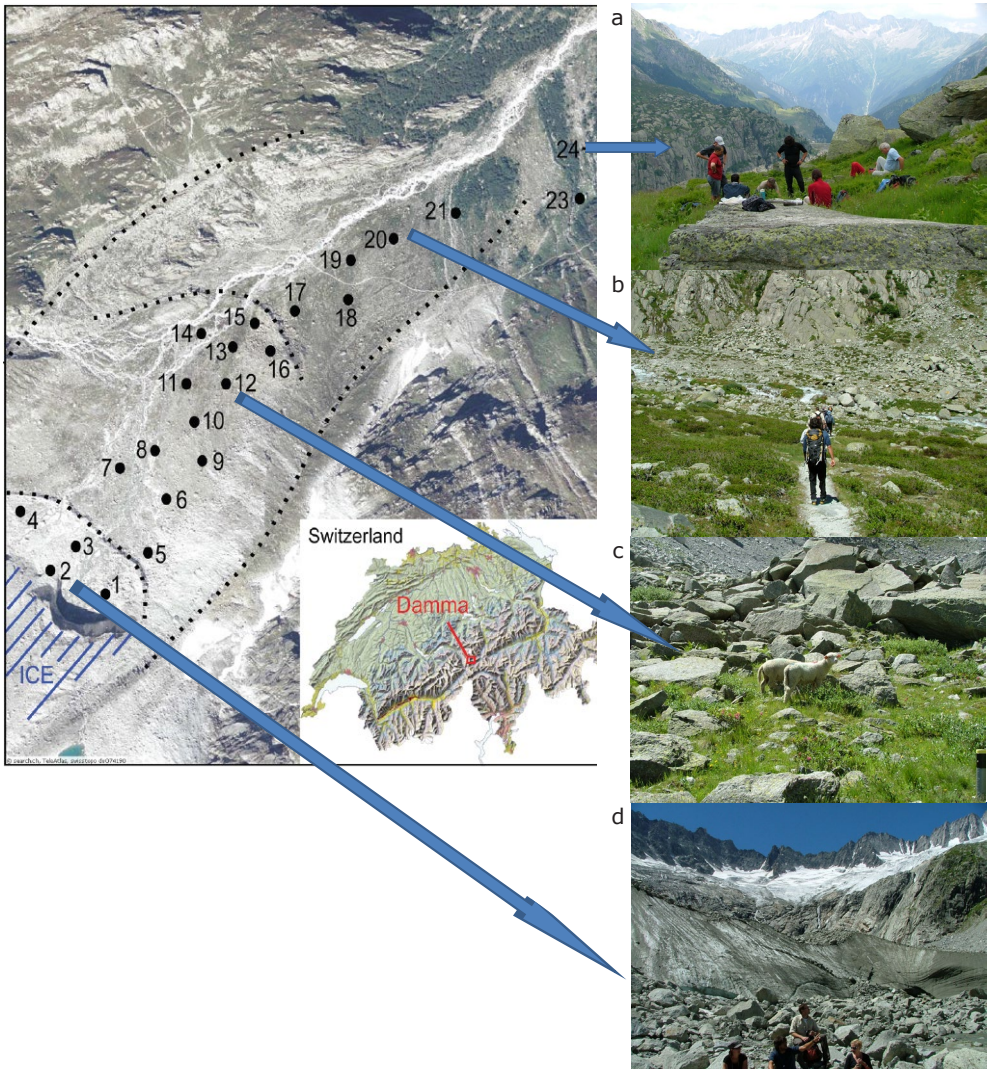

Figure 2.2 Proglacial area of the Damma Glacier in Switzerland. Samples were taken of soils of 10(d), 70(c) and 110(b) years old, and from a reference site of more than 3000 years old(a). Photos: Jaap Bloem.

three plots separated by 30-40 m were established. In Skaftafell we used a shovel to take mixed soil samples (ca. 1 kg) for microbial (bacteria, fungi), microfaunal (protozoa, nematodes), soil chemical and physical measurements, and small extractors (19.5 cm^3) for bulk density determination (cf. Vilmundardóttir et al. (2014)). In Damma, we took mixed soil samples (ca. 1 kg) from the edge of a soil profile pit for microbial (bacteria, fungi), microfaunal (protozoa, nematodes) and soil chemical and physical measurements, and used a 5 cm diameter corer for the mesofauna (enchytraeids and microarthropods). The Icelandic soils and the first stage of the Damma Glacier forefield in Switzerland did not allow for sampling of the microarthropods because of the gravelly nature of the soils.

Vegetation cover and composition were measured in 0.50 x 0.50 m quadrants at all plots using the Braun-Blanquet scale in Skaftafell, while for Damma vegetation data was taken from an earlier study at the same locations (Bernasconi et al., 2011; Zumsteg et al., 2012).

2.2.3 Soil physicochemical measurements

Soil particle size distribution (clay content) was determined with a combined sieve and pipette method for the successional stages in Damma Glacier. Therefore, first organic matter was removed with hydrogen peroxide and dispersd by reciprocal shaking with sodium metaphosphate solution for 12 h (Burt, 1992). Soil pH was measured electrochemically (Microprocessor pH Meter pH196 WTW, Weilheim, Germany) in H_2O at a soil:solution ratio of 1:2.5 (Burt, 1992). Total carbon (TC) and nitrogen (TN) in bulk soil were quantified by dry combustion using an elemental analyser (Carlo Erba N 1500 analyser). For the analysis, 5 g of sieved (<2 mm) soil without visible roots and litter was ground to size < 63 µm for homogenization and thereof 1–1.5 mg soil was used for the analysis. Total organic carbon (TOC) was calculated as the difference of total and inorganic C, measured as carbonate C by treating 0.5–2 g of fine-ground soil material with 10% HCl acid and quantifying the evolved CO_2. Hot-water-extractable carbon (HWC) was measured as the C present in solution after 16 h at 80°C, while water-soluble carbon (WSC) was measured after 30 min at 20°C (Ghani et al., 2003). Labile carbon was defined as HWC, while recalcitrant carbon was determined as the difference between TOC and labile carbon. Potentially mineralisable nitrogen (PMN) was measured as the increase in NH4 during 1 week of anoxic incubation in slurry at 40°C (Canali and Benedetti, 2006). Potential carbon and nitrogen mineralisation were measured by incubation of 200 g of homogenised and sieved soil for 6 weeks at 20°C (Bloem et al., 1994). Results of the first week (disturbance) were not used. N mineralisation was calculated from the increase in mineral N (nitrate and ammonium) between week 1

and week 6. Total concentrations of O_2 and CO_2 were measured weekly using a gas chromatograph (Carlo Erba GC 6000) equipped with a hotwire detector (HWD 430) and helium as carrier gas, and weekly respiration rates were calculated from that. Only bottles in which O_2 concentration dropped below 15% within the 6-week period, were flushed and reset to environmental concentrations to prevent O_2 limitation. For the statistical analyses, we took the average of weekly rates over the 5-week period after the first week.

2.2.4 Soil biological measurements

Soil biological measurements included the presence and abundance of the major taxonomic groups of soil organisms: microbes (bacteria, fungi) and soil fauna (protozoa, nematodes and microarthropods). Within these taxonomic groups we defined 'trophic groups' based on diet and life-history traits, following the method of Moore et al. (1988). Abundances were transformed into estimates of biomass based on body-size information, and expressed in units of micrograms of carbon per gram dry soil.

Bacterial biomass, fungal biomass, leucine incorporation, and protozoa were measured after a pre-incubation period of 2 weeks at 20°C. Bacterial numbers and cell volumes, and fungal hyphal lengths were measured in microscopic slides (Bloem and Vos, 2004). Bacterial cell numbers and volumes were determined using confocal laser scanning microscopy combined with an image analysis system. The data were transformed into bacterial biomass, taking a specific carbon content of 3.20×10^{-13} g C µm^{-3} (Bloem et al., 1995). For the transformation of fungal hyphal lengths to fungal biomass we described fungal volume as a cylinder with spherical ends ($V = (\pi/4)\ W^2\ (L - W/3)$, where V = volume in µm³, L = length in µm, and W= diameter in µm), with a mean hyphal diameter of 2.5 µm and a specific carbon content of 1.30×10^{-13} g C µm^{-3}. Bacterial growth activity was estimated by measuring incorporation rates of [^{14}C] leucine (Bloem et al., 2006a).

Two trophic groups of protozoa (flagellates and amoebae) were measured using the most-probable-number method (Bloem et al., 1994). Numbers were converted to biomass assuming a spherical shape with diameters of 4.6 µm and 9.1 µm for flagellates and amoebae, respectively, and a volume to C conversion factor of 1×10^{-13} C µm^{-3} (Bloem et al., 1994).

Soil nematodes were counted in 9 mL soil solution extracted by Oostenbrink elutriators from 100 g of soil. Numbers per trophic group (bacterivore, fungivore, herbivore, omnivore, predaceous) were derived from species composition in the samples (Bongers, 1988). Nematode biomasses were calculated using fresh weight data from Didden et al. (1994), and taking a moisture content of 75% and a carbon content of 40% (Didden et al., 1994).

Enchytraeid numbers were obtained through a (wet) extraction using Baermann funnels with increasing light and heat each 30 min after the start of the extraction during a total extraction time of 3 h. Enchytraeid numbers were converted into biomass C by measuring the average fresh weight and taking a moisture content of 85% and a carbon content of 50% of the dry weight (Didden et al., 1994).

Microarthropods were extracted from four soil cores of 196 mL per replicate, during a 1-week period with Tullgren funnels, and processed using the gel-based sub-sample methodology (Jagers op Akkerhuis et al., 2008). Total numbers were recorded, while species composition was assessed in subsamples of 100 individuals following Jagers op Akkerhuis et al. (2008), and references therein. Microarthropod biomass C was calculated based on individual weights, moisture contents and C contents from Didden et al. (1994).

Regarding the taxonomic species richness in the microarthropods in Damma we used three metrics, i.e. the absolute number of taxa present, the Shannon diversity index (H), and the Pielou evenness index (J). For the Shannon diversity index (H) we used the following formula:

$$H = -\sum_{i=1}^{N} (p_i \ln(p_i))$$

in which p_i is the fraction of the total biomass present in species i, i.e. the relative biomass, of species i, and n the total number of taxa present. A higher index value corresponds to a more diverse community. For the Pielou evenness index (J) we used the formula: $J = H / \ln(n)$, in which H represents the Shannon diversity index, and n the total number of taxa present. A higher value for the evenness index corresponds to a more even distribution of the biomass over the taxa present.

Calculation of food web metrics, including connectance, link density, complexity, stability, mean and maximum trophic level and maximum chain length is explained in detail in Appendix 2.1.

2.2.5 Statistics

We analysed the data using a Kruskal-Wallis non-parametric analysis of variance with successional stage as factor, which is the most applicable test due to the low number of replicates. The advantage of using a Kruskal-Wallis test is that due to its robustness, differences found will also be significant when tested using other techniques. Additionally, we used linear and polynomial (quadratic) regression to analyse the relation of food web metrics to age of succession. Principal Component Analysis was used to test the correlation of soil food web data with vegetation composition and C and N measurements. Statistical analyses were carried out using Canoco 5 and R (3.1.3;R Core Team (2015)).

Table 2.1 Soil physicochemical properties and biologically mediated processes at the chronosequences and reference sites studied in Skaftafell (Iceland) and Damma (Switzerland). All values represent mean and standard deviation (between brackets), measured in the topsoil (0-5 cm); [-]: not sampled/tested. The p-values represent significance levels from a Kruskal-Wallis non-parametric analysis of variance with age group as factor, where the superscript letters (within rows, per country) denote statistically significant differences between sites ([a]smallest, [ab]intermediate, [b]highest).

Chronosequence	Skaftafell					Damma				p-value S	p-value D
Stage	1	2	3	4	5	1	3	4	5		
Age	1 yr	30 yr	65 yr	120 yr	ref	10 yr	70 yr	110 yr	ref		
Clay content (%)	[-]	[-]	[-]	[-]	[-]	1.1[a] (0.36)	2.54[ab] (1.43)	2.62[ab] (0.66)	8.03[b] (2.71)	[-]	0.033
pH (H_2O)[1]	8.93	8.06	6.78	6.46	5.71	4.96 (0.38)	4.62 (0.05)	4.41 (0.18)	4.67 (0.14)	[-]	0.139
HWC[2] ($g\ C\ kg^{-1}$)	-0.043[a] (0.015)	-0.001[ab] (0.012)	0.081[ab] (0.038)	0.18[ab] (0.017)	0.62[b] (0.21)	0.034[a] (0.030)	1.33[ab] (0.30)	1.71[ab] (0.19)	9.03[b] (1.81)	0.003	0.019
TOC[3] ($g\ C\ kg^{-1}$)	2.56[ab] (0.18)	1.63[a] (0.12)	3.43[ab] (0.67)	5.96[ab] (0.72)	26.5[b] (11.9)	1.62[a] (0.59)	24.7[ab] (10.7)	60.2[ab] (31.4)	122[b] (11.9)	0.003	0.023
PMN[4] ($mg\ N\ kg^{-1}$)	-0.21[a] (0.072)	0.47[ab] (0.25)	5.05[ab] (2.50)	6.26[ab] (0.60)	19.4[b] (9.01)	2.55[a] (2.35)	53.7[ab] (11.2)	68.0[ab] (6.23)	274[b] (24.7)	0.004	0.019
Total N ($kg\ N\ ha^{-1}$)	0.11[a] (0.006)	0.11[ab] (0.000)	0.17[ab] (0.023)	0.26[ab] (0.015)	0.98[b] (0.36)	0.19[a] (0.031)	1.51[ab] (0.91)	3.13[ab] (1.43)	8.90[b] (3.28)	0.003	0.019
C:N ($g\ C\ g^{-1}\ N$)	24.1[ab] (2.08)	14.8[a] (1.12)	20.4[ab] (1.25)	23.2[ab] (1.50)	27.4[b] (1.90)	8.41[a] (1.78)	15.6[ab] (3.02)	18.1[b] (1.92)	13.3[ab] (1.39)	0.006	0.027
N min[5] ($mg\ N\ kg^{-1}\ yr^{-1}$)	6.59[ab] (2.62)	17.3[b] (10.3)	5.89[ab] (4.20)	1.04[ab] (0)	0.52[a] (0.87)	36.4 (22.5)	230 (296)	64.0 (55.7)	683 (299)	0.006	0.084
C min[6] ($g\ C\ kg^{-1}\ yr^{-1}$)	0.43[ab] (0.010)	0.21[a] (0.038)	0.26[a] (0.081)	0.36[ab] (0.031)	0.59[b] (0.17)	0.14[a] (0.097)	2.72[ab] (0.085)	2.95[ab] (0.34)	8.7[b] (0.17)	0.006	0.022
Vegetation cover (%)[7]	0	5	15	30	100	4	60	90	100	[-]	[-]
Plant sp. richness (#)[8]	0[a] (0)	10[ab] (4.6)	11[ab] (3.5)	15[b] (2.6)	12[ab] (2.1)	6	16	24	19	0.037	[-]

[1] Samples not replicated in Skaftafell, hence differences not statistically tested; [2] Hot water extractable Carbon; [3] Total Soil Organic Carbon; [4] Potential mineralisable Nitrogen; [5] Nitrogen mineralisation rate; [6] Carbon mineralisation rate; [7] for Damma taken from Zumsteg et al. (2012); [8] for Damma taken from Bernasconi et al. (2011).

2.3 Results

2.3.1 Skaftafell

Vegetation cover increased from a small pioneer vegetation with a cover <5% at the second stage (no vegetation was present at stage 1) to a fully covering dwarf shrub vegetation in the reference site, whereas plant species richness reached a peak with 15 species per plot at the stage 4 (Table 2.1).

Soil pH decreased from basic (8.9) to neutral (5.7) with age in the Skaftafell glacier forefield (Table 2.1). All measured carbon and nitrogen pools showed an increase with age. TOC correlated positively with TN, while HWC correlated positively with PMN (Spearman's rho=0.94, $p<0.001$, and rho=0.89, $p<0.001$, respectively). In contrast, potential N mineralisation reflecting net mineralisation, and relative microbial respiration (qCO_2 , calculated as g CO_2–C g^{-1} microbial C, as measure for microbial efficiency), showed a statistically significant decrease with age ($p=0.006$ and $p=0.005$, respectively). Both C mineralisation rate and the ratio of TOC to TN (indicative for organic matter quality) were high in the youngest stage, but showed an increase from the second stage towards the reference sites ($p=0.006$ and $p=0.006$, respectively).

Based on the biomass measurements of trophic groups of organisms, we constructed soil food web diagrams for all stages (Figure 2.3). At the youngest stage fungi were not yet present, while fungal biomass increased towards 105 kg C ha^{-1} at the reference site ($p=0.003$, Table 2.2). Also bacterial biomass increased with age ($p=0.010$). Due to the steeper increase in fungal biomass, the fungal to bacterial biomass ratio increased with age ($p=0.005$, Table 2.2). At the youngest two successional stages nematode presence was below the detection limit, subsequently peaked at intermediate stages especially for herbivorous ($p=0.003$) and omnivorous nematodes ($p=0.003$), and decreased again at the oldest stages, whereas fungivore nematodes increased with age, following the increase in fungal biomass ($p=0.011$, Table 2.2).

Total soil food web biomass increased with age ($p=0.004$). Biomasses at the 1st and 2nd trophic level increased with age ($p=0.004$ and $p=0.006$), whereas biomass at the 3rd trophic level decreased again after 65 years ($p=0.009$; Table 3). The number of trophic groups in the soil food web increased from 5 at the initial stages and stabilized after 65 years at around 11 trophic groups present ($p=0.006$; Table 2.3; Figure 2.3). Similarly, maximum trophic chain length stabilized after 120 years at maximum 6 groups (the longest possible chain contains trophic groups 1-3-4-5-9-10), also indicated by the best fitting polynomial regression line (Figure 2.4A). Mean and maximum trophic level peaked after 120 years ($p=0.012$).

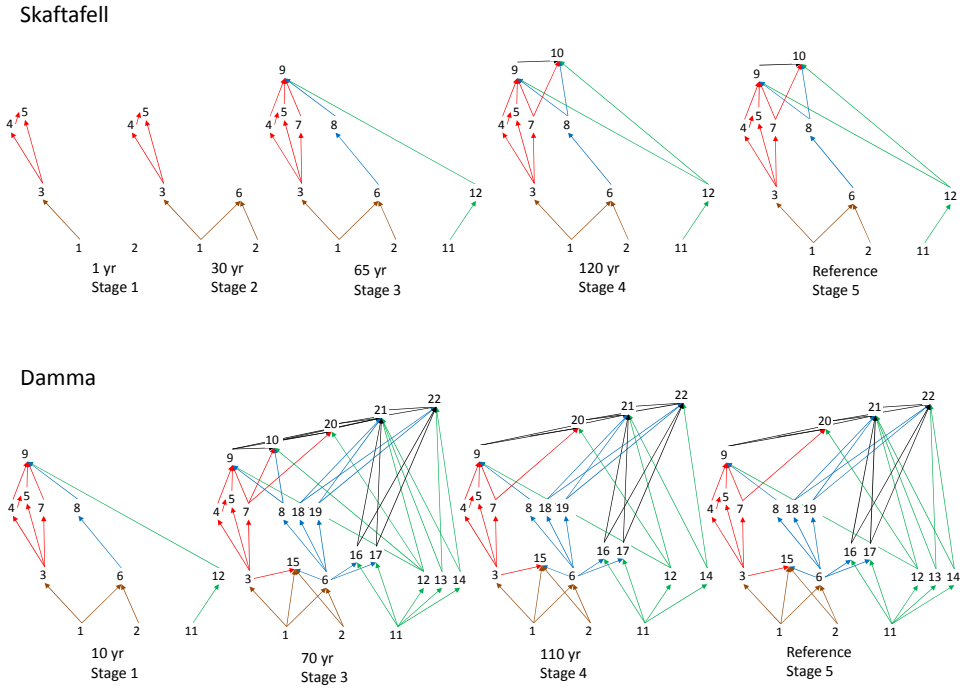

Figure 2.3 Food web assembly in the glacial fore fields of Skaftafell (Iceland) and Damma (Switzerland), of the successional stages. The numbers refer to the trophic groups: 1, labile detritus; 2, recalcitrant detritus; 3, bacteria; 4, flagellates; 5, amoebae; 6, fungi; 7, bacterivorous nematodes; 8, fungivorous nematodes; 9, omnivorous nematodes; 10, predatory nematodes; 11, plant roots; 12, herbivorous nematodes; 13, herbivorous mites; 14, herbivorous collembolans; 15, enchytraeids; 16, herbofungivorous mites; 17, herbofungivorous collembolans; 18, fungivorous mites; 19, fungivorous collembolans; 20, nematovorous mites; 21, omnivorous mites; 22, predatory mites. Years refer to soil age. Microarthropods (trophic groups 13-22) were not sampled in Skaftafell and stage 1 in Damma. Colours represent energy source (brown: detritus, red: bacteria, blue: fungi, green: plant roots, black: omnivorous).

Connectance of the food webs decreased with age (p=0.007; Table 2.3), whereas link density peaked after 65 years with inclusion of more omnivorous trophic groups (omnivorous and predatory nematodes) (p=0.005; Figure 2.4C). Complexity of the soil food webs, calculated according to Neutel et al. (2007), peaked at intermediate age (p=0.003; Figure 2.4E). Food web stability, quantified as the maximum real part of the eigenvalue of the interaction matrix, did not show a consistent trend with successional age, although was significantly lower after 65 years compared to the first stage (p=0.006). Stability was negatively correlated to maximum trophic chain length (Rho=0.66, p=0.003) and link density (Rho=0.61, p=0.008), while no significant correlation was found with complexity (Rho=0.47, p=0.05; Figure 2.5). Presence of predatory nematodes had a large negative effect on the stability of the soil food web: the only replicate in which predatory nematodes were present was

Table 2.2 Biological parameters at the chronosequences and reference sites studied in Skaftafell (Iceland) and Damma (Switzerland): biomasses (µg C g^{-1}) of the trophic groups in the soil food webs, bacterial activity and microarthropod diversity. All values represent mean and standard deviation (between brackets), measured in the topsoil (0-5 cm); [-]: not sampled. The p-values represent significance levels from a Kruskal-Wallis non-parametric test with age group as factor, where the superscript letters (within rows, per country) denote statistically significant differences between sites (^{a}smallest, ^{ab}intermediate, ^{b}highest).

Site	Skaftafell					Damma				p-value S	p-value D
Age	1 yr	30 yr	65 yr	120 yr	ref	10 yr	70 yr	110 yr	ref		
Bacteria	8.71^{ab} (1.20)	7.43^{a} (2.72)	11.6^{ab} (3.48)	10.9^{ab} (2.72)	19.6^{b} (3.75)	2.21^{a} (1.25)	15.0^{ab} (6.27)	13.6^{ab} (5.22)	36.3^{b} (9.45)	0.010	0.025
Leu1 (pmol g^{-1} h^{-1})	54.8 (70.7)	147 (14.1)	113 (16.4)	52.8 (20.8)	107 (44)	7.91^{a} (9.68)	246^{ab} (64.2)	220^{ab} (74.0)	714^{b} (84.5)	0.078	0.024
Fungi	0^{a} (0)	2.35^{ab} (2.09)	29.7^{ab} (12.1)	14.9^{ab} (2.64)	105^{b} (11.9)	13.6^{a} (12.1)	111^{b} (37.8)	77.3^{ab} (19.1)	33.9^{ab} (7.05)	0.003	0.026
Microbial biomass	8.71^{a} (1.20)	9.78^{a} (4.81)	41.3^{ab} (11.0)	25.8^{ab} (5.33)	124^{b} (9.98)	15.8^{a} (13.3)	126^{b} (42.3)	90.9^{ab} (20.4)	70.1^{ab} (10.6)	0.004	0.038
Fungal:bacterial biomass ratio	0^{a} (0)	0.28^{ab} (0.16)	2.90^{ab} (1.91)	1.40^{ab} (0.14)	5.55^{b} (1.55)	3.09 (3.34)	1.78 (0.94)	1.72 (0.86)	0.25 (0.14)	0.005	0.099
qCO_2 (g $C^{2}g^{-1}$ yr^{-1})	49.7^{b} (8.30)	26.1^{ab} (13.3)	6.45^{ab} (1.23)	14.1^{ab} (2.27)	4.76^{a} (1.47)	12.0^{a} (8.65)	23.5^{ab} (8.27)	33.5^{ab} (8.18)	124^{b} (10.8)	0.005	0.027
Amoebae	0.002^{a} (0.002)	0.019^{ab} (0.009)	0.023^{ab} (0.012)	0.086^{ab} (0.040)	0.112^{b} (0.074)	0.14 (0.072)	0.99 (0.33)	0.15 (0.08)	0.49 (0.24)	0.006	0.030*
Flagellates	0.005 (0.007)	0.002 (0.002)	0.037 (0.018)	0.38 (0.29)	0.28 (0.092)	0.15^{a} (0.11)	2.9^{b} (1.7)	0.44^{ab} (0.35)	0.46^{ab} (0.016)	0.007*	0.031
Bacterivore nematodes	0 (0)	0 (0)	0.031 (0.019)	0.020 (0.012)	0.034 (0.015)	0.039 (0.017)	0.10 (0.018)	0.26 (0.14)	0.31 (0.19)	0.013*	0.043*
Fungivore nematodes	0^{a} (0)	0^{a} (0)	0.007^{ab} (0.005)	0.011^{ab} (0.001)	0.013^{b} (0.005)	0.004 (0.002)	0.053 (0.028)	0.050 (0.052)	0.054 (0.080)	0.011	0.218
Herbivore nematodes	0^{a} (0)	0^{a} (0)	0.031^{b} (0.009)	0.024^{ab} (0.007)	0.024^{ab} (0.012)	0^{a} (0)	0.29^{ab} (0.17)	0.17^{ab} (0.12)	0.92^{b} (0.16)	0.015*	0.022
Omnivore nematodes	0^{a} (0)	0^{a} (0)	0.16^{b} (0.041)	0.076^{ab} (0.039)	0.008^{ab} (0.013)	0.001 (0.002)	0.45 (0.34)	0.73 (0.34)	0.36 (0.34)	0.005	0.095

Table 2.2 Continued

Predaceous nematodes	0 (0)	0 (0)	0 (0)	0.004 (0.006)	0.002 (0.004)	0 (0)	0.11 (0.20)	0 (0)	0 (0)	0.585	0.392
Total nematode biomass	0^{a} (0)	0^{a} (0)	0.23^{b} (0.057)	0.13^{ab} (0.048)	0.080^{ab} (0.018)	0.044^{a} (0.017)	1.01^{ab} (0.49)	1.22^{ab} (0.19)	1.65^{b} (0.21)	0.004	0.022
Enchytraeids	[-]	[-]	[-]	[-]	[-]	[-]	1.71 (1.01)	1.88 (1,77)	2.20 (0.40)	[-]	0.083
Fungivore Mites	[-]	[-]	[-]	[-]	[-]	[-]	0.004 (0.002)	0.002 (0.002)	0.032 (0.010)	[-]	0.061
Herbofungivore Mites	[-]	[-]	[-]	[-]	[-]	[-]	0.011 (0.010)	0.003 (0.003)	0.100 (0.080)	[-]	0.079
Herbivore Mites	[-]	[-]	[-]	[-]	[-]	[-]	0.0002 (0.0003)	0 (0)	0.004 (0.0005)	[-]	0.199
Nematovore Mites	[-]	[-]	[-]	[-]	[-]	[-]	0.023 (0.020)	0.016 (0.027)	0.024 (0.020)	[-]	0.668
Omnivore Mites	[-]	[-]	[-]	[-]	[-]	[-]	0.42 (0.44)	0.34 (0.28)	0.88 (0.95)	[-]	0.393
Predaceous Mites	[-]	[-]	[-]	[-]	[-]	[-]	0.028 (0.020)	0.074 (0.027)	0.14 (0.070)	[-]	0.079
Fungivore Collembola	[-]	[-]	[-]	[-]	[-]	[-]	0.054 (0.044)	0.15 (0.023)	0.34 (0.15)	[-]	0.039*
Herbofungivore Collembola	[-]	[-]	[-]	[-]	[-]	[-]	0.079 (0.077)	0.011 (0.002)	0.085 (0.075)	[-]	0.733
Herbivore Collembola	[-]	[-]	[-]	[-]	[-]	[-]	0.038 (0.043)	0.006 (0.007)	0.045 (0.079)	[-]	0.417

Table 2.2 Continued

Microarthropod biomass	[-]	[-]	[-]	[-]	[-]	[-]	0.65 (0.52)	0.60 (0.35)	1.65 (1.12)	[-]	0.288
Acari biomass	[-]	[-]	[-]	[-]	[-]	[-]	0.48 (0.48)	0.44 (0.32)	1.18 (1.00)	[-]	0.430
Collembola biomass	[-]	[-]	[-]	[-]	[-]	[-]	0.17 (0.10)	0.17 (0.030)	0.47 (0.14)	[-]	0.066
Microarthropod taxa richness	[-]	[-]	[-]	[-]	[-]	[-]	21.00 (4.58)	21.00 (4.58)	31.00 (4.58)	[-]	0.176
Shannon H index	[-]	[-]	[-]	[-]	[-]	[-]	2.12 (0.51)	2.12 (0.16)	2.72 (0.23)	[-]	0.061
Pielou evenness	[-]	[-]	[-]	[-]	[-]	[-]	0.69 (0.12)	0.70 (0.01)	0.80 (0.06)	[-]	0.561

[1] Samples not replicated in Skaftafell, hence differences not statistically tested; [2] Hot water extractable Carbon; [3] Total Soil Organic Carbon; [4] Potential mineralisable Nitrogen; [5] Nitrogen mineralisation rate; [6] Carbon mineralisation rate; [7] for Damma taken from Zumsteg et al. (2012); [8] for Damma taken from Bernasconi et al. (2011).

Table 2.3 Food web characteristics in the soil food webs at the chronosequences and reference sites studied in Skaftafell (Iceland) and Damma (Switzerland): number of trophic groups of micro- and mesofauna, connectance, link density (LD), total biomass (kg C ha^{-1}), biomasses of the separate trophic levels (kg C ha^{-1}), mean and maximum trophic level, maximum chain length, and stability (maximum real eigenvalue). All values represent mean and standard deviation (between brackets), measured in the topsoil (0-5 cm). The p-values represent significance levels from an Kruskal-Wallis non-parametric test with age group as factor, where the superscript letters denote statistically significant differences between sites (^{a}smallest, ^{ab}intermediate, ^{b}highest).

	Trophic groups micro-fauna	Trophic groups meso-fauna	Mean trophic level	Max trophic level	Max chain length	Total bio-mass	Biomass			Connec-tance	LD	Com-plexity	Stability
							Level 1	Level 2	Level 3				
Skaftafell													
1 yr	4.33^{a} (0.58)	[-]	1.39^{a} (0.10)	2.00 (0.001)	3.33^{a} (0.58)	8.72^{a} (1.20)	8.71^{a} (1.20)	0.005^{ab} (0.007)	0.002^{a} (0.002)	0.36^{b} (0.05)	0.86^{a} (0.34)	0.02^{a} (0.005)	0.000^{a} (0.000)
30 yr	6.67^{ab} (0.00)	[-]	1.56^{ab} (0.10)	2.33 (0.58)	3.67^{ab} (0.58)	9.80^{a} (4.82)	9.78^{a} (4.81)	0.002^{a} (0.002)	0.02^{ab} (0.009)	0.31^{ab} (0.02)	1.56^{ab} (0.10)	0.20^{ab} (0.26)	0.0000^{a} (0.0001)
65 yr	11.00^{ab} (0.00)	[-]	2.22^{ab} (0.02)	3.45 (0.18)	5.00^{ab} (0.58)	41.6^{ab} (11.1)	41.3^{ab} (11.0)	0.08^{ab} (0.03)	0.19^{b} (0.04)	0.24^{ab} (0.000)	2.36^{b} (0.000)	1.78^{b} (0.43)	0.011^{ab} (0.008)
120 yr	11.33^{b} (0.58)	[-]	2.29^{b} (0.10)	3.72 (0.45)	5.33^{b} (0.58)	26.4^{ab} (5.37)	25.8^{ab} (5.32)	0.42^{ab} (0.28)	0.17^{ab} (0.04)	0.24^{ab} (0.001)	2.44^{b} (0.13)	1.14^{b} (0.48)	0.28^{b} (0.27)
reference	10.50^{ab} (0.55)	[-]	2.17^{ab} (0.08)	3.30 (0.35)	4.67^{ab} (0)	125^{b} (10.0)	124^{b} (9.98)	0.33^{b} (0.08)	0.12^{ab} (0.07)	0.22^{a} (0.013)	2.09^{ab} (0.23)	0.27^{ab} (0.14)	0.05^{ab} (0.02)
p-value	0.006		0.012	0.015*	0.013	0.004	0.004	0.006	0.009	0.007	0.005	0.006	0.003
Damma													
10 yr	7.33 (0.58)	[-]	2.32 (0.12)	3.35 (0.53)	4.33 (0.58)	16.2^{a} (13.2)	15.8^{a} (13.3)	0.19^{a} (0.09)	0.001 (0.001)	0.28^{b} (0.014)	2.03^{a} (0.26)	0.35 (0.08)	0.088 (0.090)
70 yr	9.33 (0.58)	11.67 (0.58)	2.51 (0.03)	3.98 (0.06)	8.33 (0.58)	133^{b} (43.0)	126^{b} (42)	4.90^{b} (0.74)	0.44 (0.25)	0.20^{ab} (0.004)	3.89^{ab} (0.10)	0.62 (0.22)	0.19 (0.15)
110 yr	9 (0)	12 (1)	2.46 (0.03)	4.00 (0.03)	7.33 (0.58)	95.2^{ab} (19.3)	91.1^{ab} (20.3)	2.80^{ab} (2.16)	0.29 (0.10)	0.20^{a} (0.003)	3.58^{b} (0.19)	1.11 (0.77)	0.11 (0.14)
reference	8.33 (1.15)	11 (1)	2.42 (0.04)	3.85 (0.12)	7.33 (1.15)	76.6^{ab} (10.1)	71.1^{ab} (10.6)	3.58^{ab} (0.32)	0.20 (0.12)	0.20^{ab} (0.006)	3.65^{ab} (0.26)	0.42 (0.17)	0.21 (0.10)
p-value	0.264	0.431	0.09	0.075	0.069	0.038	0.038	0.037	0.075	0.041	0.033	0.099	0.108

*Significant in Kruskal-Wallis, but no differences found in pairwise comparisons with Dunn correction

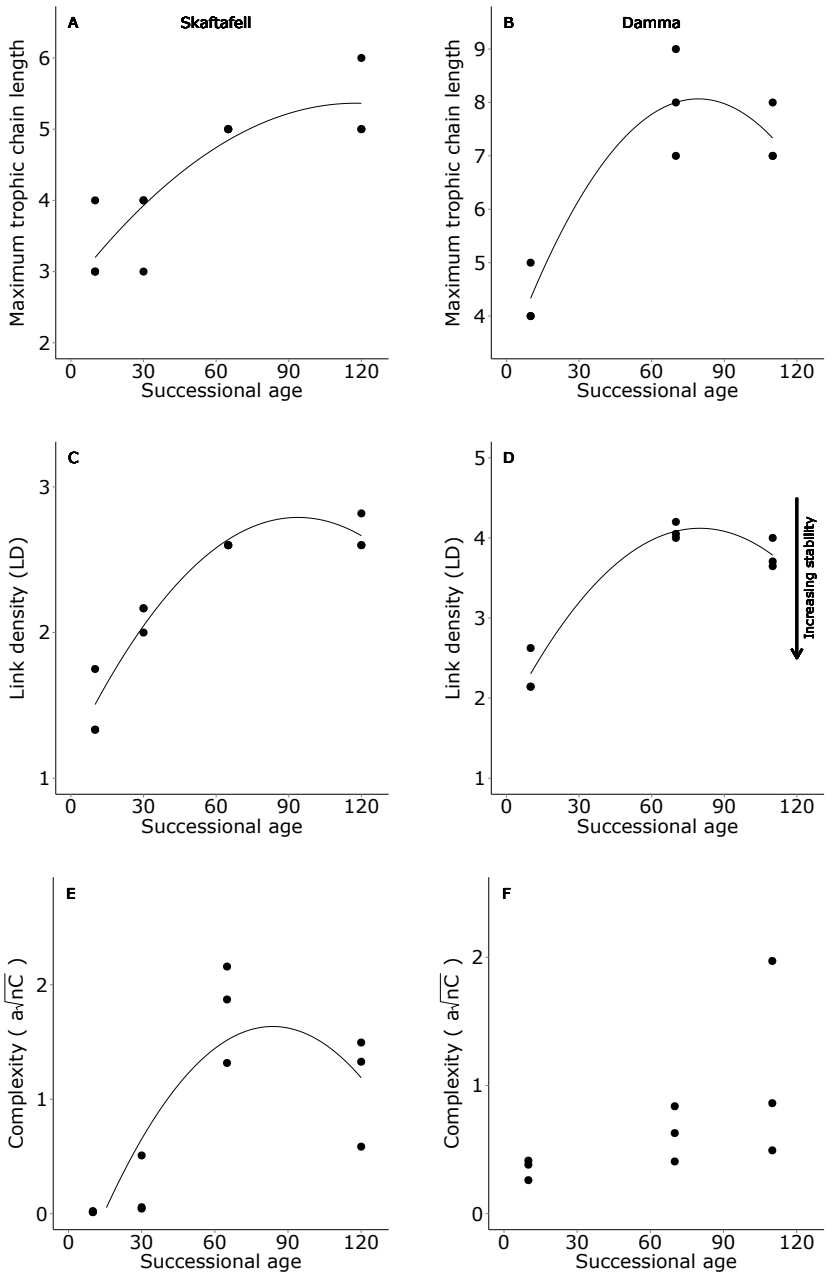

Figure 2.4 Food web metrics in the glacial fore fields of Skaftafell (Iceland) and Damma (Switzerland) in relation to successional age: maximum trophic length (A, B), link density (LD; C, D), and complexity (a√nC; E, F). Reference stages are not included in the graphs. Polynomial or linear regression: A: $y = 4.33 + 2.78x - 0.82x^2$, $r^2adj=0.73$, $p=0.001$; B: $y = 6.56 + 4.00x - 2.62x^2$, $r^2adj=0.83$, $p=0.002$; C: $y = 2.21 + 1.44x - 0.80x^2$, $r^2adj=0.92$, $p<0.001$; D: $y = 3.39 + 1.97x - 1.25x^2$, $r^2adj=0.94$, $p<0.001$; E: $y = 0.78 + 1.70x - 1.50x^2$, $r^2adj=0.66$, $p=0.003$; F: no significant regression fit.

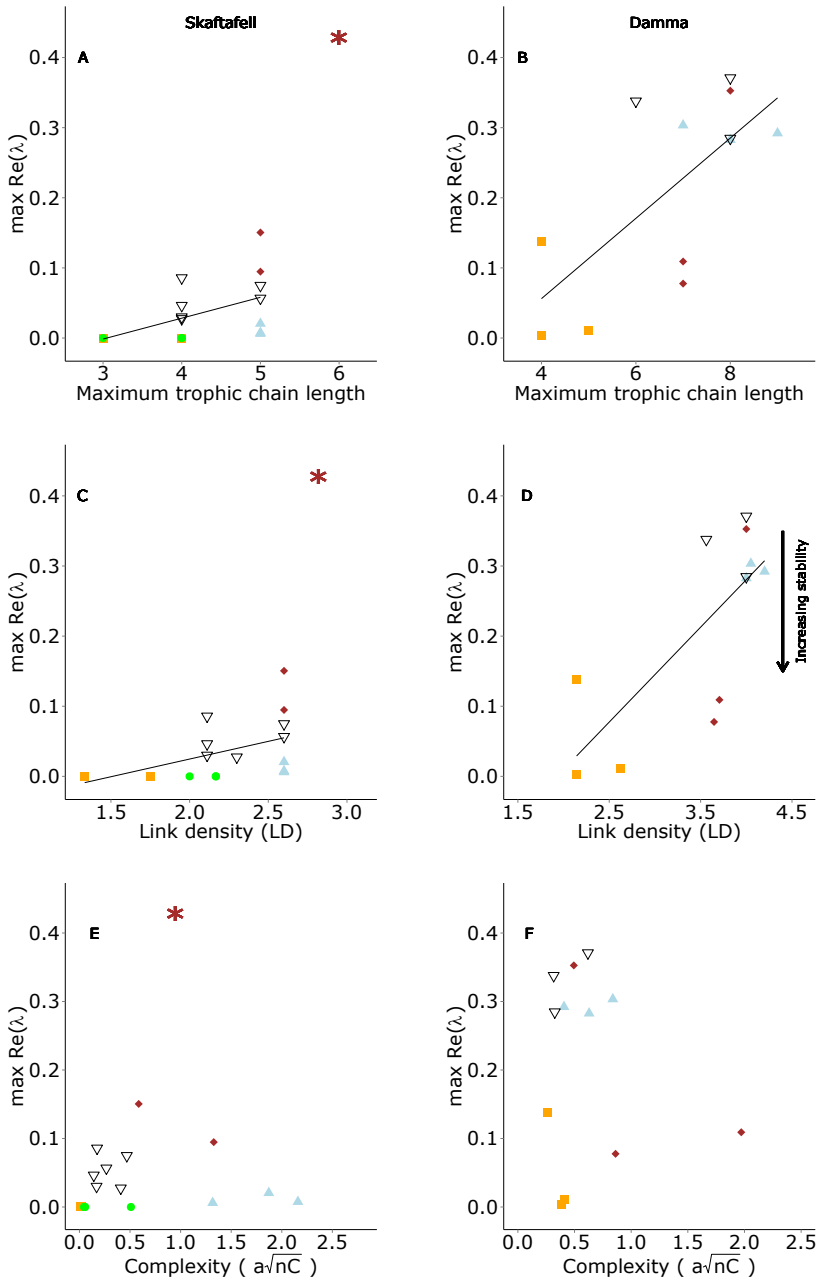

Figure 2.5 Food web stability (quantified as the maximum real part of the eigenvalue) in the glacial fore fields of Skaftafell (Iceland) and Damma (Switzerland) in relation to food web metrics: maximum trophic length (A, B), link density (LD; C, D), and complexity (a√nC; E, F). Colours represent successional stages (1, yellow squares; 2, green dots; 3, light blue triangles; 4, brown diamonds; 5, black open triangles). Spearman rank correlations: A: Rho=0.67, p=0.003; B: Rho=0.59, p=0.04; C: Rho=0.62, p=0.008; D: Rho=0.63, p=0.02; E: Rho=0.47, p=0.05; F: Rho=-0.05, p=0.89. One of the replicates in successional stage 4 in Skaftafell (shown as brown star in A, C, E) was considered an outlier, and not included in the correlations.

much less stable than the others (maximum real part of the eigenvalue was 0.59, where other replicates had values between 0 and 0.1). This replicate was therefore excluded from the correlations as an outlier.

Principal Component Analysis revealed a clear separation of successional stages in Skaftafell when based on soil food web data (Figure 2.6A, B), with a close correlation of stage 4 with the reference site. Apart from N mineralisation rate, all C and N parameters and vegetation richness and cover, were correlated with age towards the reference site. Successional stage 3 deviates from the other stages, driven by the higher biomass of especially omnivorous nematodes.

2.3.2 Damma

Vegetation cover increased from 5% cover by a species rich (18 species) community of small pioneer species in stage 1, to 95% cover, dominated by *Agrostis gigantea* at the reference site (Bernasconi et al., 2011; Zumsteg et al., 2012).

In the Damma glacier forefield, clay content increased from 1.1% at the youngest soil towards 8% at the reference site ($p=0.033$, Table 2.1). Because no carbonate was present in the soils, soil pH in Damma stayed between 4.5 and 5.

All measured carbon and nitrogen pools showed an increase with age (Table 2.1). TOC correlated positively with TN, while HWC correlated positively with PMN (Spearman's $rho=0.98$, $p<0.001$ and $rho=0.96$, $p<0.001$, respectively). C mineralisation rate and, as a result of that, also relative microbial respiration (qCO_2 , calculated as kg CO_2–C kg^{-1} microbial C, indicative for microbial efficiency) increased with age ($p=0.022$ and $p=0.027$, respectively), whereas the ratio of TOC to TN peaked at stage 4 ($p=0.027$). In contrast, N mineralisation rate showed no clear pattern.

Based on the biomass measurements of trophic groups of organisms, we constructed soil food web diagrams for all stages. A complete soil food web was already present after 70 years (Figure 2.3), and total biomass peaked at this intermediate site (Table 2.2). Bacteria, bacterial activity, nematodes and microarthropods increased in biomass with age. Some trophic groups peaked at an intermediate stage, this peak was significant for fungi ($p=0.026$) and flagellates ($p=0.031$). No consistent pattern was found in fungal to bacterial biomass ratio. Microarthropod biomass, taxonomic richness, Pielou evenness, and Shannon diversity did not differ statistically between the three successional stages in which they were determined (70, 110 and more than 3000 year old soils in Damma; Table 2.2). No clear patterns were found in the microarthropod taxa present at the three stages (Appendix 2.2).

The number of trophic groups in the soil food web increased from 11 at the initial stage without microarthropods and stabilized after 70 years at around 20 trophic groups present. Similarly, maximum trophic chain length peaked after 70 years at 9 groups (the longest possible chain contains trophic groups 1-3-4-5-9-10-20-21-22), also shown by the quadratic regression fit (Figure 2.4B). Also mean and maximum trophic level tended to be highest at intermediate age. Likewise, total biomass and biomass at the 1st and 2nd trophic levels peaked after 70 years (Table 2.3).

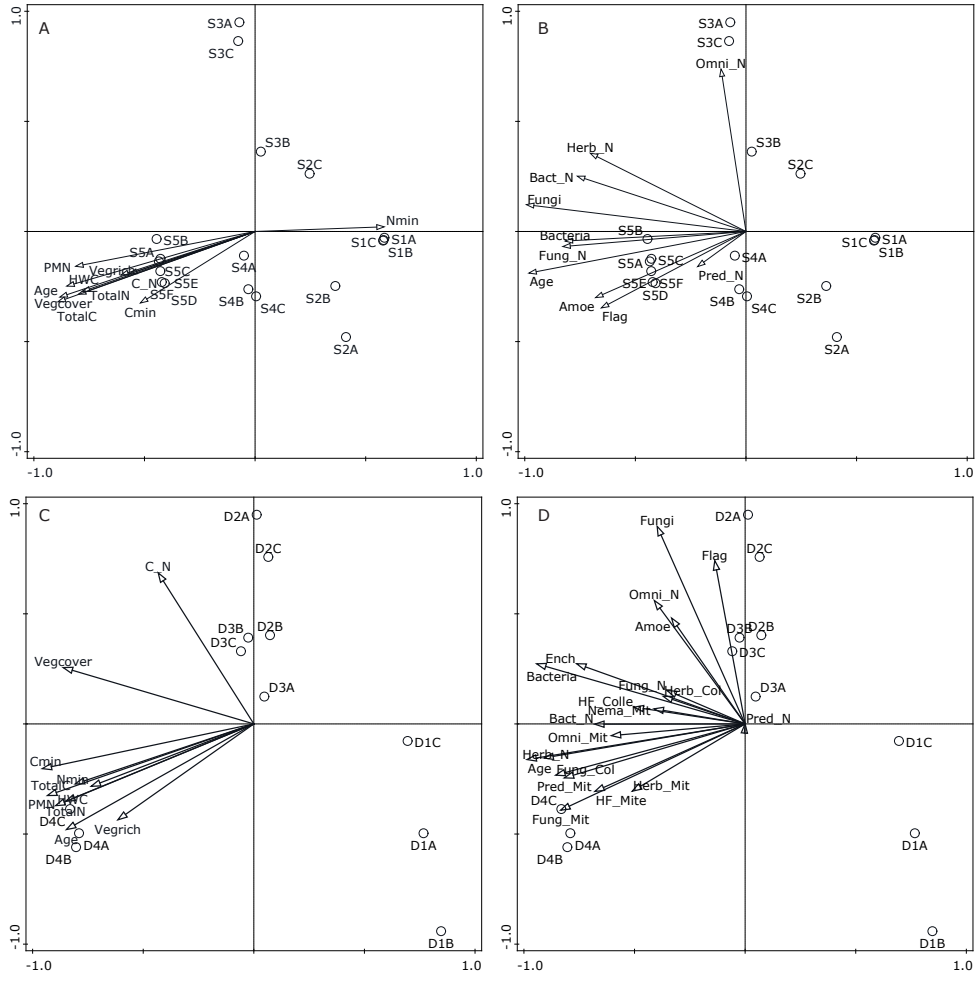

Figure 2.6 Samples from Skaftafell arranged based on PCA-scores of soil food web data with soil C and N pools and fluxes, and vegetation richness and cover as supplementary variables (with arrows representing soil parameters (A) and arrows representing trophic groups (B)), and samples from Damma arranged based on PCA-scores of soil food web data with soil C and N pools and fluxes, and vegetation richness and cover as supplementary variables (with arrows representing soil parameters (C) and arrows representing trophic groups (D)). Both chronosequences show similar patterns in biotic drivers and trophic group biomass distributions.

Connectance of the food webs decreased with age (p=0.041; Table 3), whereas link density increases with inclusion of more omnivorous trophic groups (omnivorous and predatory nematodes, omnivorous and predatory mites) (p=0.033; Figure 2.4D). Complexity of the soil food webs (Figure 2.2F), calculated according to Neutel et al. (2007) and food web stability, quantified as the maximum real part of the eigenvalue of the interaction matrix, did not show a consistent trend with successional age. Stability was negatively correlated to maximum trophic chain length (Rho=0.59, p=0.043) and link density (Rho=0.63, p=0.029), but not correlated to complexity (Rho=-0.05, p=0.89; Figure 2.5). Because soil microarthropods were not sampled at stage 1 due to the gravelly nature of the soil, we checked the effect of absence or presence of these organisms on the food web metrics. Only small effects were found: when microarthropods were not included in the food webs, differences between stages disappeared for maximum chain length and maximum trophic level, whereas all other food web metrics showed similar results with or without microarthropods.

Principal Component Analysis revealed a clear separation of successional stages in Damma when based on soil food web data (Figure 2.6C, D), with a close correlation of the intermediate stages, while the first stage and reference stage were clearly separated. Apart from C: N ratio, all C and N parameters were correlated with age towards the reference site. Vegetation richness was closely correlated with all C and N parameters, opposed to vegetation cover. Carbon to nitrogen ratio was strongly correlated with biomass of fungi, flagellates, amoebae and omnivorous nematodes, all peaking in the second successional stage. PCA analysis was only slightly influenced by the absence of microarthropods in the first stage, when microarthropods were excluded, all scores were similar.

2.4 Discussion

Soils are under threat as soils form much slower than they erode due to human activities. It is important that we understand processes that build up and regenerate soil. Therefore we studied soil ecosystem development, and the assembly of the soil food web in particular, in soils without human interferences, i.e. chronosequences in forefields of retracting glaciers. We expected that with age soil food webs in glacial forefields will increase in biomass of the trophic groups, diversity, complexity and stability, along with increases in nutrient content, vegetation cover and plant species richness.

Along both chronosequences we indeed saw soil ecosystem development according our expectations, i.e. an increase in soil carbon and nitrogen, vegetation cover and plant species richness, soil total biomass (total and per trophic groups), micro-arthropod diversity (only measured in the Damma forefield), number of trophic groups, and trophic chain length.

We did not find such an increase in food web complexity and stability. Also no significant time-lag was present between below- and aboveground ecosystem development, such as reported from secondary successions.

2.4.1 Vegetation

Vegetation cover and plant species richness increased steadily at both glacial forefields. In Skaftafell, the first stage on the edge of the glacier was still bare, whereas in Damma the first colonizing pioneer species were already present at the youngest successional stage. The relatively low vegetation cover in Skaftafell compared to Damma might have been due to extensive grazing by sheep (until 1967), the relatively low vegetation cover in the surroundings due to geographic isolation (Gunnlaugsdóttir, 1985), and the cold climatic conditions.

Nitrogen fixing plant species can speed up the soil ecosystem development (Bormann and Sidle, 1990), but all three N-fixing plant species at Skaftafell and Damma had a very low cover, indicating that here plant-based N-fixing can be discarded as an important mechanism in soil ecosystem development. In Damma also N fixation by free-living cyanobacteria has been shown to only have a minor influence (Brankatschk et al., 2011), while isotope data suggest precipitation as the origin of N in the system (Smittenberg et al., 2012). In contrast, in Skaftafell N deposition is low, whereas N fixation by cyanobacteria is supposed to be high (Vilmundardóttir et al., 2015).

2.4.2 Soil biochemical parameters

Soil pH, as a plant and weathering related factor, is an important driver for soil community development, especially determining shifts in bacterial and fungal communities (Knelman et al., 2012). In the present study we saw that soil pH in the Skaftafell forefield showed a decrease in pH from 8.9 at the youngest stage to 5.7 in the reference sites, comparable to an earlier study in Skaftafell (Vilmundardóttir et al., 2014). Due to the siliceous bedrock in the Damma forefield no carbonate was present, resulting in a low pH of around 4.5.

All carbon and nitrogen pools, both total (TOC, TN) and labile (HWC, PMN), showed an increase along the successional stages in both glacial chronosequences. This increase has been found in many other studies on other primary chronosequences (Chapin et al., 1994; Egli et al., 2010; Egli et al., 2001; He and Tang, 2008; Insam and Haselwandter, 1989; Stevens and Walker, 1970), and within the range of values in Skaftafell (Vilmundardóttir et al., 2015) and Damma (Smittenberg et al., 2012). The increase of total organic C in Damma was much steeper compared to the trend in Skaftafell. Possible explanations for the slower soil development in Skaftafell are probably related to the abiotic (climatic) conditions at

high latitudes, including frequent freeze/thaw cycles and low summer temperatures. Studies based on longer time scale chronosequences suggest that the total amount of N in the soil will stabilise after 1500 years under favouring climatic conditions (Stevens and Walker, 1970), which indicates that even our reference site in Skaftafell could be still in a developing phase. Also in comparison with other soils in Iceland (Lehtinen et al., 2015), total N content in the reference sites was still low. The increase in mineralisable N with age in Skaftafell on one hand and the decrease in potential N mineralisation by microbes on the other, imply a strong immobilisation of N by microbes in the soil, hence a strongly N limited system with a low N availability for plants. The more than 3000 year old reference site sampled in Damma should be fully developed and C and N values are comparable to other, older, Swiss alpine grasslands (Niklaus and Körner, 1996).

Microbial efficiency (indicated by qCO_2) showed a remarkable difference between the two chronosequences. Microbial efficiency increased with age in Skaftafell, which could be related to the increase in the fungal to bacterial biomass ratio with age and a higher efficiency of fungi compared to bacteria (Six et al., 2006). The same mechanism could also explain the opposite trend we found in Damma Glacier, where efficiency decreased with age, together with a negative trend in the fungal to bacterial biomass ratio. This decrease could be related to a decrease in ecosystem productivity as a result of for example accumulation of less decomposable organic matter or nutrient limitation. However, both bacterial biomass and potential nitrogen mineralisation increased with age together with contents of carbon and nitrogen, indicating a nutrient rich system.

2.4.3 Soil food web characteristics

Overall, soil organism biomass for most trophic groups increased with age in both chronosequences. Exceptions were the nematodes in Skaftafell, that peaked at the third successional stage (65 years old), and the fungi and flagellates in Damma, that also peaked at the third successional stage (70 years old). These intermediate peaks were unexpected, because soil organic carbon and nitrogen pools, as well as cover and to a lesser extent also diversity of the aboveground vegetation showed an increase towards the oldest stages. There are also in contrast to earlier studies on the same forefield in Damma (Brankatschk et al., 2011), and observations on nematode biomass in the Franz Josef glacial forefield in New Zealand showing an increase until an age of 5000 years (Doblas-Miranda et al., 2008). For the present findings, the highly dynamic processes shaping the glacial environment, with glacial stream and wind depositions as important factors (Brankatschk et al., 2011), cause strong spatial heterogeneity, which could have resulted in an incidentally locally elevated soil organism biomass in intermediate stages.

Microarthropod biomass, taxonomic richness, Pielou evenness, and Shannon diversity did not differ between the three stages in which they were sampled (70, 110 and >3000 year old soils in Damma). Thus, the assembly of the microarthropod part of the soil food web seems to take place in the first 70 years of development. This is in agreement with other studies showing strong development of aboveground invertebrate communities in the first decades, with little or no progress thereafter (Ingimarsdóttir et al., 2013b; Kaufmann, 2001).

In addition to increases in total and trophic group biomasses, we expected increases in maximum trophic chain length, connectance, link density, and complexity as the soil food webs develop with age, following the hypotheses posed by Odum (1969).

In our glacier forefields, the number of trophic groups in the food webs indeed increased with age, but these values already stabilised after 65-70 years in both chronosequences. Trophic chain length increased with age initially, but stabilized after 70 years in Damma, and 120 years in Skaftafell, which was also the case for mean and maximum trophic level. In Damma, the number of trophic groups doubled from the first to second successional stage by the inclusion of microarthropods in the sampling.

In both Skaftafell and Damma soil food web connectance decreased with age, whereas link density increased especially in the later stages. No comparable studies discussing soil food web metrics are available from other glacial forefields, but in a secondary succession on post-mining sites connectance did not correlate with successional age (Frouz et al., 2013). Link density also increases with age in a primary succession in the dune chronosequence of Schiermonnikoog, but shows no pattern in Hulsthorsterzand in the same study (Neutel et al., 2007).

Complexity of the soil food web did not differ between successional stages in Damma and peaked at an intermediate stage in Skaftafell, in line with the results of Neutel et al. (2007) in sand dune chronosequences. It should be noted that some of the calculated food web metrics, such as link density and complexity, could be sensitive to the small web size in the first successional stages, especially in Skaftafell, which might have affected the comparison with other food webs in literature (Banasek-Richter et al., 2009).

All of these metrics have often been linked with food web stability, although there is a strong debate about the relationship of stability with diversity. Also, this relationship can strongly vary between randomly assembled and empirical food webs. In randomly assembled food webs complexity and stability are negatively correlated (May, 1973), while in empirically based food webs the relationship between complexity and stability is not found

(Banasek-Richter et al., 2009; Holtkamp, 2010; Neutel et al., 2007). A positive correlation between connectance and stability was already stipulated by De Angelis (1975) for hypothetical food webs and later also found in terrestrial and aquatic food webs by Dunne et al. (2002). Stability in the present food webs from the glacial forefields showed no consistent trend with successional age and was very high in comparison with randomly generated webs, especially in the successional stages in Skaftafell. Such high levels of stability have also been found in a study on secondary succession on farmland (Holtkamp, 2010) and on primary succession in sand dune ecosystems (Neutel et al., 2007). The present study on the glacier forefield chronosequences confirmed that during food web assembly, food web structure in terms of food web topology and biomass distributions ensure food web stability. Stability in a developing system is therefore merely showing that despite principle changes in food web structure the community possesses an adequate resistance to disturbance, in a way analogue to embryonic development: the topology is continuously changing, but in a stable manner.

2.4.4 Below- and aboveground linkages

A recent paradigm in literature dictates that a successional increase in plant cover leads to an increase in the input of C and N in the soil, which is the resource for a growing soil microbial and faunal community, hence plants are the drivers of ecosystem development (facilitation-model) (Chapin et al., 1994; Knelman et al., 2012; Ohtonen et al., 1999). In the case of secondary succession this might result in a time-lag in response of belowground organisms to aboveground vegetation succession (Holtkamp et al., 2008; Korthals et al., 2001). However, as shown previously for aboveground arthropod communities (Hodkinson et al., 2001; Ingimarsdóttir et al., 2013a; Kaufmann, 2001), also the arrival of soil organisms can precede establishment of plants, hence do not depend on plant derived organic matter inputs, and form the primary nutrients for plant uptake. In the present case of soil ecosystem development, vegetation succession, and soil food web assembly at the glacial forefield chronosequences, we found that the development of the soil ecosystem followed parallel with (in line with the findings of Hedlund et al. (2003)), or even outpaced, the aboveground vegetation development. In conclusion, our study revealed that all components of terrestrial ecosystems (soil formation, vegetation development and soil food web assembly) occur in concert, and along stable pathways of ecosystem development. The results lead to a better understanding of soil development, and will thereby contribute to improved soil quality.

Appendix 2.1 Calculating food web metrics

For calculation of food web metrics, we have used the following formulas:

Connectance (*C*) of food webs was calculated following May (1973) as:

$$C = \frac{L}{n\,(n-1)}$$

in which *L* represents the number of links present in the food web, and *n* represents the number of trophic groups.

Link density (connectivity, *LD*) was calculated following May (1973) as

$$LD = \frac{L}{n}$$

in which *L* represents the number of links present in the food web, and *n* represents the number of trophic groups.

Complexity (*Cx*) was calculated following Neutel et al. (2007) as

$$Cx = a\sqrt{nC}$$

in which *a* is the weighted average of the off diagonal interaction strengths in the food web, *n* is the number of trophic groups, *C* is the connectance of the food web. Weighted average interaction strength (*a*) was calculated as

$$a = \frac{|\sum_i \sum_j \alpha_{ij}|\,|\sum_i \sum_j \alpha_{ji}|}{L\sqrt[n]{\prod_{i=1}^{n} d_i}}$$

in which *i* refers to prey, *j* refers to predator, a_{ij} is the per capita interaction strength of predators on prey, a_{ji} is the per capita interaction strength of prey on predators, *L* is the total number of feeding links, *d* is the natural death rate, and *n* represents the number of trophic groups present in the food web.

Stability of the soil food webs was calculated as the maximum real part of the eigenvalues of the interaction matrix (filled by interaction strengths a_{ij} and a_{ji}). For this calculation only the non-zero values were taking into account. Since the diagonal of the matrix (elements a_{ii}) was filled with zeros to avoid arbitrary values to be placed, the stability values in the graphs are all positive and in fact represent the amount of instability. Higher values represent less stable food webs.

Trophic level (mean and max) of the groups in the food web was calculated (slightly adjusted) following Holtkamp et al. (2008) as

$$TL = 1 + \sum_{j}^{n} \frac{F_{ij}}{\sum_{1}^{k} F_{ij}}$$

in which TL_i represents trophic level of the prey, F_{ij} represents the feeding rate of predator *j* on prey *i* calculated according to de Ruiter et al. (1995), *k* is the total number of trophic groups consumed by predator *j*, and *n* the total the number of trophic groups in the food web. Base trophic levels of labile detritus, recalcitrant detritus and roots were set to 1.

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Appendix 2.3 Abundance (m^{-2}) of the microarthropod taxa in the soil food webs at the chronosequences and reference site in the Damma glacier forefield (70 - >3000 yr). Trophic groups: Omnivorous mites (Ommi), Bacterivorous mites (Bami), Fungivorous mites (Fumi), Nematovorous mites (Nemi), Predatory mites (Prmi), Herbofungivorous mites (HFmi), Herbofungivorous collembolans (HFco), Fungivorous collembolans (Fuco) and Diplurans (Dipl). Numbers represent mean and standard deviation (between brackets), measured in the topsoil (0-5 cm).

Age		70	110	reference
Mesostigmata	Ommi	109 (189)	0 (0)	0 (0)
Alliphis	Nemi	0 (0)	271 (469)	83 (143)
Arctoseius	Prmi	0 (0)	121 (210)	0 (0)
Arctoseius cetratus	Prmi	185 (190)	325 (562)	0 (0)
Cheiroseius	Prmi	109 (189)	0 (0)	0 (0)
Lysigamasus	Prmi	0 (0)	1055 (984)	1658 (512)
Lysigamasus runcatellus	Prmi	0 (0)	0 (0)	166 (287)
Lysigamasus vagabundus	Prmi	0 (0)	0 (0)	83 (143)
Parazercon	Nemi	0 (0)	0 (0)	356 (617)
Pergamasus	Prmi	127 (220)	121 (210)	0 (0)
Pseudoparasitus	Prmi	116 (200)	0 (0)	0 (0)
Rhodacarus	Prmi	109 (189)	0 (0)	0 (0)
Trachytes aegrota	Fumi	0 (0)	0 (0)	296 (323)
Veigaia nemorensis	Prmi	0 (0)	0 (0)	83 (143)
Zercon	Nemi	0 (0)	0 (0)	356 (616)
Zercon triangularis	Nemi	1521 (717)	677 (1171)	379 (336)
Zercoseius spathuliger	Prmi	0 (0)	121 (210)	213 (369)
Oribatida	Hemi			
Achipteria		0 (0)	0 (0)	793 (879)
Brachychthoniidae	HFmi	1093 (1893)	460 (489)	15264 (12739)
Brachychthonius	HFmi	58 (100)	406 (703)	1352 (1176)
Ceratozetidae	Ommi	0 (0)	1302 (565)	1292 (1172)
Damaeidae	Fumi	0 (0)	135 (234)	0 (0)
Dissorhina ornata	Fumi	0 (0)	0 (0)	83 (143)
Hemileius initialis	Fumi	0 (0)	0 (0)	522 (535)
Liochthonius	HFmi	3298 (4297)	108 (187)	2849 (4934)
Microppia minus	Fumi	0 (0)	135 (234)	1079 (836)
Mycobatidae	HFmi	236 (206)	0 (0)	0 (0)
Oppiella	Ommi	300 (269)	0 (0)	0 (0)
Oppiella nova	Ommi	109 (189)	0 (0)	0 (0)
Oppiidae	Ommi	0 (0)	1326 (2022)	9272 (9364)
Oribatula	Ommi	0 (0)	271 (469)	0 (0)
Oribatulidae	Fumi	0 (0)	217 (375)	213 (369)
Pantelozetes	Fumi	0 (0)	0 (0)	5902 (7089)
Platynothrus	HFmi	0 (0)	0 (0)	8912 (15007)

Appendix 2.2 Continued

Sellnickochthonius	HFmi	0 (0)	0 (0)	356 (617)
Spatiodamaeus verticillipes	Fumi	824 (421)	0 (0)	0 (0)
Tectocepheus	Ommi	823 (1278)	9348 (6396)	30647 (37920)
Prostigmata	Ommi	0 (0)	0 (0)	356 (617)
Bdellidae	Prmi	0 (0)	135 (234)	439 (559)
Cunaxidae	Prmi	109 (189)	108 (187)	213 (369)
Eriophyidae	Hemi	109 (189)	0 (0)	0 (0)
Erythraeidae	Prmi	0 (0)	196 (203)	0 (0)
Eupodes	Ommi	962 (951)	2654 (890)	3197 (2787)
Eupodidae	Ommi	109 (189)	0 (0)	166 (287)
Microtydeus	Ommi	27291 (20848)	24021 (18040)	16866 (17792)
Nanorchestes	Ommi	311 (399)	0 (0)	1565 (1604)
Pachygnathidae	HFmi	0 (0)	0 (0)	331 (574)
Pyemotes	Prmi	109 (189)	1353 (1690)	652 (410)
Pygmephorus	Fumi	463 (801)	412 (234)	1314 (826)
Rhagidia	Prmi	185 (190)	169 (163)	414 (717)
Scutacarus	Ommi	5215 (3742)	677 (1172)	1636 (1982)
Stigmaeidae	Prmi	0 (0)	242 (419)	0 (0)
Tarsonemus	Ommi	1423 (986)	5965 (4815)	7640 (7837)
Tydeidae	Ommi	5807 (2910)	473 (155)	723 (1044)
Entomobryomorpha *Entomobrya*	HFco	2678 (2062)	398 (349)	213 (369)
Isotomiella minor	Fuco	0 (0)	135 (234)	853 (768)
Lepidocyrtus lignorum	HFco	0 (0)	135 (234)	2334 (3031)
Parisotoma notabilis	Fuco	352 (27)	560 (244)	3695 (1350)
Pseudisotoma sensibilis	Fuco	1279 (1313)	6341 (3107)	1244 (607)
Neelipleona *Megalothorax minimus*	HFco	0 (0)	0 (0)	331 (574)
Poduromorpha *Ceratophysella denticulata*	Fuco	0 (0)	0 (0)	83 (143)
Hypogastrura	Fuco	0 (0)	108 (187)	0 (0)
Neanura muscorum	Fuco	0 (0)	0 (0)	795 (1168)
Onychiurus	Fuco	0 (0)	0 (0)	604 (548)
Paratullbergia	Fuco	231 (401)	135 (234)	1495 (1613)
Protaphorura	Fuco	109 (189)	0 (0)	0 (0)
Xenylla	Fuco	984 (1704)	0 (0)	0 (0)
Symphypleona *Arrhopalites*	Fuco	0 (0)	0 (0)	356 (617)
Sminthuridae	Fuco	1093 (1893)	135 (234)	0 (0)
Sminthurus viridis	Fuco	300 (269)	60 (105)	356 (617)
Sphaeridia pumilis	Fuco	656 (1136)	0 (0)	356 (317)

Chapter 3

Soil food web structure and functioning under different agricultural practices in productive soils in Iceland and Austria

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Abstract

Intensive agricultural production can be an important driver for the loss of long-term soil quality. For this reason, the European Critical Zone Observatory (CZO) network adopted four pairs of agricultural CZO sites that differ in their management: conventional or organic. The CZO sites include two pairs of grassland farms in Iceland and two pairs of arable farms in Austria. Conventional fields differed from the organic fields in the use of artificial fertilizers and pesticides.

Soils of these eight farms were analysed in terms of their physical, chemical, and biological properties, including soil aggregate size distribution, soil organic matter contents, abundance of soil microbes and soil fauna, and taxonomic diversity of soil microarthropods.

In Icelandic grasslands, organically farmed soils had larger mean weight diameters of soil aggregates than the conventional farms, while there were no differences on the Austrian farms. Organic farming did not systematically influence organic matter contents or composition, nor soil carbon and nitrogen contents. Also soil food web structures, in terms of presence of trophic groups of soil organisms, were highly similar among all farms, indicating a low sensitivity of trophic structure to land use or climate. However, soil organism biomass, especially of bacteria and nematodes, was consistently higher on organic farms than on conventional farms. Within the microarthropods, taxonomic diversity was systematically higher in the organic farms compared to the conventional farms. This difference was found across countries, and farm, crop and soil types. The results do not show systematic differences in physical and chemical properties between organic and conventional farms, but confirm that organic farming can enhance soil biomass and that microarthropod diversity is a sensitive and consistent indicator for land management.

3.1 Introduction

Soil is considered to be one of the most important natural resources for life on Earth. Soil processes govern a wide array of ecosystem services, such as the provision of food, feed and fibre, carbon sequestration, hydrological regulation, and contaminant attenuation (Costanza et al., 1997).

Mostly due to human activities, soil quality, here defined in terms of the soil's ability to deliver ecosystem services, is being drastically reduced in many locations worldwide (Vitousek et al., 1997). Global loss of soil ecosystem services is due to many different environmental threats, such as climate change, intensive agricultural production, and environmental pollution.

In order to come up with effective strategies to protect and enhance soil quality, the Critical Zone Observatory (CZO) network was established across the USA and Europe (Anderson et al., 2008). The CZO network is an internationally coordinated interdisciplinary research effort to better understand the chemical, physical and biological processes that shape the Earth's surface and support the terrestrial life on the planet.

As part of the CZO research effort, the European Commission has provided funding for a large multi-disciplinary research project: Soil Transformations in European Catchments (SoilTrEC). This project aims to understand and quantify the physical, chemical, and biological processes that are critical to soil ecosystem functions and services in the European CZOs (Bernasconi et al., 2011; Menon et al., 2014).

The European CZO network consists of sites along soil formation gradients (Austria, Switzerland, Iceland), along a soil degradation gradient (Greece), along a lithology gradient (Czech Republic), and of agricultural sites differing in soil managements (Austria, Iceland) (Banwart et al., 2011; Menon et al., 2014).

This paper presents the soil quality assessment as carried out for the agricultural CZO sites in Europe. The agricultural sites have been chosen as part of the CZO network, because intensive agricultural production is an important driver of loss in soil quality, e.g. due to decreased organic matter contents. Intensive agriculture may also cause environmental problems, e.g. nitrate leaching to nearby natural ecosystems, and pesticide contamination of surface and groundwater (Skinner et al., 1997). The agricultural CZO sites consist, in total, of eigth farms: four grassland farms in Iceland, of which two are conventional and two organic, and four arable farms in Austria, of which two are conventional and two organic. The organic farms differed from the conventional farms in that only organic fertilisers were applied and no pesticides were used. On the conventional grassland farms in Iceland some organic fertilisation was used in addition

to the artificial inorganic fertilisers. On the conventional arable farms in Austria only artificial inorganic fertilisers were applied together with pesticides. The central idea behind the organic farming practice is that the community of soil organisms will become more important in terms of delivering important soil ecosystem functions, especially in terms of soil structure formation, soil carbon dynamics and nutrient mineralisation, as well as the suppression of soil borne diseases (Birkhofer et al., 2008). The present study investigates biological, physical, and chemical soil quality parameters, focused on soil structure formation, soil organic matter dynamics and nutrient cycling, and the soil as a habitat for species rich communities.

Soil structure is an important attribute of soil quality. Soil aggregates and the pores between the aggregates provide space, water, and oxygen, thereby creating habitats for a large diversity in soil organisms (Anderson, 1978; Sulkava and Huhta, 1998). Soil organisms play an important twofold role in determining soil structure formation. Firstly, microorganisms produce exudates (polysaccharides) that enhance aggregation of soil particles, and fungal hyphae also physically bind soil particles (De Gryze et al., 2005; Tisdall and Oades, 1982; Wright et al., 2007). Secondly, the soil fauna plays a role in creating a stable soil pore structure through moving in the soil and the formation of faecal pellets (Jastrow and Miller, 1991; Lavelle et al., 2006; Lee and Foster, 1991; Oades, 1993). Furthermore, soil structure is strongly linked to soil organic matter (SOM) dynamics, as incorporation of SOM into the soil aggregates "protects" it from microbial decomposition, thereby stabilizing SOM content and sequestering carbon in the soil, with potentially positive effects on plant productivity (Golchin et al., 1994).

Soil organic matter is an essential component of soil quality, governing processes like carbon sequestration, nutrient cycling, water retention, and soil aggregate turnover. Soil organic matter dynamics are driven by land use through root turnover, deposition of plant residues, and decomposition by the soil microbial populations. Soil organisms are known to play important roles in SOM dynamics (de Ruiter et al., 1994; Lavelle et al., 2006; Wardle et al., 2004) by decomposing SOM. This process mineralises carbon (C) and nutrients like nitrogen (N), making these available for plant uptake. To understand the role of soil organisms in decomposition processes, SOM has been defined in terms of fractions based on decomposability (Golchin et al., 1994). The idea behind this fractioning is that the labile fractions, such as dissolved and particulate organic matter, are better available for biological decomposition, contribute more to soil structure formation, and are more sensitive to soil management than more stable fractions such as lignin (Beare et al., 1994; Tisdall and Oades, 1982).

The soil as habitat for species rich communities has increasingly received attention for the intrinsic and functional value of soil biodiversity. High levels of biodiversity are thought to enhance stability of soil functions and services against perturbations and disturbances, and aid in the suppression of soil-borne pests and diseases (Altieri, 1999; Barrios, 2007; Griffiths et al., 2000). Soil biodiversity is also recognised as a sensitive biological indicator for effects of environmental change and disturbance (Pattison et al., 2008; Ponge et al., 2006; Ritz et al., 2009; Wardle, 1995). One of the key indicator groups is the soil microarthropods, because these are abundant, functionally diverse, and respond to a variety of ecological and environmental factors (Gardi and Parisi, 2002; Parisi et al., 2005). In addition, the area covered during the lifecycle is representative of the examined site, and their life histories permit insights into soil ecological conditions (Gardi et al., 2009).

The results presented in this paper are from a field survey on all agricultural CZO sites, in which soil was analysed in terms of its physical, chemical, and biological properties. Soil physical and chemical measurements included soil aggregate size fractions (<20, 20–250, 250–5000 µm); soil organic matter contents and distribution (based on different organic matter fractions); nutrient contents, including nitrogen (N), phosphorus (P), and potassium (K); and soil pH. Soil biological measurements included the presence and abundance of soil microbes (bacteria, fungi) and soil fauna (protozoa, nematodes and microarthropods), representing the main taxonomic groups and trophic levels in the soil food web. In addition we measured the taxonomic richness and diversity within the group of microarthropods, as well as vegetation diversity.

3.2 Methods

3.2.1 Site description

The soils analysed were sampled from the eight agricultural CZO research sites; of these, four are under sub-arctic (Iceland) and four under continental (Austria) climatic conditions (Figure 3.1). The four farms in Iceland were grassland farms, and the four in Austria were arable farms practicing crop rotations (Table 3.1, 3.2). In each country two farms applied "organic" practices and two farms applied "conventional" practices. The organic farms differed from the conventional farms in that only organic fertilisers were applied and no pesticides. On the conventional grassland farms in Iceland, some organic fertilisation was used in addition to the artificial inorganic fertilisers. The organic fields in Iceland were ploughed the first three consecutive years when grasslands were renewed to apply green manure, whereas conventional fields were ploughed only once. On

Table 3.1 Characteristics of the farms studied in Iceland (conventional farms IceHaAcon and IceHiAcon, organic farms IceHaAorg and IceHiAorg), including vegetation richness (values represent mean and standard deviation (between brackets)).

Country	Iceland	Iceland	Iceland	Iceland
Type	Conventional	Organic	Conventional	Organic
Farm	IceHaAcon	IceHaAorg	IceHiAcon	IceHiAorg
Coordinates	N 64°02'33.78 W 20°12'18.06	N 64°03'0.2 W 20°10'44.4	N 64°20'32.82 W 21°34'54.42	N 64°20'42.90 W 21°36'14.22
Average temperature (°C)[1]	3.6	3.6	4.3	4.3
Average rainfall (mm)[1]	1120	1120	800	800
Soil type	Haplic andosol	Haplic andosol	Histic andosol	Histic andosol
Land use type	Grassland	Grassland	Grassland	Grassland
Last tillage	1995	2003	1998	1996
Conversion to organic	-	1996	-	1994
Organic Fertilizers - Manure (t ha^{-1})	20 (spring)	35 (spring)	30 (spring)	30 (spring)
- Compost (t ha^{-1})		35 (fall)		10 (fall)
- Cattle urine (t ha^{-1})		50 (spring)		
- Total N (kg N ha^{-1})	40	970	60	260
- Total C (t C ha^{-1})	0.8	8.6	1.2	3.2
Inorganic fertilizers - Total N (kg ha^{-1})	80 (spring)		80 (spring)	
- Total P (kg ha^{-1})	20 (spring)		20 (spring)	
- Total K (kg ha^{-1})	20 (spring)		30 (spring)	
Vegetation richness	4	7 (0)	4 (0)	8 (1.73)

[1] Icelandic Meteorological Office database, 2012

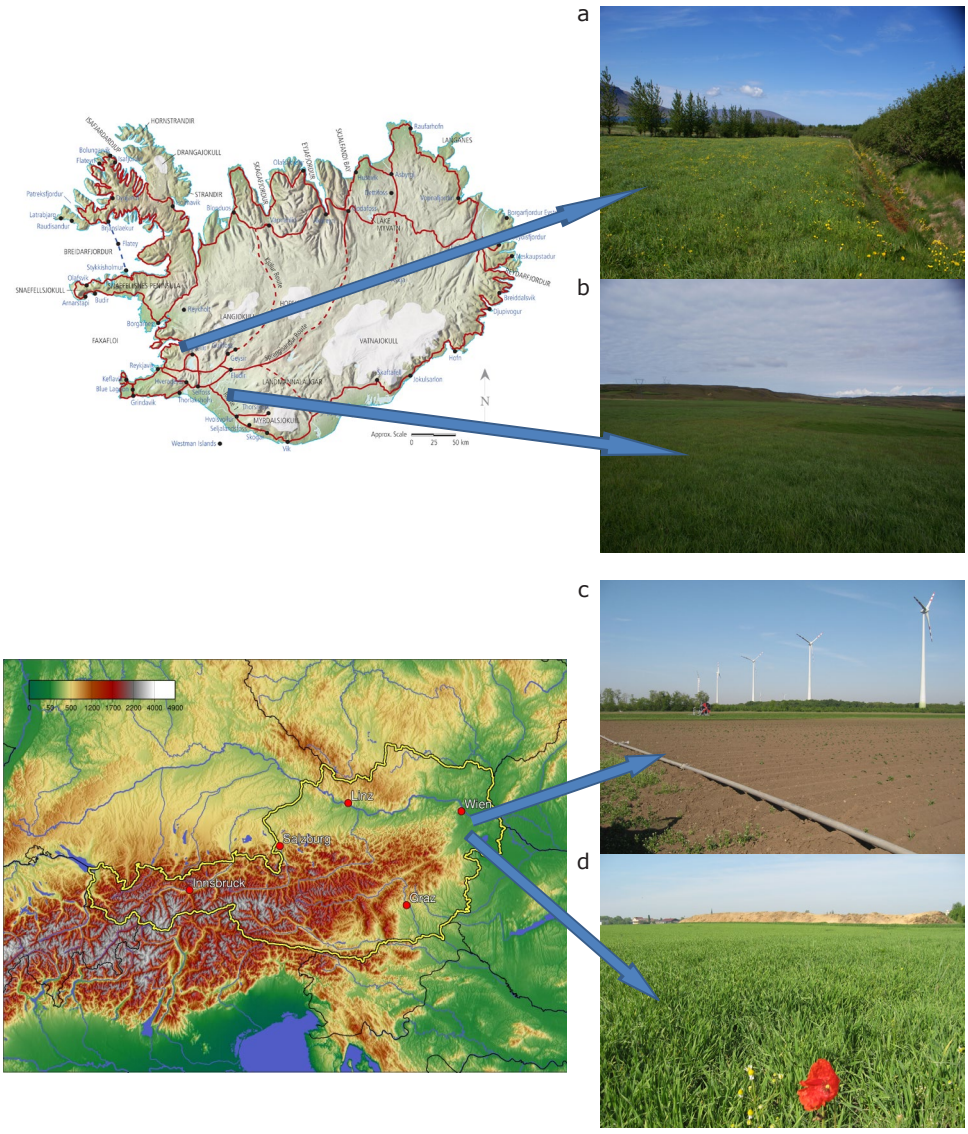

Figure 3.1 Location of the studied farms in Iceland and Austria: IceHiAorg (a), IceHaAcon (b), AusPOTorg (c) and AusWWorg (d).

Table 3.2 Characteristics of the farms studied in Austria (organic farms AusPOTcon and AusWWorg, conventional farms AusPOTcon and AusWWcon). Crop rotation before 2001 was similar to the crop rotation presented in the table.

Country	Austria	Austria	Austria	Austria
Type	Organic	Conventional	Organic	Conventional
Farm	AusPOTorg	AusPOTcon	AusWWorg	AusWWcon
Coordinates	N 48°17'08.7 E 16°41'24.5	N 48°17'09.3 E 16°41'20.9	N 48°13'55.6 E 16°50'05.1	N 48°14'15.3 E 16°50'09.0
Average temperature (°C)1	9.5	9.5	9.5	9.5
Average rainfall (mm)1	525	525	525	525
Soil type	Chernozem	Chernozem	Chernozem	Chernozem
Land use type	Crop rotation (potato)	Crop rotation (potato)	Crop rotation (winter wheat)	Crop rotation (winter wheat)
Last tillage	2010	2010	2010	2010
Conversion to organic	1976	-	1995	-

Crop rotation history	Crop	Biowaste compost (t ha^{-1})[2]	Crop	Fertilizer (kg ha^{-1})	Crop	Horse manure (t ha^{-1})[3]	Crop	Fertilizer (kg ha^{-1})
2011	Potato	10	Potato	N 95, P 50, K 130	Winter wheat		Winter wheat	N 138, P 21, K 21
2010	Soy bean	10	Sugar beet	N 118, P 46, K 60	Sugar beet	20	Corn	N 150, P 40, K 40
2009	Soy bean	10	Winter wheat	N 120	Spring barley		Sugar beet	N 126
2008	Winter wheat	10	Onion		Winter wheat		Winter wheat	N 138, P 40, K 40
2007	Potato	10	Winter wheat	N 120	Peas		Sun flowers	N 60
2006	Soy bean	10	Potato		Spring barley & potato		Winter wheat	N 128, P 24, K 24
2005	Corn	10	Winter wheat	N 120	Sugar beet	20	Corn	N 137
2004	Winter wheat	10	Sugar beet		Winter wheat		Durum wheat	N 129, P 19, K 19
2003	Potato		Winter wheat	N 120	Clover mix		Sugar beet	N 133, P 30, K 30
2002	Poppy		Potato		Clover mix		Winter wheat	N 130, P 25, K 25
2001	Winter wheat		Winter wheat	N 120	Corn		Corn	

[1] Zentralanstalt für Meteorologie und Geodynamik, 2014; [2] Total nitrogen content: 115-164 kg N ha^{-1}; [3] Total nitrogen content: 200-400 kg N ha^{-1}.

the conventional arable farms in Austria only artificial inorganic fertilisers were applied together with pesticides. In Iceland, one pair of organic and conventional farms (in the southwest) were on Histic Andosols; the other pair (southern Iceland) was on Haplic (Brown) Andosols. In Austria, one pair of organic and conventional farms grew potatoes as current crop; the other pair grew winter wheat. All Austrian farms were situated in the Marchfeld, southeast of Vienna, on Haplic Chernozems. Farm properties are listed in Tables 3.1 and 3.2.

3.2.2 Sampling scheme

Samples were taken in May-June 2011 (0–10 cm in Iceland, 0–15 cm in Austria). On each farm, three plots were selected at which all measurements were carried out; the plots were approximately 30–40 m apart. At each plot, mixed soil samples (ca. 1 kg, from 10 to 15 cores) were taken by use of a 8 cm diameter corer for microbial (bacteria, fungi), microfaunal (protozoa, nematodes), soil chemical and physical measurements, and a 5 cm diameter corer for the mesofauna (enchytraeids and microarthropods). In the grasslands on Iceland, vegetation diversity was estimated by application of four 2 m line transects at all farms, except for the conventional farm in southern Iceland, for which the vegetation data were supplied by the farmer. A line-intercept method was applied and four 2 m length tapes were laid out from the sampling point, each tape separated by 90°. Species were recorded each time a plant species intercepted the tape, or when a group of equally mixed plant species occurred (e.g. Kent and Coker (1992)). Vegetation richness was calculated as the total number of plant species present on the transects.

3.2.3 Soil physicochemical measurements

Particle size distribution (clay content) was determined with a combined sieve and pipette method after removal of organic matter with hydrogen peroxide and dispersion by reciprocal shaking with sodium metaphosphate solution for 12 h (Burt, 1992). Soil pH was measured electrochemically (microprocessor pH Meter pH196 WTW, Weilheim, Germany) in H2O at a soil : solution ratio of 1 : 2.5 (Burt, 1992). Calcium (Ca) content was measured by flame atomic absorption spectrophotometry (Perkin-Elmer 2100). Plant available P and K were determined by calcium acetate-lactate (CAL) extraction (ÖNORM L1087).

A three-step procedure was carried out to fractionate soil aggregates and organic matter. Free particulate organic matter (fPOM, 20–5000 µm) was separated using sodium polytungstate solution (density of 1.8 g cm^{-3}). To obtain particulate organic matter occluded in aggregates (oPOM, 20–5000 µm), the heavy fraction of soil aggregates (>1.8 g cm^{-3}) was treated by ultrasound (8 J ml^{-1}), which disrupted the macroaggregates and protected

the microaggregates (Lehtinen et al., 2014). With a subsequent density fractionation step (sodium polytungstate solution, 1.8 g cm^{-3}), the oPOM floating on the suspension was obtained after centrifugation (10 minutes at 4350 rpm). POM fractions were washed with deionised water until the electric conductivity dropped below 5 µS cm^{-1} (Steffens et al., 2009). The residue of the density fractionation procedure – mineral particles and organo-mineral associations – was sieved at 250 and 20 µm to obtain macroaggregates (250–5000 µm) and microaggregates (20–250 and < 20 µm). All aggregate fractions were washed with deionised water until the electronic conductivity dropped below 5 µS cm^{-1}; subsequently they were oven dried at 100°C and weighed. The weights of aggregates were corrected for the sand content of the same size (for aggregates 20–250, and > 250 µm), in order to exclude a sand particle from being weighed as an aggregate (Lehtinen et al., 2014; Six et al., 2000). Mean weight diameter (MWD) of the sand-corrected aggregates was calculated according to Kemper and Rosenau (1986) as the sum of the geometric means of aggregate sizes multiplied by the respective fraction.

Total organic carbon (TOC) and nitrogen (TN) contents, hot-water-extractable carbon (HWC), potentially mineralisable nitrogen (PMN), and C and N mineralisation rates were determined as described in chapter 2.

3.2.4 Soil food web measurements

Soil biological measurements included the presence and abundance of the major taxonomic groups of soil organisms: microbes (bacteria, fungi) and soil fauna (protozoa, nematodes and microarthropods). Within these taxonomic groups we defined 'trophic groups' based on diet and life-history traits, following the method of Moore et al. (1988). Abundances were transformed into estimates of biomass based on body-size information, and expressed in units of kilograms of carbon per hectare for the 0–10 cm top soil layer. The laboratory techniques used to analyse the biological parameters are described in chapter 2.

Regarding the taxonomic species richness in the microarthropods in Damma we used three metrics, i.e. the absolute number of taxa present, the Shannon diversity index (H), and the Pielou evenness index (J).

$$H = -\sum_{i=1}^{N} (p_i \ln(p_i))$$

For the Shannon diversity index (H) we used the following formula: in which p_i is the fraction of the total biomass present in species i, i.e. the relative biomass, of species i, and n the total number of taxa present. A higher index value corresponds to a more diverse community. For the Pielou evenness index (J) we used the formula: $J = H / \ln(n)$, in which H represents the Shannon diversity index, and n the total number of taxa present. A higher value for the evenness index corresponds to a more even distribution of the biomass over the taxa present.

Table 3.3 Soil physicochemical properties and biologically mediated processes on the farms studied in Iceland (conventional farms IceHaAcon and IceHiAcon, organic farms IceHaAorg and IceHiAorg) and Austria (conventional farms AusPOTcon and AusWWcon, organic farms AusPOTcon and AusWWorg). Values represent mean and standard deviation (between brackets) per farm, measured in the topsoil (0-10 cm). Significance values of the factors farming (organic vs. conventional), country (Iceland vs Austria) and the interaction-effect are shown.

Country	Iceland	Iceland	Iceland	Iceland	Austria	Austria	Austria	Austria	Effect	Effect	Effect
Type	Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic	Farming	Country	Interaction
Farm	Ice-HaAcon	Ice-HaAorg	Ice-HiAcon	Ice-HiAorg	Aus-POTcon	Aus-POTorg	Aus-WWcon	Aus-WWorg	p-value	p-value	p-value
Clay content (%)	5.23 (1.28)	5.43 (1.12)	15.93 (10.04)	13.72 (2.66)	17.02 (0.94)	16.70 (1.62)	13.93 (0.24)	14.40 (1.76)	0.995	0.165	0.997
pH (H2O)	5.76 (0.22)	5.88 (0.17)	5.07 (0.13)	5.17 (0.17)	7.92 (0.04)	7.95 (0.02)	8.04 (0.02)	8.12 (0.03)	0.757	0.001	0.934
Ca (kg ha^{-1})	74.8 (7.29)	96.2 (10.2)	129 (20.0)	190 (38.9)	37868 (6963)	42176 (1102)	110542 (5014)	107955 (10926)	0.955	0.001	0.999
P (kg ha^{-1})	3.75 (1.42)	3.43 (0.68)	8.10 (2.23)	4.79 (1.17)	180.77 (38.54)	164.88 (40.63)	124.17 (8.12)	123.07 (13.58)	0.785	0.001	0.859
K (kg ha^{-1})	15.86 (14.88)	28.04 (21.70)	7.97 (6.64)	6.02 (1.16)	317.86 (69.63)	109.30 (19.53)	161.01 (38.62)	281.92 (24.77)	0.758	0.026	0.698
Bulk density (g cm^{-3})	0.79 (-)	0.74 (-)	0.46 (-)	0.63 (-)	1.45 (-)	1.54 (-)	1.40 (-)	1.38 (-)			
MWD[1] (mm)	8.27 (3.75)	19.91 (4.10)	4.75 (1.20)	11.70 (0.84)	9.98 (4.57)	7.64 (2.37)	4.50 (2.41)	3.82 (2.24)	0.236	0.169	0.125
fPOM[2] (g kg^{-1})	33.12 (15.45)	23.46 (1.31)	444.12 (142.39)	358.52 (66.42)	2.20 (0.97)	2.20 (0.59)	2.63 (0.22)	3.54 (0.49)	0.867	0.185	0.865
oPOM[3] (g kg^{-1})	5.69 (1.43)	7.22 (1.71)	72.67 (50.11)	29.74 (18.37)	2.01 (0.32)	2.06 (0.37)	1.69 (0.35)	2.32 (0.42)	0.595	0.203	0.584

Table 3.3 Continued

Country	Iceland	Iceland	Iceland	Iceland	Austria	Austria	Austria	Austria	Effect	Effect	Effect
Type	Conven-tional	Organic	Conven-tional	Organic	Conven-tional	Organic	Conven-tional	Organic	Farming	Country	Interaction
Farm	Ice-HaAcon	Ice-HaAorg	Ice-HiAcon	Ice-HiAorg	Aus-POTcon	Aus-POTorg	Aus-WWcon	Aus-WWorg	p-value	p-value	p-value
TOC[4] (kg ha^{-1})	47354 (7192)	51597 (6967)	88317 (21026)	78723 (5452)	22093 (799)	27792 (6113)	28004 (968)	25654 (2503)	0.893	0.010	0.876
HWC[5] (kg ha^{-1})	716.8 (123.1)	904.1 (76.05)	1931 (564.9)	2135 (243.0)	502.4 (110.6)	488.5 (58.10)	565.8 (34.08)	702.5 (59.91)	0.696	0.072	0.905
WSC[6] (kg ha^{-1})	11.77 (21.23)	36.79 (1.44)	55.85 (5.29)	65.21 (8.28)	-	-	-	-			
Total N (kg ha^{-1})	3128 (676)	3439 (554)	5615 (1333)	5476 (477)	1990 (101)	2232 (171)	2074 (279)	2093 (116)	0.782	0.020	0.968
PMN[7] (kg ha^{-1})	58.46 (16.83)	71.21 (5.71)	152.45 (62.55)	162.33 (18.22)	13.67 (10.49)	12.46 (4.20)	21.37 (7.39)	42.70 (14.45)	0.577	0.022	0.839
C min[8] (kg ha^{-1} y^{-1})	5069 (238.6)	5113 (353.9)	2654 (641.8)	2157 (1601)	3263 (506.4)	4467 (282.3)	4412 (148.9)	4544 (261.5)	0.914	0.507	0.572
N min[9] (kg ha^{-1} y^{-1})	282.6 (96.90)	215.9 (52.40)	745.9 (280.7)	1010 (82.75)	89.46 (67.10)	26.21 (65.86)	90.98 (28.47)	97.41 (10.82)	0.680	0.032	0.624

[1] Aggregate size distribution (mean weight diameter); [2] Free particulate organic matter; [3] Occluded particulate organic matter; [4] Total Soil Organic Carbon; [5] Hot water extractable Carbon; [6] Water soluble Carbon; [7] Potential mineralisable Nitrogen; [8] Carbon mineralisation rate; [9] Nitrogen mineralisation rate.

3.2.5 Statistics

The data were from eight farms that differed in various ways: climate, soil type, soil management, and crop. There were no real replicates, as the triplicate measurements for all variables were from plots on the same farm. Hence, we performed a nested two-way ANOVA with two factors: country (Iceland–Austria) and farm management (organic–conventional), and farm as a random nested factor. By taking country as a factor, we separated the grassland (Iceland) from the cropland (Austria) farms. By including farm as a random nested factor, we accounted for the variation among farms. We tested the differences between soil types separately using an one-way ANOVA with soil as factor. All data were log-transformed to obtain homogeneity of variances. Statistical analyses were carried out using SPSS (20.0.0) and R (2.15.2).

3.3 Results

3.3.1 Soil physicochemical measurements

Many physicochemical soil characteristics varied strongly over farms, as a consequence of different soil types, soil management, and climatic conditions (countries) (Table 3.3). The most pronounced differences were found between the soils from the two different countries. Clay content was lowest in the Haplic Andosols in Iceland ($p = 0.001$). Soils in Austria were alkaline (pH 8) as a result of the much higher calcium content of the Chernozems, whereas the Andosols in Iceland had a lower pH (pH 5–6). Plant available nutrients (P, K) were much higher on the farms in Austria than in Iceland, due to the strong nutrient retention in Andosols ($p = 0.001$ and $p = 0.026$, Table 3.3).

For the MWD of soil aggregates we found a difference between farm management: on the organic farms in Iceland the MWD was more than twice as high as on the conventional farms, although the difference was not statistically significant ($p = 0.173$). The opposite was found in Austria, although here the differences were relatively small (Table 3.3). Mean weight diameter was positively correlated to fungal (Pearson test, $r = 0.739$, $p = 0.006$) and bacterial biomass (Pearson test, $r = 0.664$, $p = 0.019$), whereas no significant correlations were found with organic matter parameters. The content of free particulate organic matter (fPOM) and occluded particulate organic matter (oPOM) varied strongly between the different countries and between soil types within countries. The fPOM content in the Icelandic Histic Andosols (358–444 g kg^{-1}) was higher than in the Icelandic Haplic Andosols (23–33 g kg^{-1}), and all Austrian soils (2–3 g kg^{-1}, $p<0.001$). The oPOM content showed a similar pattern. The high contents of particulate organic matter in Iceland, especially in the Histic Andosols, reflect the very high content of organic carbon (contents of

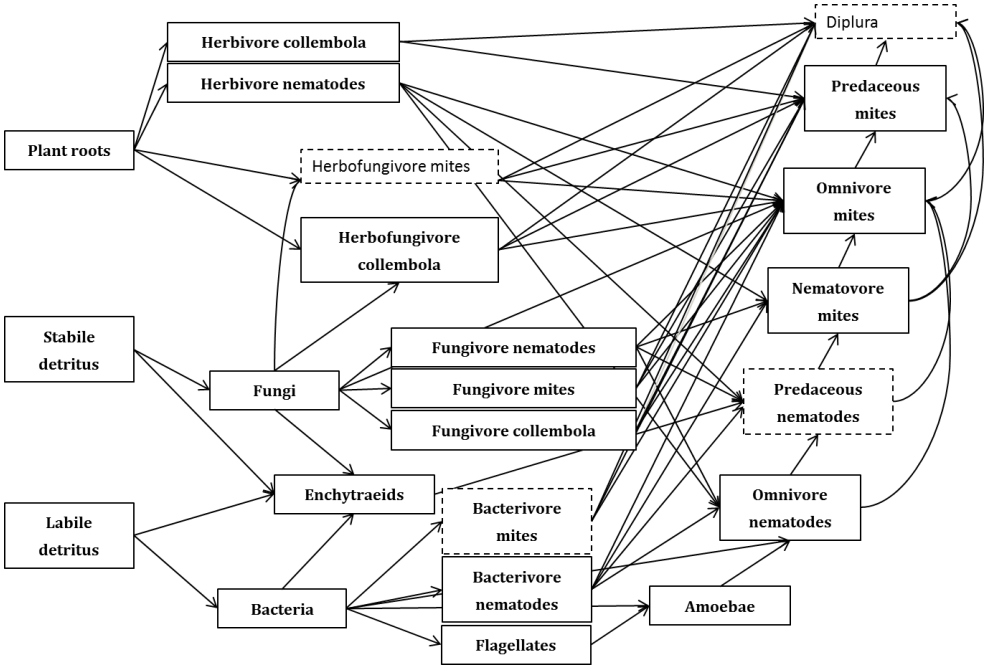

Figure 3.2 Soil food web diagram representative for all eight farms. Boxes represent the presence of trophic groups in the soil food web, arrows represent feeding interactions based on diet information. Solid groups were present at all farms, dashed groups were only present at some farms.

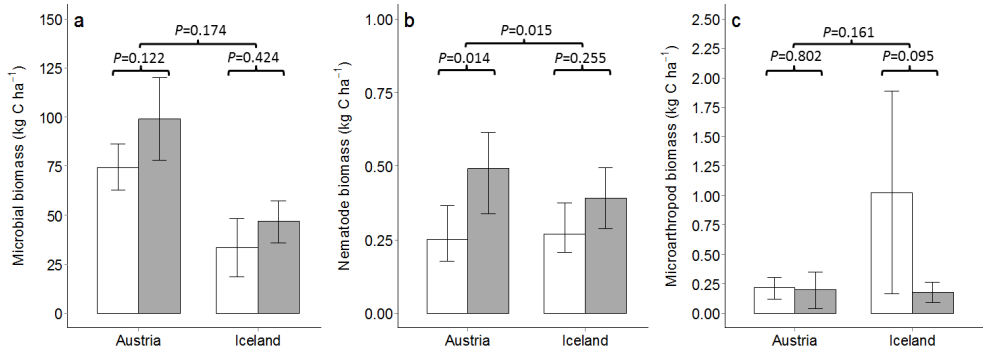

Figure 3.3 Biomass in kg C ha^{-1} of microbes (bacteria+fungi) (a), nematodes (b) and microarthropods (c) on organic and conventional farms in Austria and Iceland. Bars are means ± standard deviation (n = 6), measured in the topsoil (0-10 cm). P values are the results of a nested univariate analysis of variance (ANOVA), with type (conventional (white bars) or organic (grey bars)) and country (Austria or Iceland) as factors.

TOC, HWC, and WSC) and nitrogen (both total N and PMN) in these soils: TOC (p = 0.010), HWC (p = 0.072), total N (p = 0.020), and PMN (p = 0.022) were all higher in Iceland compared to Austria. The farms on Histic Andosols in Iceland had a lower C mineralisation rate (2157–2654 kg ha^{-1} yr^{-1}), but a much higher potential N mineralisation rate (746–1010 kg ha^{-1} yr^{-1}) than the farms on Haplic Andosols in Iceland; these differences were even more pronounced compared to the farms in Austria (p = 0.032).

The way organic carbon (OC) and nitrogen (N) were distributed over aggregate sizes and organic matter fractions, was also different between farms. On the organic farm on Haplic Andosol in Iceland, macroaggregates >250 µm contributed the greatest quantities of OC and N to bulk soil (65% OC, 65% for N). On both farms on Histic Andosols in Iceland, the fPOM fraction contributed the largest quantities of OC and N to bulk soil (61 and 69% for OC and 56 and 62% for N, respectively). On the winter wheat farms in Austria, microaggregates of 20–250 µm contributed the greatest quantities of OC and N to bulk soil (46 and 50% for OC and 45 and 45% for N, respectively), while on the potato farms in Austria the microaggregates <20 µm contributed the greatest quantities of OC and N to bulk soil (51 and 46% for OC; 51 and 47% for N, respectively).

3.3.2 Soil food web measurements

Based on presence-absence data of the soil organisms, we constructed soil food web diagrams for all farms (Figure 3.2). These diagrams were very similar; despite differences in climatic conditions, crop type, soil type, and soil management, most of the trophic groups were present on all farms. Some of the trophic groups were only present at some farms, including predaceous nematodes, bacterivore mites, herbofungivore mites and Diplura (Fig. 3.2, Table 3.4).

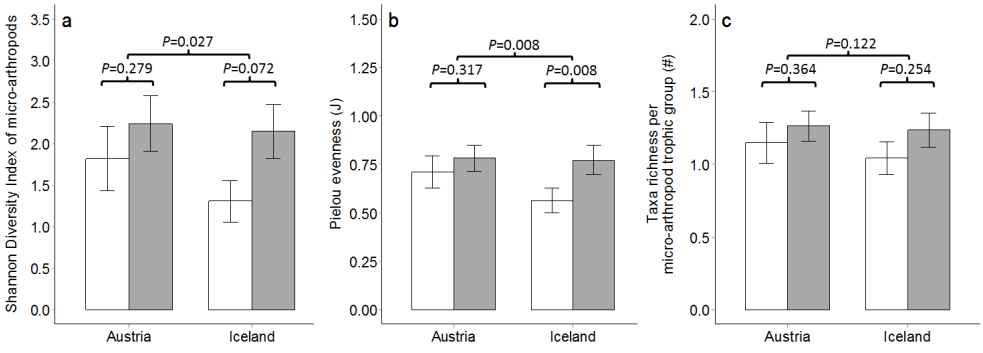

Figure 3.4 Shannon diversity index on microarthropod taxa (a), Pielou evenness on microarthropod taxa (b) and absolute microarthropod taxa richness (c) on organic and conventional farms in Austria and Iceland. Bars are means ± standard deviation (n = 6), measured in the topsoil (0-10 cm). P values are the results of a nested univariate analysis of variance (ANOVA), with type (conventional (white bars) or organic (grey bars)) and country (Austria or Iceland) as factors.

Table 3.4 Biological parameters on the farms studied in Iceland (conventional farms IceHaAcon and IceHiAcon, organic farms IceHaAorg and IceHiAorg) and Austria (conventional farms AusPOTcon and AusWWcon, organic farms AusPOTcon and AusWWorg): biomasses (kg C ha^{-1}) of the trophic and taxonomic groups in the soil food webs, bacterial activity and microarthropod diversity. Numbers represent mean and standard deviation (between brackets), measured in the topsoil (0-10 cm), nd: not detected. Significance values of the factors farming (organic vs. conventional), country (Iceland vs Austria) and the interaction-effect are shown.

Country	Iceland	Iceland	Iceland	Iceland	Austria	Austria	Austria	Austria	Effect	Effect	Effect
Type	Conven-tional	Organic	Conven-tional	Organic	Conven-tional	Organic	Conven-tional	Organic	Farming	Country	Interaction
Farm	Ice-HaAcon	Ice-HaAorg	Ice-HiAcon	Ice-HiAorg	Aus-POTcon	Aus-POTorg	Aus-WWcon	Aus-WWorg	p-value	p-value	p-value
Bacteria	27.70 (3.41)	38.00 (3.51)	17.89 (6.32)	30.04 (9.62)	55.49 (14.14)	68.48 (15.49)	53.80 (9.86)	94.88 (22.20)	0.062	0.005	0.920
Leu (pmol g-1 h^{-1})[1]	-10.27 (15.01)	45.44 (27.95)	126.5 (64.52)	133.8 (37.45)	163.5 (78.72)	90.41 (9.38)	101.8 (8.30)	152.7 (50.74)	0.509	0.294	0.476
Fungi	16.93 (8.70)	16.61 (2.79)	4.33 (1.82)	8.67 (1.76)	18.34 (2.54)	19.54 (6.94)	21.14 (9.32)	15.00 (3.02)	0.736	0.187	0.522
Amoebae	0.63 (0.24)	1.03 (0.37)	0.82 (0.49)	4.03 (2.03)	3.02 (1.28)	1.63 (0.09)	2.68 (1.26)	3.04 (2.47)	0.315	0.101	0.122
Flagellates	0.62 (0.35)	0.31 (0.06)	0.21 (0.02)	1.85 (1.43)	0.53 (0.26)	0.49 (0.18)	1.08 (0.30)	0.78 (0.12)	0.655	0.587	0.448
Bacterivore nematodes	0.07 (0.03)	0.08 (0.02)	0.12 (0.01)	0.19 (0.02)	0.15 (0.09)	0.20 (0.09)	0.13 (0.03)	0.13 (0.04)	0.411	0.348	0.945
Fungivore nematodes	0.003 (0.003)	0.014 (0.013)	0.001 (0.001)	0.023 (0.007)	0.021 (0.019)	0.033 (0.028)	0.055 (0.032)	0.030 (0.008)	0.589	0.049	0.262
Herbivore nematodes	0.11 (0.03)	0.13 (0.05)	0.07 (0.03)	0.12 (0.03)	0.09 (0.03)	0.21 (0.02)	0.03 (0.02)	0.15 (0.02)	0.035	0.700	0.169
Omnivore nematodes	0.13 (0.03)	0.13 (0.08)	0.02 (0.02)	0.02 (0.03)	0.02 (0.02)	0.06 (0.11)	0.01 (0.02)	0.17 (0.09)	0.403	0.811	0.337
Predaceous nematodes	Nd	nd	nd	0.09 (0.11)	nd	nd	nd	nd	0.374	0.374	0.374
Total nematode biomass	0.33 (0.02)	0.35 (0.03)	0.21 (0.01)	0.44 (0.14)	0.28 (0.10)	0.51 (0.22)	0.23 (0.05)	0.48 (0.10)	0.015	0.606	0.335

Table 3.4 Continued

Enchytraeids	0.79 (0.94)	0.13 (0.07)	0.09 (0.09)	0.33 (0.26)	0.15 (0.21)	nd	nd	0.01 (0.01)	0.538	0.153	0.896
Bacterivore mites	Nd	nd	nd	nd	0.002 (0.002)	0.007 (0.009)	nd	nd	0.562	0.275	0.562
Fungivore mites	0.001 (0.001)	0.033 (0.040)	0.004 (0.003)	0.003 (0.003)	0.001 (0.001)	0.001 (0.001)	0.009 (0.008)	0.025 (0.036)	0.310	0.914	0.710
Herbofungivore mites	Nd	nd	0.001 (0.002)	0.001 (0.001)	nd	nd	nd	0.002 (0.001)	0.466	0.868	0.732
Nematovore mites	0.006 (0.011)	0.030 (0.027)	nd	0.003 (0.004)	nd	0.001 (0.001)	0.005 (0.005)	0.002 (0.003)	0.434	0.316	0.350
Omnivore mites	0.52 (0.78)	0.01 (0.01)	0.80 (0.56)	0.02 (0.01)	0.07 (0.05)	0.03 (0.01)	0.16 (0.04)	0.04 (0.004)	0.012	0.050	0.037
Predaceous mites	0.058 (0.074)	0.013 (0.013)	0.076 (0.044)	0.029 (0.014)	0.008 (0.006)	0.005 (0.002)	0.037 (0.005)	0.060 (0.027)	0.357	0.393	0.177
Total Acari	0.88 (0.60)	0.06 (0.02)	0.58 (0.85)	0.07 (0.06)	0.08 (0.05)	0.03 (0.01)	0.21 (0.02)	0.13 (0.06)	0.023	0.069	0.053
Fungivore collembola	0.134 (0.071)	0.058 (0.041)	0.305 (0.186)	0.112 (0.057)	0.052 (0.085)	0.021 (0.015)	0.039 (0.012)	0.188 (0.014)	0.606	0.274	0.192
Herbofungivore collembola	0.009 (0.009)	nd	0.101 (0.106)	0.009 (0.004)	0.010 (0.008)	0.003 (0.005)	0.010 (0.012)	0.012 (0.009)	0.307	0.416	0.357
Herbivore collembola	0.02 (0.027)	0.034 (0.022)	0.023 (0.029)	0.004 (0.004)	0.014 (0.008)	0.001 (0.001)	0.002 (0.004)	nd	0.569	0.131	0.750
Total Collembola	0.43 (0.31)	0.12 (0.05)	0.16 (0.08)	0.09 (0.06)	0.08 (0.08)	0.03 (0.01)	0.05 (0.02)	0.20 (0.01)	0.649	0.191	0.369
Diplura	Nd	nd	nd	nd	nd	nd	0.002 (0.004)	nd	0.374	0.374	0.374

Table 3.4 Continued

Total microarthropod biomass (kg C ha^{-1})	0.75 (0.92)	0.18 (0.12)	1.31 (0.87)	0.18 (0.07)	0.15 (0.09)	0.07 (0.01)	0.27 (0.04)	0.33 (0.07)	0.161	0.239	0.290
Microarthropod taxa richness (# taxa)	10.33 (1.15)	20.67 (2.08)	10.33 (4.04)	12.67 (1.53)	12.00 (5.29)	14.67 (3.21)	15.33 (4.16)	21.00 (1.00)	0.122	0.449	0.707
Shannon H index	1.33 (0.11)	2.38 (0.09)	1.28 (0.37)	1.91 (0.29)	1.60 (0.30)	2.07 (0.33)	2.05 (0.37)	2.41 (0.29)	0.027	0.176	0.311
Pielou evenness J	0.57 (0.06)	0.79 (0.03)	0.56 (0.08)	0.75 (0.11)	0.66 (0.04)	0.77 (0.06)	0.76 (0.08)	0.79 (0.08)	0.008	0.049	0.069
# taxa / trophic group	1.03 (0.12)	2.07 (0.21)	1.03 (0.40)	1.27 (0.15)	1.20 (0.53)	1.47 (0.32)	1.53 (0.42)	2.10 (0.10)	0.122	0.449	0.707

[1] bacterial activity: Leucine incorporation rate

Trophic groups showed differences in abundances (Table 3.3) and species composition (see microarthropod diversity). Bacterial biomass was consistently higher on organic farms in both countries, although the differences were not statistically significant. Bacterial activity, measured as the incorporation rate of [^{14}C]leucine, did not differ significantly between farms. Fungal biomass did not show a consistent pattern over all farms, although fungal biomass tended to be lower on the farms on Histic Andosols. Protozoa (amoebae, flagellates) and enchytraeids showed no clear pattern in biomass (Table 3.4).

Nematode biomass was consistently higher on organic farms than in conventional farms, regarding all trophic groups, although differences were only statistically significant for herbivorous nematodes ($p = 0.035$) and total nematode biomass ($p = 0.015$, Fig. 3.3b).

Microarthropod abundance varied strongly from just over 12 000 m^{-2} to over 200 000 m^{-2}. We did not find systematic differences between country or management type. Total microarthropod biomass was much higher on the conventional farms in Iceland compared to all other farms (Fig. 3.3c). Total Acari biomass was significantly higher on conventional farms compared to organic farms ($p = 0.023$, Table 3.4). The higher biomass of omnivorous mites ($p = 0.012$) and, to a lesser extent, also the consistently higher Acari biomass ($p = 0.023$) on conventional farms was fully accounted for by the high biomass of the astigmatid mite *Tyrophagus similis*. *T. similis* accounted for 98.1 and 99.7% of the total omnivorous mite biomass, and 59.8 and 69.7% of the total microarthropod biomass in the conventional grasslands in Iceland, while this species was (nearly) absent at all other farms (Appendix 3.1). In Iceland, Collembolan biomass was higher on conventional farms compared to organic farms (Table 3.4).

3.3.3 Microarthropod species identity and diversity

In total, 82 taxa of microarthropods were found in our study sites, with an overall larger diversity in Austria than Iceland. All farms showed striking differences in the microarthropod species composition: only three taxa out of the 82 taxa were present on all farms (the mesostigmatid *Arctoseius cetratus* and the prostigmatids *Eupodes sp.* and *Pygmephorus sp.*). In Iceland, 27 taxa were found that did not occur in Austria, and 37 taxa were found only in Austria, while only 18 taxa were found in both countries. The number of taxa only occurring on organic farms amounted to a total of 33, either in Iceland (14 taxa) or in Austria (18 taxa), while 1 taxon (*Tyrophagus sp.*) was found on organic farms both in Iceland and in Austria. Moreover, 12 taxa were found only on conventional farms, of which 5 were in Iceland and 7 in Austria. The organic wheat farm in Austria had a remarkably high microarthropod taxonomic richness, with 34 taxa present, of which 12 were unique for that farm. The conventional

grasslands in Iceland in particular had low taxonomic richness of only 18 taxa (HiAcon) and 17 taxa (HaAcon).

Organic farms had a significantly higher microarthropod diversity measured according to all diversity measures; for the Shannon index ($p = 0.027$, Fig. 3.4a) and the Pielou index for evenness ($p = 0.008$, Fig. 3.4b), differences were statistically significant; for taxonomic richness it was not statistically significant ($p = 0.122$, Fig. 3.4c).

3.4 Discussion

In this study we investigated soil quality parameters (physical, chemical, and biological) on the organically and conventionally managed farms that are part of the European CZO network.

3.4.1 Soil aggregate formation, soil organic matter, and soil nutrient cycling

Regarding soil structure formation and soil organic matter, the different farming practices, organic versus conventional, did not reveal systematic differences in many physical and chemical soil properties. The soil aggregate size distributions were consistently higher on organic than on conventional farms in Iceland, but no differences were found in Austria. Other management practices such as tillage (Beare et al., 1994) or crop rotation history may have obscured effects of organic amendments. For example, the arable farms in Austria applied a crop rotation with a yearly tillage. As soil aggregates are sensitive to soil tillage (Beare et al., 1997; Beare et al., 1994; Six et al., 2000), it could be expected that the differences between organic and conventional arable farms are comparably small. In contrast, the Icelandic grasslands had not been tilled for 8–16 years (Table 3.1). Also the addition of higher quantities of organic amendments was expected to have a positive effect through enhanced soil biological activity, in terms of aggregate-forming substances. However, the observed higher mean weight diameters on the organic farms on Iceland could not be linked to higher organic matter contents, e.g. in terms of total carbon, or a difference in organic matter composition. However, mean weight diameter of aggregates was significantly correlated with fungal and bacterial biomass. Both bacteria and fungi produce soil-binding compounds like polysaccharides, which are important for production of relatively small aggregates (De Gryze et al., 2005; Wright et al., 2007). Soil fungi are assumed to be especially more important for the formation of larger soil aggregates through entanglement by hyphae (Tisdall and Oades, 1982).

Regarding the soil carbon and nitrogen, we also did not detect systematic differences between organic and conventional farming. C and N mineralisation rates as well as the measured C and N pools (TOC, HWC, Total N, PMN; Table 3.3) were quite similar on organic and conventional farms. Furthermore, bacterial activity was similar on organic and conventional farms. The present results partly confirm the results reported from earlier studies (Bloem et al., 2006b; van Diepeningen et al., 2006).

In summary, C and N contents and dynamics between organic and conventional farms have been studied in three different ways: factorial field experiments on a single farm, pairwise comparisons of farms (as in our study), and comparisons across larger number of farms (n = 10–20). In a factorial field experiment on an arable farm, the Lovinkhoeve in the Netherlands, Bloem et al. (1994) found a higher C and N mineralisation in an integrated field compared to a conventional field, probably as a result of organic amendments. Similarly, on a grassland farm in the Netherlands, a higher N mineralisation and potentially mineralisable N has been measured when organic fertiliser was applied, while no difference has been found in C mineralisation (van Eekeren et al., 2009). Also, Poudel et al. (2002) found a higher potential N mineralisation in organically managed crop rotation fields than in conventional fields in California, but here the organic fields also grew legumes between growing seasons, enhancing N availability. In Switzerland, Birkhofer et al. (2008) observed a lower N mineralisation when only mineral fertiliser was used, while C mineralisation did not show differences between the fields. Also, in this study, no differences were found between organic fields and fields that received both artificial fertilisers and organic manure, similar to the Icelandic grasslands in the present study. Thus, in factorial experiments on a single farm, the effects of organic management on soil N dynamics are quite clear, while the effects on C dynamics are not consistent.

In an example of a pairwise comparison between organic and conventional arable farms in the Netherlands, van Diepeningen et al. (2006) observed lower nitrate levels on organic farms, with no differences in total organic C, organic N, or total N. Conventional farms in that study also applied organic manure in addition to artificial fertilisers, which is comparable to the grasslands in Iceland, where we also did not find differences in total organic C and total N. In an example of a comparison across larger number of farms in the Netherlands (n=10–20), Bloem et al. (2006b) showed higher C and N mineralisation rates in organic grasslands compared to conventional grasslands, but not in the comparison between organic and conventional arable farms. Thus, our study confirms the notion that more factors are variable and differences between organic and conventional farming are less prominent when C and N dynamics are studied on a larger scale with more farms involved.

3.4.2 Soil food web structure

The trophic structure of the soil food webs showed a high similarity; nearly all trophic groups were present on all farms. This indicates that the trophic structure of the soil food webs was neither very sensitive to management nor to climate, soil type, and farm type. Biomass of the different organisms, however, differed between farms.

Microbial biomass, as the sum of bacteria and fungi, was consistently higher on organic farms, although not statistically significant. The higher microbial biomass, especially bacterial biomass, is in line with previous studies that have compared organic and conventional farms (Birkhofer et al., 2008; Bloem et al., 2006b; Gunapala and Scow, 1998; Haubert et al., 2009; Hole et al., 2005; Mäder et al., 2002; van Diepeningen et al., 2006). Other studies also have reported a higher microbial activity (Bloem et al., 2006b; Hole et al., 2005), which we did not find in our study. We did not find differences in fungal biomass, in contrast with some previous results (De Vries et al., 2006; Yeates et al., 1997), but in line with others (Shannon et al., 2002). These results might be due to the fact that added organic amendments in organic farming are generally easily degradable and therefore enhance mainly bacterial biomass and activity (Hole et al., 2005).

We observed a significantly higher total nematode biomass on organic farms. Although a higher biomass was observed for all trophic groups of nematodes, the difference was mostly counted for by herbivorous nematodes. This is in agreement with the higher nematode abundance that was found after addition of organic manure to wheat fields in Switzerland, where herbivorous nematodes were also the dominant group (Birkhofer et al., 2008). It is also in agreement with the higher nematode abundance (although dominated by fungivores) found in organic grasslands in Wales (Yeates et al., 1997). Hence, our results support the notion that nematodes are sensitive to farming type and that they profit from the addition of organic amendments.

Microarthropod biomass measurements did not reveal systematic differences between farm types, although total microarthropod biomass was highest on the conventional farms within Iceland. We also did not find a difference between the grassland farms in Iceland and the arable farms in Austria. This is a bit unexpected, because it is frequently observed that microarthropod biomass is higher in grasslands compared to arable farms, because ploughing decreases microarthropod biomass, which is more intense for root/tuber crops such as potato (Vreeken-Buijs et al., 1998). In our study, the organic grasslands in Iceland were however ploughed in the three consecutive years when the field was renewed which, together with the colder climatic conditions, may explain why

biomass of microarthropods was not higher in the grasslands than in the arable fields (Sjursen et al., 2005).

We found a statistically higher biomass of mites (Acari) on the conventional farms compared to the organic farms. We lack an explanation for this somewhat unexpected result. For example, it is contrary to the results from an earlier study, showing higher abundances of Acari in organic grasslands compared to conventional grasslands in Wales (Yeates et al., 1997). The similar collembolan biomass on organic and conventional farms is in line with the results of Birkhofer et al. (2008) in Switzerland, but in contrast with the results of Bardgett et al. (1993), who reported higher collembolan biomass in organic fields. The two species of Collembola that are by far the most abundant in the study of Bardgett et al. (1993) were much less abundant (*Onychiurus procampatus*) or even absent (*Folsomia quadrioculata*) in our data, which may explain the difference between the studies.

3.4.3 Microarthropod diversity

The most systematic difference we found in the comparison between organic and conventional farming, was the higher microarthropod diversity on the organically managed farms. This difference was found across countries, farm types (grassland versus arable), and crop and soil type. This finding is in agreement with Doles et al. (2001) and Macfadyen et al. (2009).

Factors known to enhance soil microarthropod diversity include plant litter diversity leading to a higher microhabitat and resource diversity (Hansen and Coleman, 1998) and plant species identity (Wardle, 2005). In Iceland, organic grasslands had a higher plant diversity than conventional grasslands, which supports the hypothesis that plant diversity enhances belowground microarthropod diversity. On the arable farms in Austria, where plant diversity does not play a role, the application of artificial fertilisers may have reduced the microarthropod diversity (Siepel and van de Bund, 1988).

Soil microarthropod diversity is described as a sensitive biological indicator for effects of environmental change and disturbance on soil quality (Gardi et al., 2009; Gardi and Parisi, 2002; Parisi et al., 2005). Our results confirm that the taxonomic diversity of the soil microarthropods was sensitive to differences in farm type and management system.

If we look at these findings in terms of the role of biodiversity in ecosystem functioning, we see that the higher microarthropod diversity on organic farms did not result in differences in the food web structure, nor did it yield higher ecosystem services, such as soil fertility or C sequestration. This is in agreement with Setälä et al. (2005), who argue that the functional

importance of individual groups is rather high at coarse (trophic group) level but low at species level, and that effects of species diversity on ecosystem functioning are most likely found in studies with a very low species richness and therefore a low functional redundancy. Nevertheless, in our study microarthropod diversity was found to be a sensitive and consistent indicator for land management. At present, determining microarthropod diversity is a relatively intensive activity, but when the current progresses in methodology lead to faster and cheaper analyses, such as barcoding extracted microarthropods, soil microarthropod diversity will become more cost-effective and an even more valuable indicator for soil quality.

3.4.4 Conclusions

In this study we investigated soil biological, chemical and physical parameters for soil quality on organically and conventionally managed farms. The chosen farms were part of the European Critical Zone Observatory network. Factors that vary across farms, such as climate, soil type, and farm type, and the limited number of replicates taken, have made it difficult to find clear patterns or draw general conclusions. On the other hand, we did observe that the organic farms showed higher biological parameters, in particular the diversity in soil micro-arthropods, despite these limitations. Physical and chemical parameters showed no clear differences between the organic and conventional farms. Our results therefore do support the use of micro-arthropod diversity as a soil quality indicator, although physical and chemical soil properties are indispensable for a complete assessment and understanding of soil quality.

Appendix 3.1 Biomass (kg C ha^{-1}) of the microarthropod taxa in the soil food web on the farms studied in Iceland (conventional farms IceHaAcon and IceHiAcon, organic farms IceHaAorg and IceHiAorg) and Austria (conventional farms AusPOTcon and AusWWcon, organic farms AusPOTcon and AusWWorg). Trophic groups: omnivorous mites (Ommi), bacterivorous mites (Bami), fungivorous mites (Fumi), nematovorous mites (Nemi), predatory mites (Prmi), herbofungivorous mites (HFmi), herbofungivorous collembolans (HFco), fungivorous collembolans (Fuco) and diplurans (Dipl). Numbers represent mean and standard deviation (between brackets), measured in the topsoil (0–10 cm).

Country		Iceland	Iceland	Iceland	Iceland	Austria	Austria	Austria	Austria
Type		Conventio-nal	Organic	Conven-tional	Organic	Conventio-nal	Organic	Conventio-nal	Organic
Farm		IceHaAcon	IceHaAorg	IceHiAcon	IceHiAorg	AusPOTcon	AusPOTorg	AusWWcon	AusW-Worg
Astigmata									
Acaridae	Ommi		0.0063 (0.011)						
Astigmata	Ommi						0.0010 (0.0018)		
Histiostoma	Bami					0.0002 (0.0002)	0.0007 (0.0009)		
Rhizoglyphus	Fumi		0.0314 (0.0395)						
Schwiebea	Fumi								0.0205 (0.0347)
Tyrophagus	Ommi		0.0003 (0.0005)						0.0020 (0.0017)
Tyrophagus similis	Ommi	0.5194 (0.7805)		0.7801 (0.5551)	0.0002 (0.0004)		0.0003 (0.0005)		
Mesostigmata									
Alliphis siculus	Nemi	0.0020 (0.0034)	0.0094 (0.0086)		0.0003 (0.0005)		0.0001 (0.0002)	0.0012 (0.0011)	0.0006 (0.0010)
Arctoseius	Prmi				0.0007 (0.0012)				
Arctoseius cetratus	Prmi	0.0320 (0.0553)	0.0031 (0.0054)	0.0207 (0.0256)	0.0186 (0.0130)	0.0032 (0.0056)	0.0015 (0.0015)	0.0067 (0.0117)	0.0169 (0.0118)

Appendix 3.1 Continued

Arrhopalites caecus	Prmi				0.0011 (0.0020)				
Dendrolaelaps	Prmi						0.0011 (0.0010)		
Dendrolaelaps rectus	Prmi								0.0101 (0.0174)
Dendrolaelaps samsinaki	Prmi								0.0034 (0.0058)
Dendrolaelaps zwoelferi	Prmi								0.0026 (0.0045)
Dinychus perforatus	Ommi		0.0010 (0.0018)						
Evimirus uropodinus	Nemi						0.0001 (0.0002)		
Hypoaspis	Prmi						0.0006 (0.001)		0.0043 (0.0075)
Hypoaspis aculeifer	Prmi							0.0025 (0.0044)	
Lysigamasus	Prmi	0.0063 (0.0059)	0.0043 (0.0048)	0.0043 (0.074)			0.0011 (0.0018)		
Lysigamasus runciger	Prmi	0.0178 (0.0263)	0.0047 (0.0042)	0.0082 (0.0142)					
Pachylaelaps karawaiewi	Prmi					0.0011 (0.0019)		0.0141 (0.0082)	
Pergamasus	Prmi				0.0008 (0.0014)			0.0021 (0.0036)	0.0014 (0.0025)
Pergamasus norvegicus	Prmi	0.0019 (0.0034)							
Prozercon	Nemi				0.0007 (0.0008)			0.0004 (0.0007)	
Rhodacarellus	Prmi						0.0006 (0.001)	0.0046 (0.0041)	

Appendix 3.1 Continued

Rhodacarellus silesiacus	Prmi						0.0045 (0.0078)	0.0115 (0.0014)
Rhodacaridae	Prmi				0.0011 (0.002)			
Uropoda	Prmi							0.0074 (0.0029)
Uropoda orbicularis	Prmi		0.001 (0.0017)					
Veigaia nemorensis	Prmi				0.0011 (0.002)			
Veigaia planicola	Prmi							0.0013 (0.0022)
Oribatida								
Liebstadia similis	HFmi				0.0001 (0.0001)			
Liochthonius	HFmi							0.0003 (0.0005)
Liochthonius propinquus	HFmi							0.0008 (0.0014)
Microppia minus	Fumi					0.0001 (0.0002)		
Oromurcia sudetica	HFmi				0.0014 (0.0009)			
Pantelozetes paolii	Fumi	0.0005 (0.0004)	0.0001 (0.0002)		0.0002 (0.0003)			
Platynothrus thori	HFmi			0.0009 (0.0016)				
Protoribates capucinus	Fumi						0.0008 (0.0008)	
Rhysotritia ardua	HFmi						0.0003 (0.0004)	0.0006 (0.0006)

Appendix 3.1 Continued

Tectocepheus velatus	Ommi					0.001 (0.0018)	0.0009 (0.0008)	0.0046 (0.004)	0.0049 (0.0043)
Trhypochthonius cladonicola	Ommi				0.0084 (0.0022)				
Prostigmata									
Eupodes	Ommi	0.0018 (0.0019)	0.0018 (0.0012)	0.0027 (0.0047)	0.0102 (0.0044)	0.0018 (0.0012)	0.0008 (0.0003)	0.0569 (0.0201)	0.0081 (0.0065)
Microtydeus	Ommi	0.0002 (0.0003)	0.001 (0.0018)		0.0013 (0.0017)		0.0003 (0.0003)	0.0088 (0.0009	0.0033 (0.0057)
Nanorchestes	Ommi					0.0539 (0.0506)	0.0143 (0.0062)	0.0909 (0.0206)	0.0229 (0.0032)
Pyemotes	Prmi				0.0023 (0.0024)				
Pygmephorus	Fumi	0.0001 (0.0002)	0.001 (0.001)	0.0039 (0.0034)	0.0025 (0.0028)	0.0012 (0.0014)	0.0006 (0.0005)	0.0079 (0.0075)	0.004 (0.0021)
Rhagidia	Prmi				0.0028 (0.0025)	0.0035 (0.0049)		0.0021 (0.0036)	
Scutacarus	Ommi				0.0016 (0.0015)	0.0007 (0.0012)	0.0002 (0.0003)		
Speleorchestes	Ommi					0.0095 (0.0029)	0.009 (0.0029)	0.0037 (0.0025)	0.0004 (0.0007)
Stigmaeidae	Prmi			0.0432 (0.0549)					
Tarsonemus	Ommi			0.004 (0.004)	0.0016 (0.0015)				
Trombidiidae	Prmi								0.0013 (0.0022)
Tydeidae	Ommi			0.0083 (0.0084)	0.0007 (0.0007)				
Collembola									
Entomobryomorpha									

Appendix 3.1 Continued

Folsomia sexoculata	HFco	0.0066 (0.0026)		0.1007 (0.1064)	0.0089 (0.0035)				
Folsomides parvulus	Fuco						0.0006 (0.0011)		0.0015 (0.0026)
Isotoma	Fuco		0.0006 (0.001)		0.0048 (0.0083)				
Isotoma anglicana	Fuco			0.0045 (0.0078)	0.0009 (0.0015)				
Isotomiella minor	Fuco	0.0006 (0.0011)	0.0056 (0.0038)	0.0416 (0.0607)	0.0307 (0.0226)	0.0032 (0.0017)	0.0005 (0.0009)		
Lepidocyrtus	HFco							0.0054 (0.0094)	
Lepidocyrtus cyaneus	HFco					0.008 (0.0097)	0.0006 (0.0011)		0.0014 (0.0024)
Parisotoma notabilis	Fuco			0.0371 (0.0643)	0.0366 (0.0434)	0.0068 (0.0118)	0.0042 (0.0046)	0.0219 (0.0022)	0.0221 (0.0093)
Proisotoma minuta	Fuco					0.0364 (0.0631)			0.0338 (0.0024)
Pseudisotoma sensibilis	Fuco								0.0036 (0.0062)
Pseudosinella alba	HFco						0.0012 (0.0021)	0.0024 (0.0041)	0.0051 (0.0054)
Neelipleona									
Megalothorax minimus	HFco					0.0016 (0.0017)	0.0012 (0.0021)	0.0027 (0.0047)	0.0051 (0.0054)
Poduromorpha									
Ceratophysella denticulata	Fuco	0.0952 (0.0525)	0.0414 (0.0481)	0.2088 (0.092)	0.0017 (0.003)		0.0105 (0.0167)	0.0024 (0.0041)	0.0959 (0.0284)
Friesea truncata	Fuco	0.0006 (0.0011)	0.0024 (0.0041)		0.0077 (0.0073)				

Appendix 3.1 Continued

Hypogastrura	Fuco							0.01 (0.0054)	
Mesaphorura	Fuco			0.0132 (0.0131)					
Mesaphorura macrochaeta	Fuco						0.0032 (0.0031)		0.0183 (0.0081)
Onychiurus	Fuco	0.0376 (0.0219)	0.0079 (0.0107)		0.0139 (0.0072)	0.0046 (0.0079)	0.0012 (0.0021)		
Paratullbergia callipygos	Fuco								0.0015 (0.0026)
Stenaphorurella quadrispina	Fuco								0.0036 (0.0062)
Tullbergia	HFco	0.0027 (0.0032)							
Symphypleona									
Sminthuridae	Heco	0.0196 (0.0271)		0.0045 (0.0078)	0.0023 (0.0022)	0.0034 (0.0059)		0.0024 (0.0041)	
Sminthurinus	Heco		0.0173 (0.0155)						
Sminthurus viridis	Heco					0.011 (0.0121)	0.0011 (0.001)		
Sphaeridia pumilis	Heco		0.017 (0.007)	0.0186 (0.0321)	0.0014 (0.0025)				
Diplura	Dipl							0.0024 (0.0041)	
Pauropoda	Fuco				0.016 (0.0163)	0.0011 (0.002)	0.0006 (0.001)	0.0048 (0.0083)	0.0078 (0.0031)

Chapter 4

Land use differences affect soil microbial communities at different soil depths in Austria

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Abstract

Human activities, such as land use and –management, can strongly affect the soil's ability to provide ecosystem services. Microbes are key groups in ecosystem functioning, because of their role in nutrient cycling and soil formation. As not only microbial biomass may decrease with soil depth, but also the structure of microbial community may differ in terms of functional group abundances, it is unknown what the effects of land use are on soil ecosystem functioning below the soil surface. The present study assessed the effects of land use type on soil microbiological biomass, activity and community structure at different soil depths, in three land use types (arable, forest and grassland) under the same climatic and pedological conditions.

We measured in three soil horizons (A, AC and C) the microbial abundance through direct microscopic counts, fumigation extraction and phospholipid fatty acid (PLFA) contents, and microbial activity through the rate of leucine incorporation, epifluoresence microscopy and dimethyl sulphoxide reduction (DMS). Additionally, we measured carbon pools and mineralisation rate, nitrogen pools, soil pH, moisture and porosity as potential drivers of microbial community structure.

Microbial biomass (both bacteria and fungi) was significantly lower in the arable field. There were no significant differences between forest and grassland. Also for microbial activity we did find significant differences between land use types. However, both microbial biomass and activity decreased with soil depth in all land use types. Furthermore, Principal Component Analysis of the PLFA data suggested a differentiation of microbial community structure between land use types and with soil depth: i.e. the microbial community structure in the C horizon was similar in all three land uses, whereas the community structure in the topsoil differed strongly between arable, grassland and forest.

This study shows that land use exerts strong effects on soil microbial biomass, activity and community structure. Deeper in the soil these effects of vegetation are diluted, with microbial community structure and functioning becoming similar in all land use types.

4.1 Introduction

Sustainable land management is essential for continuation of life on Earth as we are used to, and can only be obtained if we understand the interplay between soil physical, chemical and biological processes. These processes govern a wide array of ecosystem services such as the provision of food, feed and fibre, carbon storage and sequestration, hydrological regulation and contaminant attenuation (Costanza et al., 1997). Human activities, such as land use and –management, have strong effects on soil ecosystem functioning, hence studying the soil processes under different land uses is necessary in order to protect and regenerate the soil's ability to deliver ecosystem services.

One of the basic soil biochemical processes is the build-up of soil structure. Pores between and within soil aggregates provide space, water and oxygen, and thereby create habitats for a large diversity in soil microorganisms (Anderson, 1978; Caesar-TonThat et al., 2011; Sulkava and Huhta, 1998). Soil structure is strongly linked to soil organic matter (SOM) dynamics (Regelink et al., 2015), as incorporation of SOM into the soil aggregates 'protects' it from microbial decomposition, thereby stabilizing SOM content and sequestering carbon in the soil, with potential positive effects on plant productivity (Golchin et al., 1994).

Soil microorganisms make up more than 95% of the total soil biomass (Bardgett, 2005), and play important roles in soil ecosystem functioning, given their role in soil formation processes, dynamics of SOM, and cycling of nutrients (Brussaard et al., 1997; Pulleman et al., 2005; Wright and Anderson, 2000). Soil biochemical processes mediated by soil microbes are sensitive to land use, e.g. through differences in litter composition and rooting depth and –turnover rates (Hooper and Vitousek, 1998; Stenberg, 1999).

Effects of land use are expected to be found especially in the rooted topsoil. For example, several studies have shown that microbial biomass shows a decrease with soil depth (Ekelund et al., 2001; Taylor et al., 2002). Such decreases are likely to be linked to a decreased resource (SOM) availability in the deeper soil layers (Six et al., 2006). Besides a decrease in microbial biomass with soil depth, also the structure of microbial community, in terms of functional group abundances, may differ between different soil horizons (Agnelli et al., 2004; Fierer et al., 2003). This raises the question of how such differences in soil microbial community structure may originate from land use and –management.

The Marchfeld area in the Danube basin in Austria provides an excellent opportunity to study land use effects on the soil microbial community at different depth layers. The basis consists of arable fields, forests and

grasslands, on young developing Chernozems in homogeneous river sediments, deposited approximately 400-600 years ago. Hence, all soils have been formed from the same parent material and have a similar soil age. Yet, the different land use types will differ in type of litter input, rooting depth, rhizosphere effects and land management practices. Our objectives were to assess the effects of the three land use types on soil microbiological biomass, activity and community composition at different soil depths. Hence, soil from each of the three land use types (arable, grassland and forest), from three soil horizons (A, AC and C), was analysed for its microbiological and soil physicochemical properties.

4.2 Methods

4.2.1 Site description

The Marchfeld study area is located in the Danube basin downstream of Vienna (Austria). During Alpine glaciations the Danube continuously incised into the uplifting Tertiary basin and accumulated melt-water terraces. The floodplain is morphologically subdivided into two units: the recent floodplain sensu stricto and a slightly higher area covered by older fluvial deposits. The soils are classified as former Fluvisols, in development towards Chernozems. Mean annual temperature in the sampling area is approximately 9°C and mean annual precipitation is about 550 mm with dry summers (Lair et al., 2009). To investigate the effects of land use on soil microbiological properties, samples were taken from three land use types in the floodplain sensu stricto (Figure 4.1), where soils form on the alluvial sediments deposited over the last 600 years (Lair et al., 2009). Land use history can be followed using historical maps starting in the early 18th century: a grassland (N 48°08´38.9, E 16°52´35.4), converted from forest to grassland between 1809 and 1859; an arable field under intensive agricultural production (N 48°08´27.9, E 16°41´53.2), converted in the first half of the 20th century from grassland (that already existed before 1781); and a mixed forest (N 48°08´40.0, E 16°41´37.3), which evolved semi-naturally (Fraxino-Ulmetum). The cropland is under intensive agricultural use and received mineral fertiliser according to the Austrian fertilisation recommendations depending on the cultivated crop (1997-2011: winter barley (46% of total crops), rape seed (20%), potato, sugar beet, wheat, maize, and triticale (each 6.8%)) (BMLFUW, 2006). All sites are protected against flood events of the Danube river by a dike constructed between 1882 and 1905.

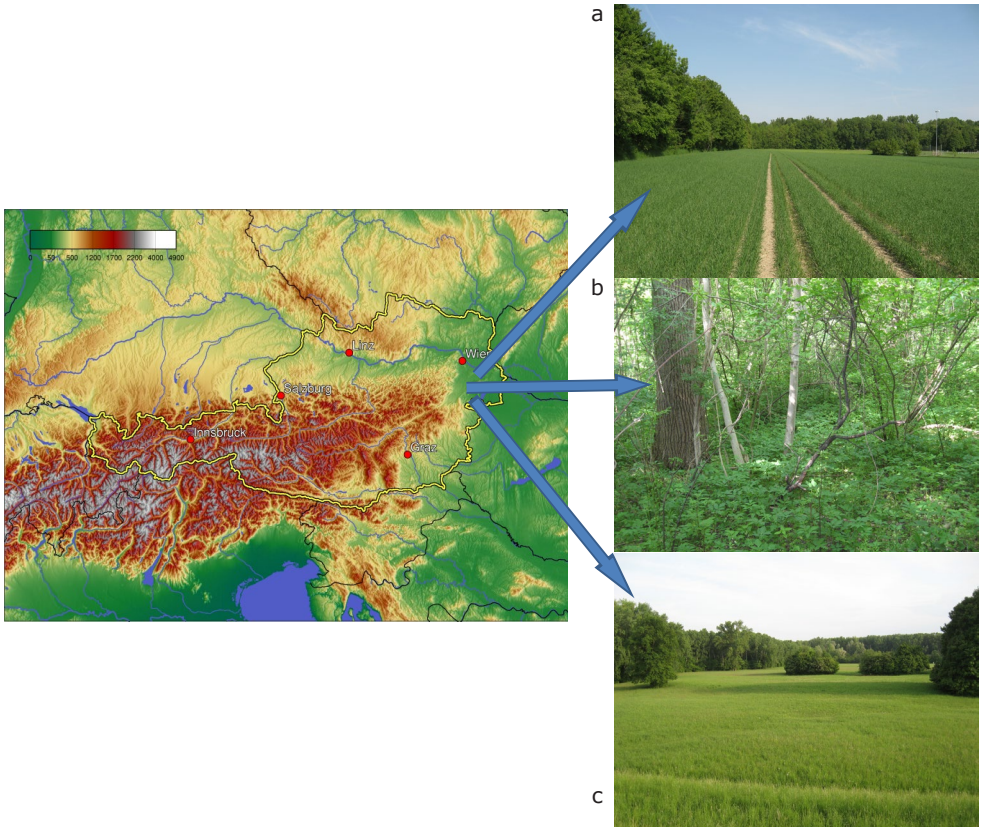

Figure 4.1 Location and impressions of the studied land use types in Austria: arable field (a), forest (b), and grassland (c).

4.2.2 Sampling

In May 2011, for each land use type three plots were selected where all measurements were carried out; the plots were separated by approximately 30-40 m. At each plot, mixed soil samples (ca. 1 kg, from 10 to 15 cores) were taken by use of a 8 cm diameter corer for microbial (bacteria, fungi) and soil physicochemical measurements. Samples were taken from three soil horizons, identified in the field: A (0-10 cm), AC (30-40 cm in grassland and cropland, 50-60 cm in forest), and C (55-65 cm in grassland and cropland, 70-80 cm in forest). For homogenisation, soil samples were gently broken by hand and sieved through a 5 mm sieve in the field. Soil samples were transported in plastic boxes, and kept at 4°C in the dark for the microbiological analyses, and air-dried in the laboratory prior to all other analyses. Samples for PLFA analyses were kept at -18°C until analysis. Prior to use, the soil samples were defrosted for 12 h at 4°C.

4.2.3 Soil microbiological analysis

Bacterial biomass, fungal biomass were measured by direct counts using epifluorescence microscopy (Bloem and Vos, 2004), and bacterial activity was estimated by measuring [^{14}C]leucine incorporation (Bloem et al., 1995) as described in chapter 2. Also the ratios of fungal to bacterial biomass and microbial biomass to total organic carbon were calculated based on direct counts. Additionally, microbial biomass C was determined by the chloroform fumigation-extraction method (Vance et al., 1987).

The soil's microbial community structure was assessed by analysing PLFA contents as described by Djukic et al. (2010). In short, PLFAs were extracted from 1.5 g of field-moist soil according to the procedure of (Bligh and Dyer, 1959). Samples were analysed on a Hewlett-Packard 5890 II gas chromatograph equipped with a flame ionisation detector and a HP Ultra 2 column (50 m x 0.2 mm, Agilent). Identification and quantification of peaks was based on comparison of retention times to the internal standards 13:0 and 19:0 and a bacterial fatty acid methyl ester mix (Supelco Bacterial Acid Methyl Esters CP Mix #47080-U, Sigma-Aldrich). We used the fatty acid nomenclature described by (Frostegård et al., 1993).Total amount of extracted microbial phospholipid fatty acids (total PLFAs; nmol (g OC)$^{-1}$) was taken as measure of total microbial biomass. We used individual PLFA markers to quantify the relative abundances of specific cell types. Iso- and anteiso-branched saturated fatty acids (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) represented Gram-positive bacteria (Kaur et al., 2005), whereas cyclopropyl (cy17:0, cy19:0), the mono-unsaturated 16:1ω7c, and the straight chain fatty acids 14:0, 15:0, 17:0 represented Gram-negative bacteria (Kourtev et al., 2002). The biomarker B18:1ω9c respresented another bacterial functional group (Frostegård and Bååth, 1996). The PLFAs 18:2ω6,9 and F18:1ω9c were used as indicator of fungal biomass. Additionally, total bacterial and fungal PLFAs were calculated. Besides absolute PLFA contents, PLFAs were also normalised using TOC content to assess abundances independent of the TOC content of the soils.

Microbial activity was measured using dimethyl sulphoxide (DMSO) reduction to dimethyl sulphide (DMS) following Alef and Kleiner (1989). We added 275 µl of DMSO solution (6.6% w/v) to 1.5 g of fresh soil and incubated at 30°C for 3 h. After incubation, 100 ml of the gas phase was removed and injected into a gas chromatograph (Hewlett-Packard 5890 GC; HP5 methyl silicon column (5 m x 0.2 mm, Agilent)), with flame ionization detector and Helium (He) as the carrier gas.

4.2.2 Soil physicochemical measurements

Soil bulk density was measured using metal cylinders, from which the contents were weighed after drying. Particle density was measured

volumetrically using pycnometers (Soil Survey Staff, 2004). Soil porosity was estimated as 1-(bulk density/particle density). Soil pH was measured electrochemically (Microprocessor pH Meter pH196 WTW, Weilheim, Germany) in H_2O at a soil:solution ratio of 1:2.5 (Burt, 1992). Content of carbonates (mineral carbon) was measured gas volumetrically (Soil Survey Staff, 2004). Total carbon (TC) and nitrogen (TN) contents were measured using dry combustion (Tabatabai and Bremner, 1991), total organic carbon (TOC) was calculated as the difference between total and carbonate carbon. Hot-water-extractable carbon (HWC) was measured as the carbon present in solution after 16 h at 80°C, potentially mineralisable nitrogen (PMN) was measured as the increase in NH_4 during 1 week of anoxic incubation in slurry at 40°C, and carbon and nitrogen mineralisation rates were determined using gas chromatography during a 6 week incubation at 20°C, as described in van chapter 2. Dissolved organic carbon (DOC) was determined by UV adsorption at 254 nm (Brandstetter et al., 1996).

4.2.5 Statistics

Differences in soil microbiological and physicochemical properties between samples were tested with an ANOVA, using land use type, soil horizon and the interaction of land use type x horizon as factors. All data were log-transformed before analysis to obtain homogeneity of variances. The concentrations of individual PLFAs were used as input values in Principal Components Analysis (PCA), to determine whether the PLFA signatures of microbial communities varied with land use and soil horizons (soil depth). Correlations of first and second Principal Component scores with potential drivers of the microbial community structure (pH, moisture, pools of C and N) were tested with Pearson's correlation test. Statistical analyses were carried out using SPSS (22.0.0) and R (2.15.2; (R Core Team, 2015)).

4.3 Results

4.3.1 Microbial biomass and activity

Biomass estimations showed coherent patterns that were independent of the used method (i.e. direct counts, fumigation extractions and PLFA contents). Bacterial, fungal and microbial biomass (sum of bacterial and fungal biomass) were lowest in the arable field. When comparing forest and grassland, only fungal biomass showed a significantly higher biomass in the grassland. Microbial biomass was highest in the topsoil: the A horizon was for all parameters significantly higher than the C horizon, with the AC horizon being the intermediate (Table 4.1). However, even in the C horizon a considerable amount of bacterial and fungal biomass was found, especially in the arable field (up to 30% of total fungal biomass and 27% of total bacterial biomass). Normalised biomass of fungi and

Table 4.1 Soil microbiological parameters at the three land use types (arable, forest and grassland) in the Marchfeld (Austria) for three soil horizons: A, AC, and C. Numbers represent mean (standard deviation, n=3). Statistical significances based on ANOVA (land use (superscript letters represent significantly different groups: [a]smallest, [b]intermediate, [c]highest) + horizon (superscript numbers represent significantly different groups: [1]smallest, [2]intermediate, [3]highest)) are shown.

	Arable			Forest			Grassland			ANOVA	
	A	AC	C	A	AC	C	A	AC	C	Land use	horizon
Direct counts:											
Bacteria (µg C g^{-1})	37.55 ^{a,3} (5.76)	34.15 ^{a,2} (8.24)	26.16 ^{a,1} (9.61)	215.6 ^{b,3} (16.48)	114.8 ^{b,2} (28.07)	60.39 ^{b,1} (17.90)	247.2 ^{b,3} (28.60)	119.3 ^{b,2} (193.8)	46.38 ^{b,1} (8.14)	<0.001	<0.001
Fungi (µg C g^{-1})	9.07 ^{a,2} (2.13)	4.75 ^{a,12} (0.45)	5.82 ^{a,1} (2.69)	20.55 ^{b,2} (1.82)	9.70 ^{b,12} (1.27)	8.31 ^{b,1} (0.84)	46.25 ^{c,2} (2.71)	48.94 ^{c,12} (4.76)	17.45 ^{c,1} (0.73)	<0.001	0.001
Fungal to bacterial biomass ratio	0.26 ^{ab} (0.08)	0.15 ^{ab} (0.02)	0.25 ^{ab} (0.14)	0.10 ^{a} (0.01)	0.09 ^{a} (0.01)	0.17 ^{a} (0.05)	0.19 ^{b} (0.01)	0.43 ^{b} (0.06)	0.41 ^{b} (0.10)	<0.001	0.337
Microbial biomass (µg C g^{-1})	46.62 ^{a,3} (9.08)	38.90 ^{a,2} (15.02)	31.98 ^{a,1} (18.04)	263.13 ^{b,3} (26.85)	124.46 ^{b,2} (50.17)	68.70 ^{b,1} (31.94)	293.41 ^{b,3} (54.05)	168.26 ^{b,2} (38.04)	63.83 ^{b,1} (13.33)	<0.001	<0.001
Cmic:Corg (%)	0.28 ^{a} (0.06)	0.25 ^{a} (0.16)	0.26 ^{a} (0.14)	0.62 ^{b} (0.08)	0.67 ^{b} (0.10)	0.47 ^{b} (0.14)	0.71 ^{b} (0.17)	0.66 ^{b} (0.18)	0.51 ^{b} (0.05)	<0.001	0.261
Fumigation:											
Microbial biomass (µg C g^{-1})	27.55 ^{a,3} (2.19)	18.25 ^{a,2} (4.78)	10.37 ^{a,1} (3.71)	99.94 ^{b,3} (4.96)	39.67 ^{b,2} (10.69)	17.98 ^{b,1} (5.81)	142.4 ^{b,3} (2.75)	63.27 ^{b,2} (7.09)	12.37 ^{b,1} (1.09)	<0.001	<0.001
Activity:											
Leu* (pmol $g^{-1}h^{-1}$)	144.83 (15.06)	33.432 (5.99)	17.411 (0.33)	158.83 (19.45)	60.842 (29.96)	30.631 (9.13)	97.873 (18.13)	38.072 (7.51)	13.961 (3.11)	0.065	<0.001
Leu / Bac C (pmol $g^{-1}h^{-1}$ g C^{-1})	3.92 ^{c,2} (1.31)	1.16 ^{c,1} (0.39)	0.86 ^{c,1} (0.29)	0.74 ^{b,2} (0.25)	0.47 ^{b,1} (0.16)	0.51 ^{b,1} (0.17)	0.39 ^{a,2} (0.13)	0.34 ^{a,1} (0.11)	0.31 ^{a,1} (0.10)	<0.001	0.002
Fungal activity (%)	4.35 (4.35)	14.00 (4.16)	35.80 (23.88)	2.60 (2.60)	3.73 (2.44)	2.67 (1.34)	0.43 (0.43)	2.24 (1.26)	1.19 (0.60)	0.552	0.202

*: Leucine incorporation rate.

Table 4.2 PLFA contents at the three land use types (arable, forest and grassland) in the Marchfeld (Austria) for three soil horizons: A, AC, and C. PLFA measures in nmol g^{-1} soil: total microbial biomass, gram positive bacteria (GrP), gram negative bacteria (GrN), B18:1ω9c, total bacterial PLFA, the fungal biomarkers 18:1ω9c,18:2ω6,9 and total fungal PLFA, and microbial activity (DMS in μg g $soil^{-1}$ h^{-1}). Numbers represent mean (standard deviation, n=3). Statistical significances based on ANOVA (land use (superscript letters represent significantly different groups: [a]smallest, [b]intermediate, [c]highest) + horizon (superscript numbers represent significantly different groups: [1]smallest, [2]intermediate, [3]highest)) are shown.

	Arable			Forest			Grassland			ANOVA	
	A	AC	C	A	AC	C	A	AC	C	Land use	horizon
Microbial biomass	48.11 ^{a,3} (4.72)	41.45 ^{a,2} (8.17)	28.71 ^{a,1} (3.96)	150.2 ^{b,3} (39.53)	44.60 ^{b,2} (11.64)	36.65 ^{b,1} (9.23)	223.5 ^{c,3} (17.21)	113.2 ^{c,2} (9.41)	34.18 ^{c,1} (4.97)	<0.001	<0.001
GrP	9.40 ^{a,3} (1.18)	10.80 ^{a,2} (3.05)	7.84 ^{a,1} (1.12)	35.44 ^{b,3} (12.99)	10.95 ^{b,2} (3.41)	10.09 ^{b,1} (3.84)	54.97 ^{c,3} (4.79)	28.28 ^{c,2} (3.64)	9.38 ^{c,1} (1.61)	<0.001	<0.001
GrN	8.01 ^{a,3} (0.79)	7.36 ^{a,2} (0.88)	6.53 ^{a,1} (1.10)	25.18 ^{b,3} (6.17)	7.43 ^{b,2} (1.81)	6.36 ^{b,1} (1.25)	35.45 ^{c,3} (1.96)	19.05 ^{c,2} (0.14)	7.87 ^{c,1} (1.09)	<0.001	<0.001
B18:1ω9c	6.04 ^{1} (0.50)	8.85 ^{1} (1.43)	11.59 ^{2} (1.29)	6.82 ^{1} (1.00)	7.34 ^{1} (2.25)	12.79 ^{2} (3.34)	6.20 ^{1} (0.29)	6.72 ^{1} (0.19)	10.44 ^{2} (1.88)	0.306	<0.001
Total bacteria	26.99 ^{a,3} (1.44)	29.97 ^{a,2} (2.86)	28.82 ^{a,1} (1.87)	79.41 ^{b,3} (13.58)	28.75 ^{b,2} (4.56)	31.93 ^{b,1} (4.95)	113.44 ^{c,3} (4.64)	60.54 ^{c,2} (2.63)	30.70 ^{c,1} (2.25)	<0.001	<0.001
F18:1ω9c	4.04 ^{a,3} (0.31)	2.64 ^{a,2} (0.37)	1.53 ^{a,1} (0.14)	11.59 ^{b,3} (1.40)	3.55 ^{b,2} (0.07)	1.78 ^{b,1} (0.12)	18.70 ^{c,3} (1.18)	9.54 ^{c,2} (0.63)	2.15 ^{c,1} (0.22)	<0.001	<0.001
18:2ω6,9	1.22 ^{a,3} (0.06)	1.34 ^{a,2} (0.05)	1.30 ^{a,1} (0.10)	1.53 ^{a,3} (0.09)	1.08 ^{a,2} (0.03)	1.17 ^{a,1} (0.11)	7.92 ^{b,3} (1.05)	4.36 ^{b,2} (0.32)	1.35 ^{b,1} (0.05)	<0.001	<0.001
Total fungi	5.25 ^{a,3} (0.31)	3.98 ^{a,2} (0.32)	2.83 ^{a,1} (0.22)	13.12 ^{b,3} (1.45)	4.63 ^{b,2} (0.10)	2.95 ^{b,1} (0.19)	26.62 ^{c,3} (1.61)	13.90 ^{c,2} (0.39)	3.50 ^{c,1} (0.23)	<0.001	<0.001
DMS	0.12 ^{a,3} (0.001)	0.14 ^{a,2} (0.001)	0.10 ^{a,1} (0.001)	0.20 ^{b,3} (0.000)	0.13 ^{b,2} (0.001)	0.13 ^{b,1} (0.001)	0.22 ^{c,3} (0.001)	0.17 ^{c,2} (0.001)	0.11 ^{c,1} (0.000)	<0.001	<0.001

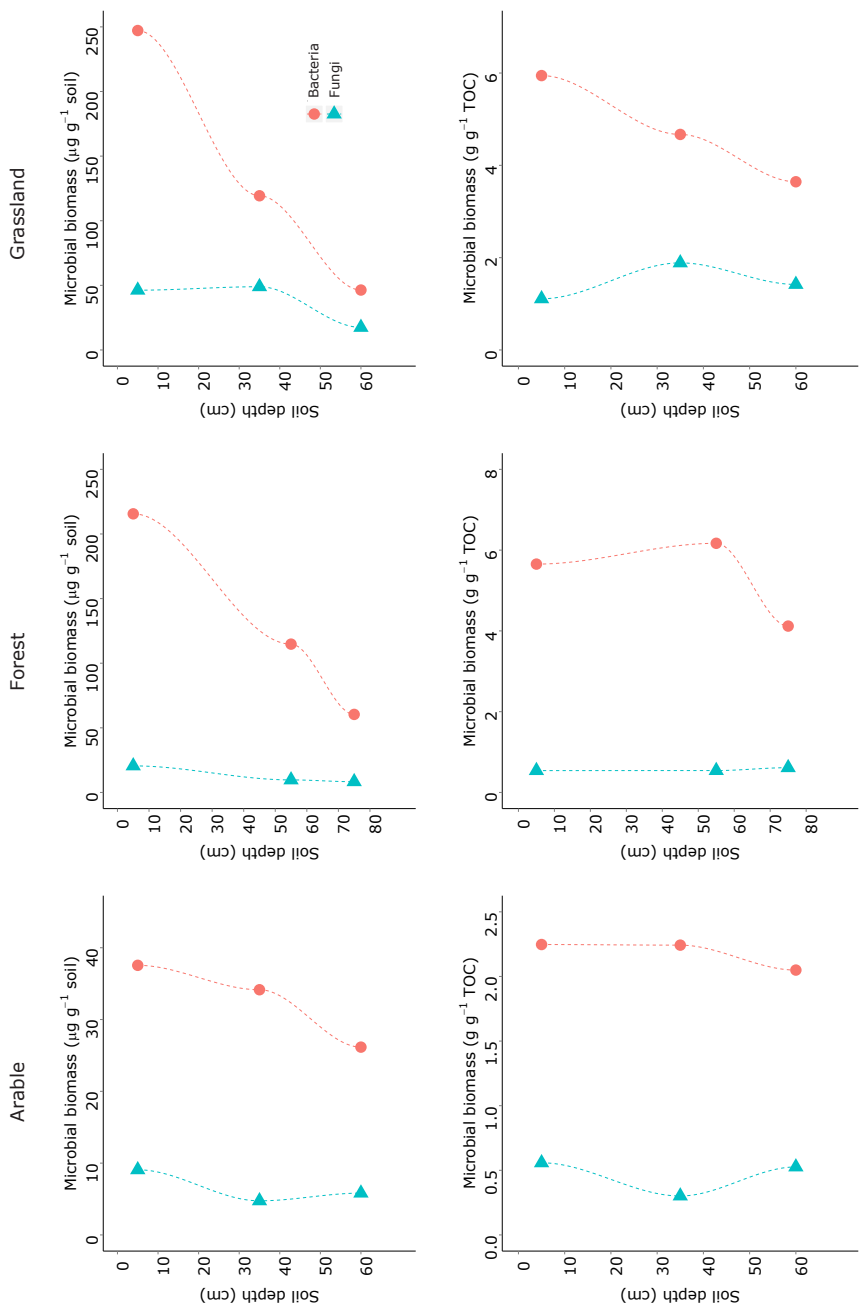

Figure 4.2 Bacterial and fungal biomass (based on direct counts) in three land use types (Arable: left, Forest: middle, Grassland: right) plotted against soil depth. Plotted are absolute abundances (µg C g^{-1} soil: upper panel) and normalized abundances (g C g^{-1} TOC: lower panel).

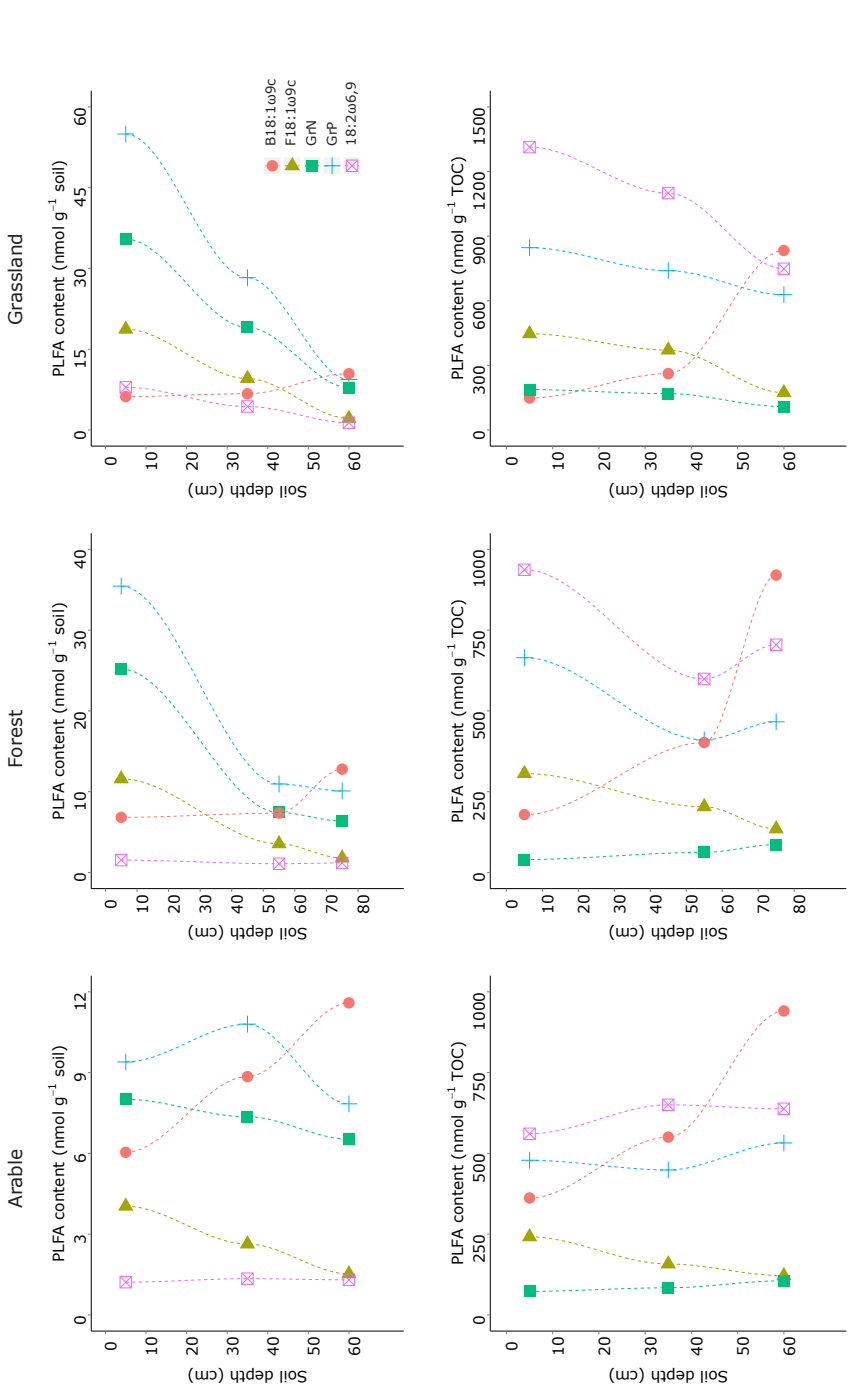

Figure 4.3 PLFA content for microbial functional groups in three land use types (Arable: left, Forest: middle, Grassland: right) plotted against soil depth. Plotted are absolute abundances (nmol g^{-1} soil: upper panel) and normalized abundances (nmol g^{-1} TOC: lower panel). GrP: Gram-positive bacteria, GrN: Gram-negative bacteria. B18:1ω9c represents another bacterial functional group. As indicator for fungal biomass the biomarkers 18:2ω6,9 and F18:1ω9c were used.

bacteria (as μg g^{-1} TOC) revealed a similar pattern as a result regarding land use effects in the order Forest=Grassland>Arable for bacteria, and Grassland>Forest=Arable for fungi. Normalised biomasses did not differ significantly with soil depth (Figure 4.2). The effect of land use type on microbial biomass decreased with soil depth, differences were strongest in the A horizon and biomass values did not differ significantly in the C horizon.

Bacterial activity, measured as Leucine incorporation rate, and fungal activity, measured as percentage of active hyphae, did not differ between land use types. DMS, however, was highest in grassland and lowest in the arable field. In contrast, the specific bacterial activity (Leucine incorporation rate per bacterial biomass) was highest in the arable field and lowest in the grassland. Leucine incorporation rate, specific incorporation rate and DMS decreased with soil depth in all land use types (Table 4.1, 4.2). As bacterial activity decreased with soil depth, also differences between land use types decreased. Microbial activity differed strongest between land use types in the A horizon, whereas it did not differ significantly in the C horizon.

4.3.2 Microbial community composition

The absolute abundance of all PLFAs were significantly different for the different land use types (Table 4.2). Contents of all fatty acids except fungal biomarker 18:2ω6,9 and biomarker B18:1ω9c were lowest in the arable field, intermediate in the forest, and highest in the grassland (Figure 4.3). The absolute abundance of fungal biomarker 18:2ω6,9 did not significantly differ between arable field and forest, but was highest in the grassland, whereas the absolute abundance of the biomarker B18:1ω9c was not different between the land use types. For all land use types, the absolute amount of all PLFAs, except for B18:1ω9c, significantly decreased with soil depth in the order A>AC>C (Table 4.2); biomarker B18:1ω9c showed a two to five fold increase with depth from A to C horizon (Figure 4.3). Differences in PLFA contents normalised for TOC were similar to those of the absolute values, but less pronounced. The patterns in PLFA contents were similar to the biomasses measured by direct counts. The gram-positive and gram-negative bacterial PLFAs were positively correlated with the microscopically estimated bacterial biomass values and the fungal biomarkers correlated with the microscopically estimated fungal biomass, both indicating the consistency in patterns produced by the different soil microbial parameters.

PCA of the PLFA data indicated a differentiation of microbial community structure between land use and with soil depth (Figure 4.4). The microbial community structure in the C horizon of the three land uses was very similar, whereas especially the community structures in the A horizons

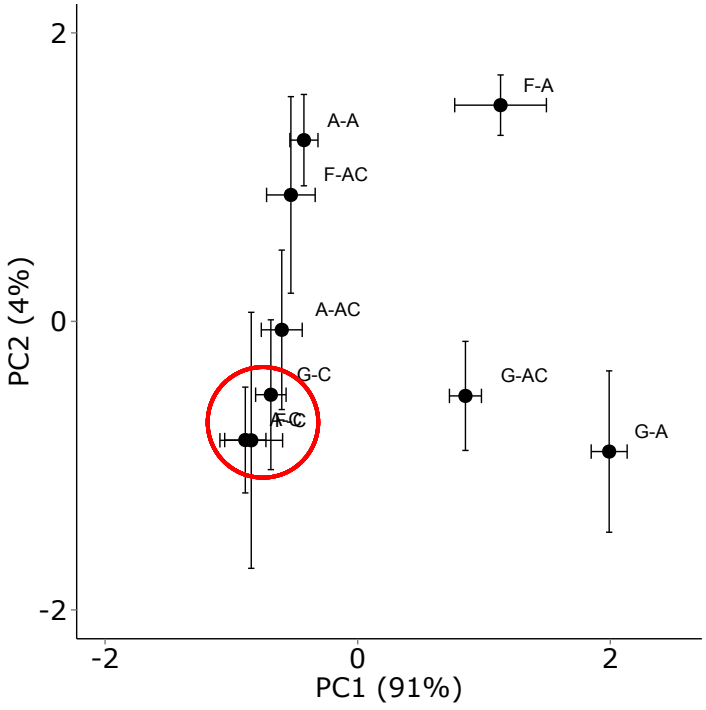

Figure 4.4 Principal component analysis (PCA) of PLFA contents from the soil samples collected in three land use types (A: arable, F: forest and G: grassland), at three soil horizons (A, AC and C). PC1 explains 91% of the variance in the data, PC2 explains only 4% of the variance. Points represent means (n=3), error bars represent ±1 SD. The red circle emphasises the small Euclidean distances between the C-horizons of the three land use types.

differed significantly between arable, grassland and forest on the first and second PC axes. The first PC-axis explained 91% of the variation in the PLFA data, while the second PC-axis only added 4% of the explained variation.

4.3.3 Soil physicochemical properties

Bulk density was highest in the arable field, and lowest in the forest. In all land use types bulk density increased with soil depth. Partly derived from bulk density, soil porosity showed the opposite pattern and was lower in the arable field than in grassland and forest (Table 4.3). In all land use types, soil porosity decreased with depth. Linked to soil porosity, soil moisture content was lower in the arable field than in grassland and forest. Soil moisture decreased with depth in forest and grassland, but not in the arable field (there was a significant interaction effect). $CaCO_3$ content was high throughout the Danube floodplain, but it was lowest in the arable field, intermediate in the forest, and highest in the grassland.

Table 4.3 Soil physicochemical properties at the three land use types (arable, forest and grassland) in the Marchfeld (Austria) for three soil horizons: A, AC, and C. Texture classification according to FAO 2015 (SiL = silt loam, SL = sandy loam, SI = silt). Numbers represent mean (standard deviation, n=3). Statistical significances based on ANOVA (land use (superscript letters represent significantly different groups: [a]smallest, [b]intermediate, [c]highest) + horizon (superscript numbers represent significantly different groups: [1]smallest, [2]intermediate, [3]highest)), and Pearson's correlation coefficients with bacterial and fungal biomass (based on direct counts) are shown (numbers represent r value (p-value)).

	Arable			Forest			Grassland			ANOVA (p-value)		Pearson r (p-value)	
	A	AC	C	A	AC	C	A	AC	C	Land use	horizon	Fungi	Bacteria
Soil texture	SiL	SiL	SiL/SL	SiL	SiL/Si	SiL	SiL/Si	SiL/Si	Si	-	-	-	-
Bulk density (g cm^{-3})	1.50 ^{c,1} (0.08)	1.49 ^{c,2} (0.02)	1.52 ^{c,2} (0.07)	1.04 ^{a,1} (0.08)	1.19 ^{a,2} (0.04)	1.39 ^{a,2} (0.17)	1.13 ^{b,1} (0.03)	1.38 ^{b,2} (0.06)	1.37 ^{b,2} (0.03)	<0.001	<0.001	-0.59 (0.094)	-0.92 (<0.001)
Porosity (%)	50.1^{a,2} (4.2)	41.4 ^{a,1} (1.0)	42.7 ^{a,1} (0.2)	60.8 ^{b,2} (1.3)	52.5 ^{b,1} (1.3)	48.9 ^{b,1} (0.9)	58.3 ^{b,2} (2.0)	53.2 ^{b,1} (0.4)	51.7 ^{b,1} (4.3)	<0.001	<0.001	0.81 (0.008)	0.91 (<0.001)
Moisture content (%)	17.0 ^{b,1} (0.20)	17.3 ^{b,2} (0.00)	18.1 ^{b,2} (4.10)	26.2 ^{a,1} (4.40)	21.1 ^{a,2} (4.17)	18.3 ^{a,2} (2.54)	26.9 ^{a,1} (0.67)	21.1 ^{a,2} (0.32)	18.9 ^{a,2} (0.55)	<0.001	<0.001	0.73 (0.026)	0.94 (<0.001)
CaCO3 (%)	23.25 ^{a,1} (0.37)	23.74 ^{a,2} (0.73)	24.03 ^{a,2} (0.43)	23.27 ^{b,1} (0.29)	26.18 ^{b,2} (0.21)	25.34 ^{b,2} (0.42)	25.08 ^{c,1} (0.48)	26.21 ^{c,2} (1.39)	30.50 ^{c,2} (0.43)	<0.001	<0.001	0.31 (0.410)	-0.01 (0.987)
pH H2O	7.741 (0.08)	7.782 (0.07)	8.093 (0.13)	7.621 (0.02)	8.002 (0.09)	8.133 (0.07)	7.581 (0.03)	7.842 (0.03)	8.183 (0.02)	0.625	<0.001	-0.43 (0.249)	-0.59 (0.095)
TOC (%)	1.67 ^{a,3} (0.08)	1.66 ^{a,2} (0.19)	1.31 ^{a,1} (0.18)	3.82 ^{b,3} (0.13)	1.81^{b,2} (0.26)	1.41 ^{b,1} (0.25)	4.19 ^{b,3} (0.12)	2.59 ^{b,2} (0.15)	1.25 ^{b,1} (0.10)	<0.001	<0.001	0.72 (0.027)	0.89 (0.001)
HWC (µg g^{-1})	350.5 ^{a,1} (31.61)	230.5 ^{a,1} (72.99)	74.98 ^{a,2} (60.25)	1267 ^{b,1} (75.10)	509.6 ^{b,1} (177.7)	185.6 ^{b,2} (49.62)	1671 ^{ab,1} (48.84)	627.1 ^{ab,1} (76.81)	74.69 ^{ab,2} (34.49)	0.041	<0.001	0.41 (0.034)	0.90 (<0.001)
DOC (mg L^{-1})	2.63 ^{a,2} (0.09)	3.54 ^{a,2} (0.85)	1.96 ^{a,1} (0.33)	7.68 ^{b,2} (0.83)	4.85 ^{b,2} (0.91)	3.23 ^{b,1} (0.14)	5.14 ^{a,2} (0.33)	3.69 ^{a,2} (0.80)	1.73 ^{a,1} (0.47)	0.001	<0.001	0.73 (0.272)	0.83 (0.005)

Table 4.3 Continued

C min (µg C $g^{-1}wk^{-1}$)	69.1 ^{a,3} (2.5)	58.7 ^{a,2} (8.6)	39.9 ^{a,1} (3.3)	94.4 ^{b,3} (24.8)	66.5 ^{b,2} (11.8)	46.1 ^{b,1} (2.3)	141.2 ^{b,3} (9.8)	57.7 ^{b,2} (2.1)	44.5 ^{b,1} (1.0)	<0.001	<0.001	0.73 (0.027)	0.90 (0.001)
Total N (%)	0.13 ^{a,3} (0.00)	0.10 ^{a,2} (0.02)	0.08 ^{a,1} (0.03)	0.27 ^{b,3} (0.01)	0.14 ^{b,2} (0.03)	0.09 ^{b,1} (0.02)	0.32 ^{b,3} (0.00)	0.17 ^{b,2} (0.02)	0.07 ^{b,1} (0.00)	0.011	<0.001	0.69 (0.040)	0.89 (0.001)
PMN (µg g^{-1})	10.78^{a,3} (1.4)	5.81 ^{a,2} (2.0)	2.70 ^{a,1} (0.7)	129.4 ^{b,3}(4.4)	30.3 ^{b,2} (14.4)	9.3 ^{b,1} (2.9)	118.2 ^{b,3} (11.3)	19.5 ^{b,2} (1.1)	4.8 ^{b,1} (0.9)	<0.001	<0.001	0.58 (0.102)	0.86 (0.003)
TOC:TN	12.46 (0.65)	16.85 (1.76)	21.652 (12.72)	14.001 (0.73)	13.26 (1.48)	15.34 (2.99)	12.98 (0.63)	15.60 (1.44)	18.89 (1.04)	0.398	0.020	-0.42 (0.263)	-0.64 (0.064)

pH was similar (i.e. around 8) in the three land use types. $CaCO_3$ content, and consequently pH, increased with soil depth in all land use types. All pools of organic carbon and nitrogen (i.e. TOC, HWC, DOC, TN and PMN) were lowest in the arable field and these contents decreased with depth in all land use types. Similarly, carbon mineralisation rate was lowest in the arable field, and decreased with soil depth in all land uses (Table 4.3).

4.3.4. Correlations

Bacterial and fungal biomass estimates based on direct counts were significantly negatively correlated with bulk density, and positively with soil porosity, soil moisture and all measured pools of carbon and nitrogen. In all cases, the correlation with bacterial biomass was stronger than with fungal biomass (Table 4.3). As potential driver of the differentiation in microbial community structure, TOC showed the highest correlation ($r = 0.97$, $p < 0.001$) with the first PC-axis from the PCA analyses from the PLFA data, closely followed by TN ($r = 0.95$, $p < 0.001$), HWC ($r = 0.95$, $p < 0.001$), C mineralisation ($r = 0.95$, $p < 0.001$), soil moisture ($r = 0.90$, $p = 0.001$), PMN ($r = 0.86$, $p = 0.003$), pH ($r = -0.79$, $p = 0.012$), soil porosity ($r = 0.78$, $p = 0.013$) and DOC ($r = 0.68$, $p = 0.042$). None of these parameters correlated significantly with the second PC-axis.

4.4 Discussion

4.4.1 Effect of land use on soil microbial community

All microbial biomass estimations (direct counts, fumigation, and PLFA extractions) were lowest in the arable field, which confirm findings in other studies, e.g. (Bardgett, 2005; Six et al., 2006). It has been suggested that differences in resource availability, resulting from differences in vegetation composition and agricultural practices including tillage, cause low microbiological parameters in arable soils (Six et al., 2006). This finding was confirmed by our results, as all carbon and nitrogen pools and carbon mineralisation rate were lowest in the arable field. When comparing forest and grassland, only fungal biomass differed statistically significantly with a higher fungal biomass in grassland. It has also been suggested that high organic carbon contents in grassland and forest will have a strong effect on the microbial community, both in abundance and community structure (Steenwerth et al., 2002). This was confirmed by the present PCA analyses, in which the first PC axis, explaining nearly all variation within the microbial PLFA data, correlated strongly with all measured carbon pools. In all sites bacteria dominated soil biomass and process rates, and all measured bacterial parameters correlated positively with organic matter and carbon mineralisation.

In contrast to the coherent set of observed patterns in biomass parameters over different land use types, patterns in microbial activity were not consistent. Bacterial activity was measured as leucine incorporation rate, and fungal activity as percentage of active hyphae, and for both these parameters we did not find differences between land use types. Also in other studies no differences in microbial activity between land use types were found (Bossio et al., 2005; Yao et al., 2000). Specific bacterial activity was highest in the arable field, while microbial activity measured as DMS was lowest in the arable field. The high specific activity is linked to low biomass with a high turnover at the arable field, via a higher disturbance and acceleration of decomposition of organic matter due to ploughing (Nsabimana et al., 2004).

4.4.2 Effects of soil depth on soil microbial community

In all land use types, microbial biomass decreased with soil depth: the A horizon had significantly higher values (up to an order of magnitude) for all microbial groups than the C horizon, with the AC horizon being the intermediate. Similarly, microbial activity decreased with soil depth in all land use types. These results are consistent with those obtained in several other studies e.g. (Federle et al., 1986; Fierer et al., 2003; Taylor et al., 2002). The differences between soil depths are most likely the result of decreased C and N availability at the deeper soil horizons (Hansel et al., 2008). However, when normalising with TOC data, the decrease of microbial biomass with soil depth was still present in many groups. This indicates that not only the quantity of carbon was important, but also quality plays an important role.

Within the soil profile we measured (depending on the land use up to 65 or 80 cm), not only a decrease in microbial biomass, but also shifts in the relative abundances of functional groups were found. PLFA contents of most functional groups decreased with depth, whereas microbes containing biomarker B18:1ω9c showed an increase with soil depth. The origin of the biomarker B18:1ω9c is often not clear because both bacteria and fungi contain these fatty acids in their membranes (Frostegård and Bååth, 1996). However, as all our samples were dominated by bacteria, it is most likely that this biomarker represents a bacterial functional group. That in our study both gram-negative and gram-positive bacteria decreased with soil depth was in contrast with studies in grassland by Fierer et al. (2003) and in arable fields by Blume et al. (2002), who found gram-negative bacteria to dominate at the surface while gram-positive bacteria were dominant in deeper soil layers.

There are some hypothesised drivers for the changes in soil microbial communities with soil depth, such as pH, soil moisture, soil porosity, and resource availability. In our study $CaCO_3$ content and consequently pH increased with soil depth in all three land uses. Generally, a high pH favours bacterial over fungal biomass (Blagodatskaya and Anderson, 1998). However, in our study sites pH was relatively high in all sites, and did not correlate statistically significant with bacterial or fungal biomass. This makes it unlikely that pH was the dominant factor determining the differences in soil community structure in the Chernozems we studied. Moisture content though was highly correlated with bacterial and fungal biomass and also correlated with the scores of the microbial communities on the first PC-axis. Hence moisture content should be considered as potential driver for both the microbial biomass as well as for community structure. Also soil porosity has been shown to affect soil microbes (Torbert and Wood, 1992), and indeed, soil porosity in our soils was significantly correlated with bacterial and fungal biomass as well as with the scores of the microbial communities on the first PC-axis. However, the correlations with soil moisture and soil porosity were less strong compared to the correlations of microbial biomass and microbial community structure with TOC and TN. Therefore, resource availability seems the most prominent driver for the differences in microbial biomass and community structure, which is in agreement with Fierer et al. (2003) and Hansel et al. (2008).

Microbial community structure differentiated between land use types and with soil depth, based on PCA analyses. The microbial communities in the C horizons of the three land use types were closely related, whereas especially the A horizons differed strongly between arable, grassland and forest on the first and second PC axes. Also, all biomass measures and microbial activity in the C horizon were similar in the different land use types. This could be explained by their similar soil type, developed in the same river sediment under the same climatic and pedological conditions, this makes the design of our study excellent for the evaluation of the effect of land use on the soil microbial community structure, without confounding factors. The effects of land use were especially clear at the topsoil, in the zone where rooting is most dense and the influences of vegetation and land management are strongest (Griffiths et al., 1999). In the topsoil, carbon is readily supplied through root exudates and fresh litter, supporting higher microbial biomass. Indeed we found that differences in carbon contents between the land use types correlated with differences in microbial biomass. In contrast, in the C horizon below the rhizosphere differences in land use had little effect on the microbial biomass, activity and community structure, which therefore depended purely on the soil pedological conditions.

4.5 Conclusions

Many soil ecological studies attempted to link soil organism abundance and richness to ecosystem productivity and functioning. However, most effort went into the rich topsoil, while lower soil horizons were generally understudied, especially in relation to land use effects. This study shows that land use has strong effects on the soil microbial community in the rooted zone in the upper soil horizons, while in deeper soil layers pedological conditions are more important as drivers.

Chapter 5

Biological soil properties under risk of degradation in semi-arid Crete

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Abstract

Land use and soil management practice can have strong effects on soil quality, defined in terms of soil fertility, carbon sequestration and conservation of biodiversity. In this study, we investigate whether ecological soil quality parameters are adequate to assess soil quality under harsh conditions, and are able to reflect different land uses and intensities of soil management practices.

We selected three sites as main representatives for the dominant types of land use in the region: an intensively cultivated olive orchard (annually tilled), an extensively used olive orchard (not tilled) and a heavily grazed pasture site in the Koiliaris catchment (Crete/Greece). Soil quality was analysed using an ecosystem approach, wherein soil biological properties (soil organism biomass and activity, taxonomic diversity of soil microarthropods) were studied in relation to abiotic soil parameters (including soil organic matter contents, and soil aggregate stability).

The intensively cultivated olive orchard had a much lower aggregate water stability than the extensive olive orchard and the pasture. Contents of soil organic C and N were higher in the extensively used olive orchard than in the intensively cultivated orchard, with intermediate concentrations in the pasture. This was mainly caused by the highest input of organic matter, combined with the lowest organic matter decomposition rate. Soil organism biomasses in all sites were relatively low compared to values reported from less harsh systems, while microarthropod richness was highest in the pasture compared to both the intensive and extensive olive orchards.

From the present results we confirm that microarthropod taxonomic richness is a very useful indicator for ecological soil quality, because it is not only able to separate harsh sites from other systems, but it is also sensitive enough to show differences between land management practices under harsh conditions. Microbial biomass and especially microarthropod biomass were much lower in our harsh study sites than reported from less affected areas, and have therefore also potential as biological indicators for degradation.

5.1 Introduction

Land use and land use changes have a strong effect on the provision of soil ecosystem services, such as carbon sequestration (Muñoz-Rojas et al., 2012; Novara et al., 2012), nutrient cycling and biodiversity. In large areas in the Mediterranean region, soil quality, defined as the ability of soil to provide ecosystem services, is adversely affected by overgrazing and overharvesting of natural vegetation, ultimately leading to soil degradation, erosion, and desertification (Milgroom et al., 2007; Muñoz-Rojas et al., 2012). Such losses in soil quality impose a severe and increasing risk for the local populations in semi-arid regions, because climate predictions indicate decreasing precipitation in the near future for the Mediterranean region (Chartzoulakis and Psarras, 2005).

In order to understand the interrelationships between land use and soil quality, the Critical Zone Observatory (CZO) network was established across the USA and Europe (Anderson et al., 2008). The CZO network is an internationally coordinated interdisciplinary research effort, including chemical, physical, and biological processes that govern soil ecosystem services. As part of the CZO research effort, the European Commission has provided funding for a large multi-disciplinary research project: Soil Transformations in European Catchments (SoilTrEC) (Bernasconi et al., 2011; Menon et al., 2014). The European CZOs represent different stages in the soil life, including sites along soil formation gradients (Austria, Switzerland, Iceland), along a soil degradation gradient (Greece), along a lithology gradient (Czech Republic), and of agricultural sites differing in soil management (Austria, Iceland) (Banwart et al., 2011; Menon et al., 2014).

The aim of the present study is to investigate biological soil properties under different land management at the Koiliaris CZO sites in Crete (Greece) that are considered to be at risk of potential soil degradation and desertification. This is characterized by loss of vegetation, inducing water erosion, and subsequently loss of soil (Tsiafouli et al., 2005), which will be intensified by the predicted desiccation for the region over the next century (Chartzoulakis and Psarras, 2005). Koiliaris CZO is representative for the soils in the Mediterranean region impacted by a strong climatic gradient, steep upland slopes, and anthropogenic intensification, which make these soils sensitive to degradation (Fernández-Romero et al., 2014). More than 40% of the Koiliaris CZO has a slope steeper than 5% (Moraetis et al., 2014), and large areas are grazed rangelands formed by shrub degradation formations (54%) or cultivated through olive and orange orchards, and annual crops (29%) (Kourgialas et al., 2012; Nikolaidis et al., 2010; Stamati et al., 2011). The climatic gradient is influenced by the extreme elevation relief, extending from 0 to 1300 m

elevation (Kourgialas et al., 2012; Naoum and Tsanis, 2004).The sites we sampled in the Koiliaris CZO (Crete, Greece) include three dominant land management types: an intensively cultivated olive orchard, an extensively used olive orchard, and a pasture site.

Loss of soil fertility and soil degradation have mostly been approached from an abiotic perspective, with emphasis on soil structure (Celik, 2005), water erosion (Kosmas et al., 1997), nutrient cycling (Solomon et al., 2000), and organic matter dynamics (Wu and Tiessen, 2002). Here, in addition to the abiotic parameters, we look specifically at biological soil quality parameters. The role of soil organisms has received less attention in assessments of land degradation and desertification, although the importance of biology in soil quality and fertility is more and more acknowledged (Ashford et al., 2013; Brussaard et al., 1997; Brussaard et al., 2007; Buchan et al., 2013; Cole et al., 2006; De Deyn et al., 2003; Holtkamp et al., 2008; Hunt et al., 1987; Moore, 1994; Oades, 1993; Setälä and Huhta, 1991; Wardle et al., 2004). Up till now, soil quality assessments from an ecological perspective have mostly been carried out in soils that were not prone to severe losses of soil in terms of degradation, erosion and desertification, while focusing on soil quality for sustainable agricultural fertility or for habitat preservation of biodiversity in natural ecosystems (Bending et al., 2004; Birkhofer et al., 2008; Carpenter-Boggs et al., 2000; Doran and Zeiss, 2000; chapter 2). In this study we investigate whether ecological soil quality parameters are also adequate to assess soil quality under harsh conditions, and are able to reflect different land uses and intensities of soil management practices.

The present paper investigates biological soil properties in relation to abiotic soil parameters in semi-arid conditions, i.e. we look at soil structure, soil organic matter, nutrient availability, and soil as a habitat for species-rich communities. These soil properties are all considered to be important aspects of soil quality and are inextricably linked. For example, soil structure affects soil organic matter decomposition and the biological habitat function of the soil, soil organic matter is the most important resource for the soil food web, and soil organisms play a role in soil structure formation, organic matter decomposition and incorporation, and nutrient mineralisation (chapter 2). Soil physical and chemical measurements included soil aggregate size distribution, soil organic matter contents and quality, nitrogen (N) content, and soil pH. Soil biological measurements included the presence and abundance of microbes (bacteria, fungi) and soil fauna (protozoa, nematodes and microarthropods), representing the main taxonomic groups and trophic levels in the soil food web. In addition we measured the taxonomic richness and diversity within the group of microarthropods.

5.2 Methods

5.2.1 Site description

Koiliaris CZO is situated 25 km east from the city of Chania, Crete, Greece (Moraetis et al., 2014). The total watershed area is 130 km² and the main supply of water originates in the White Mountains. The main outcropping lithology includes thick bedded limestone, metamorphic rock, neogene limestone, and alluvium sediments. Samples were taken at three land management types (Table 5.1, Figure 5.1). Site I was an intensively cultivated olive orchard (20 year old trees, tree density 100 ha-1) where tillage (once a year to facilitate harvesting), and litter removal (prune residues to be used as fodder for livestock) were applied on alluvium sediments in a floodplain. Site E was an extensively used, 600 year old terrace (no tillage or litter removal) with olive trees on a steep slope, while site P was formed by a 600 year old terrace, formerly utilised as cropland (until 1940), with permanent grassland and sparse tree/shrub cover, currently used as grazed pasture (see Table 5.1 for site characteristics). Sites E and P were both situated on soils developed on bedded limestone. A map of the sampled sites can be found in Moraetis et al. (2014), in which the sites are described as K2 (I), K4 (E) and K5 (P).

5.2.2 Sampling scheme

All samples were taken in May 2010. In each sampling site, three plots were selected in which all measurements were carried out; the plots were 10—20 m apart. In each plot, mixed soil samples (ca. 1 kg) were taken from the edge of a soil profile pit of about 1 m wide for microbial (bacteria, fungi), microfaunal (protozoa, nematodes) and SOM characterization, and by use of a 5 cm diameter corer for the mesofauna (enchytraeids and microarthropods). All samples were taken from the topsoil (0—10 cm), biologically the most active layer (Ekelund et al., 2001; Miura et al., 2008).

5.2.3 Soil analyses

Particle size distribution (clay content), soil pH, and calcium content were determined as described in chapter 2. Soil structure was experimentally approached by measuring the water stability of aggregates (1—3 mm in diameter), using a standard wet sieving procedure modified after Yoder (1936). Water stable aggregates (WSA) were calculated by the mass of aggregates remaining on the 1 mm sieve after wet sieving and subtracting the mass of sand > 1mm from this aggregate size fraction (e.g. Kercheva et al., 2011). WSA indicates the suitability of soil for agricultural production (Banwart et al., 2012).

Total carbon (TOC) and nitrogen (TN) contents, hot-water-extractable carbon (HWC), potentially mineralisable nitrogen (PMN), and C and N mineralisation rates were determined as described in chapter 2.

Table 5.1 Characteristics of the Koiliaris Critical Zone Observatory (CZO) at the three different sites (I = intensively cultivated olive orchard, E = extensively used olive orchard, P = pasture).

Site	I	E	P
Land use type	Intensive olive orchard	Extensive olive orchard	Pasture
Tillage	yes	no	no
Fertilisation	no	no	no
Litter removal	yes	no	no
Grazing pressure	Not grazed	Grazed	Heavily grazed
Elevation	20 m	465 m	1065 m
Average rainfall	567 mm	915 mm	1335 mm
Average temp.	19°C	18°C	14°C

Table 5.2 Soil physicochemical properties and biologically mediated processes at three different sites in the Koiliaris Critical Zone Observatory (Crete, GR) (I = intensively cultivated olive orchard, E = extensively used olive orchard, P = pasture). Values represent mean and standard deviation (between brackets). The p-values represent significance levels from an ANOVA for repeated measures, where the superscript letters denote statistically significant differences between sites, and number of ** denotes statistical significance level (*: $p < 0.05$, **: $p < 0.01$). All measurements were done in the topsoil (0-10 cm).

Site	I	E	P	rmANOVA (p-value)
Soil moisture (%)	4.09 ^{a} (2.21)	9.83 ^{b} (1.26)	14.47 ^{c}(0.55)	0.006 **
pH-H_2O	6.9 (0.96)	5.4 (0.41)	5.9 (0.11)	0.056
Clay content (%)	5.1 ^{a} (0.43)	26.6 ^{b} (10.5)	24.6 ^{b} (9.2)	0.002 **
$CaCO_3$ (g kg^{-1})	22.2 (18.2)	1.78 (0.59)	1.39 (0.25)	0.086
Bulk density (g cm^{-3})	1.22 (0.03)	1.10 (-)	1.09 (0.07)	0.054
WSA (%)[1]	38.4 ^{a} (5.4)	77.0 ^{b} (7.4)	67.1 ^{b} (5.4)	0.005 **
TOC (kg ha^{-1})[2]	21670 ^{a} (2662)	59926 ^{c} (8444)	39991 ^{b} (6319)	0.004 **
HWC (kg ha^{-1})[3]	390 ^{a} (112)	952 ^{b} (406)	700 ^{b} (28)	0.045 *
Total N (kg ha^{-1})	1557 ^{a} (249)	4246 ^{c} (363)	2843 ^{b} (421)	0.003 **
PMN (kg ha^{-1})[4]	81.26 (22.97)	66.73 (47.43)	101.8 (20.38)	0.475
TOC : Total N	13.98 (0.54)	14.14 (2.02)	14.07 (0.58)	0.994
HWC : PMN	4.80 ^{a} (0.14)	20.29 ^{b} (13.48)	7.03 ^{a} (1.18)	0.042 *
C min (kg $ha^{-1}y^{-1}$)[5]	2526 ^{a} (1131)	2418 ^{a} (103)	2818 ^{b} (1080)	0.048 *
N min (kg $ha^{-1}y^{-1}$)[6]	24.34 ^{a} (18.31)	54.11 ^{a} (20.62)	172.9 ^{b} (93.00)	0.011 *

[1]Percentage of water stable aggregates of 1-3 mm; [2]Total soil organic carbon; [3]Hot-water-extractable carbon; [4]Potential mineralisable nitrogen; [5]Carbon mineralisation rate; [6]Nitrogen mineralisation rate.

Figure 5.1 Location of the studied sites in the Koiliaris CZO in Crete: intensively cultivated olive orchard (a), extensively used olive orchard (b), and pasture (c). Photos: Jaap Bloem

Soil biological measurements included the presence and abundance of the major taxonomic groups of soil organisms: microbes (bacteria, fungi) and soil fauna (protozoa, nematodes and microarthropods). Within these taxonomic groups we defined 'trophic groups' based on diet and life-history traits, following the method of Moore et al. (1988). Abundances were transformed into estimates of biomass based on body-size information, and expressed in units of kilograms of carbon per hectare for the 0–10 cm top soil layer. The laboratory techniques used to analyse the biological parameters are described in chapter 2.

Regarding the taxonomic species richness in the microarthropods in Damma we used three metrics, i.e. the absolute number of taxa present, the Shannon diversity index (H), and the Pielou evenness index (J). For the Shannon diversity index (H) we used the following formula:

$$H = -\sum_{i=1}^{N} (p_i \ln(p_i))$$

in which p_i is the fraction of the total biomass present in species *i*, i.e. the relative biomass, of species *i*, and *n* the total number of taxa present. A higher index value corresponds to a more diverse community. For the Pielou evenness index (*J*) we used the formula: $J = H / \ln(n)$, in which *H* represents the Shannon diversity index, and *n* the total number of taxa present. A higher value for the evenness index corresponds to a more even distribution of the biomass over the taxa present.

5.2.5 Statistics

Differences in soil physicochemical and biological properties were tested with an ANOVA for repeated measures (rmANOVA), with the replicates within a site taken as repeated measures from the same object. We tested correlations between soil parameters with Pearson's correlation test. All data were log-transformed to obtain homogeneity of variances. Statistical analyses were carried out using SPSS (20.0.0) and R (2.15.2;R Core Team (2012)).

5.3 Results

5.3.1 Soil physicochemical measurements

The intensively cultivated olive orchard had a significantly lower WSA than the extensively used olive orchard and pasture ($p = 0.005$, Table 5.2).

Dynamics of soil organic matter and N cycling are biologically mediated soil quality indicators. Total organic carbon (TOC, Table 5.2) and total nitrogen (TN) were both greatest in the extensively used orchard, smallest in the intensively cultivated orchard and intermediate in the pasture ($p = 0.04$ and $p = 0.003$, respectively). As a result, TOC and TN were strongly positively correlated with each other (Pearson correlation test, $r = 1.00$, $p = 0.011$). The pool of labile C, measured as HWC, showed the same differences as TOC and TN, and was smallest in the intensively cultivated orchard ($p = 0.045$). No differences in PMN ($p = 0.475$) and the total C : N ratio of the soil (calculated as TOC : TN) were found. The C : N ratio of the labile organic matter (calculated as HWC : PMN), however, was larger in the extensively used olive orchard than in the two other sites ($p = 0.042$). C mineralisation rate and especially N mineralisation rate were greatest in the pasture site ($p = 0.048$ and $p = 0.011$, respectively).

To identify the relation of abiotic soil parameters with soil structure formation, we tested the correlations of WSA with TOC, HWC and clay content. WSA was positively correlated with TOC and HWC ($r = 0.98$, $p = 0.137$, and $r = 0.99$, $p = 0.050$, respectively) and clay content ($r = 0.99$, $p = 0.092$).

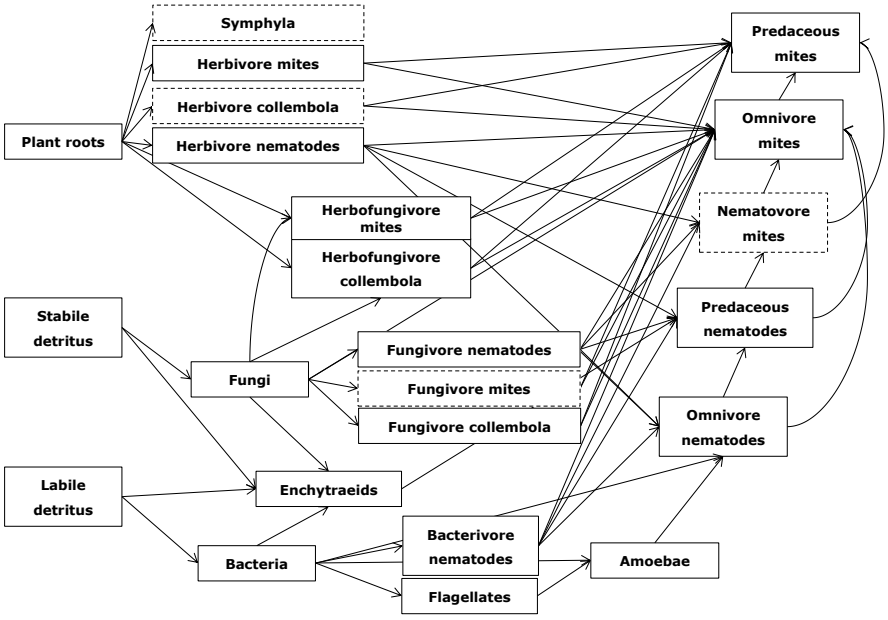

Figure 5.2 Soil food web diagram representative for all three land use types in the Koiliaris Critical Zone Observatory (Crete, GR). Boxes represent the presence of trophic groups in the soil food web, arrows represent feeding interactions based on diet information (the arrow points from the group eaten to the group that eats). Groups with drawn boxes were present at all sites, groups with dashed boxes were only present at some sites.

5.3.2 Soil biological measurements

Based on presence-absence data of the soil organisms, we constructed soil food web diagrams for the three sites (Figure 5.2). These diagrams of the three sites were highly similar and most of the trophic groups were present in all sites. A few trophic groups were missing in some of the sites: Symphyla and fungivore mites were both missing in the intensively cultivated orchards, herbivore collembolans were missing in the extensively used orchards, whereas nematovore mites were only present in the extensively used orchards (Table 5.3).

Analysis of the soil community as a whole showed the following statistically significant differences between the sites. Total soil biomass was greatest in the intensively cultivated olive orchard, followed by the pasture and smallest in the extensively used olive orchard ($p = 0.024$, Table 5.3). Bacterial biomass was greatest in the intensively cultivated olive orchard, followed by the pasture and smallest in the extensively used olive orchard ($p = 0.003$, Figure 5.2a). Fungal biomass followed the same trend, but here differences were not statistically significant ($p = 0.095$). Bacterial activity (measured as labelled thymidine (Thy) and leucine (Leu) incorporation rates) showed the same pattern, and was smallest in the extensively

Table 5.3 Biological parameters at the three different sites in the Koiliaris Critical Zone Observatory (Crete, GR) (I = intensively cultivated olive orchard, E = extensively used olive orchard, P = pasture): biomasses (in kg C ha^{-1}) of the trophic and taxonomic groups in the soil food webs, bacterial activity and microarthropod diversity. Values represent mean and standard deviation (between brackets), nd: not detected. The p-values represent significance levels from an ANOVA for repeated measures, where the superscript letters denote statistically significant differences between sites, and number of ** denotes statistical significance level (*: $p < 0.05$, **: $p < 0.01$). All measurements were done in the topsoil (0-10 cm).

Site	I	E	P	rmANOVA (p-value)
Bacteria	63.31 ^{b} (11.95)	14.60 ^{a} (3.79)	38.17 ^{b} (5.83)	0.003 **
Thy (pmol g^{-1} h^{-1})[1]	5.52 ^{b} (3.56)	0.77 ^{a} (0.46)	7.27 ^{b} (0.98)	0.026 *
Leu (pmol g^{-1} h^{-1})[2]	229.3 ^{b} (89.78)	86.26 ^{a} (21.22)	294.4 ^{b} (45.02)	0.014 *
Fungi	50.19 (10.82)	24.94 (11.73)	34.70 (10.35)	0.095
Fungal : bacterial biomass ratio	0.79 (0.05)	1.75 (0.87)	0.91 (0.23)	0.121
Flagellates	0.65 (0.32)	0.51 (0.16)	0.32 (0.26)	0.52
Amoebae	23.34 (22.30)	6.39 (4.10)	9.45 (3.22)	0.587
Fungivore nematodes	0.05 (0.02)	0.007 (0.006)	0.04 (0.02)	0.148
Bacterivore nematodes	0.10 (0.03)	0.03 (0.005)	0.06 (0.06)	0.241
Herbivore nematodes	0.18 ^{b} (0.02)	0.01 ^{a} (0.006)	0.28 ^{b} (0.12)	0.015 *
Omnivore nematodes	0.23 (0.17)	0.12 (0.04)	0.10 (0.14)	0.620
Predaceous nematodes	0.09 (0.09)	0.03 (0.03)	0.08 (0.06)	0.564
Total nematode biomass	0.65 (0.22)	0.20 (0.06)	0.57 (0.33)	0.152
Enchytraeids	0.003 (0.006)	0.03 (0.06)	0.003 (0.006)	0.545
Herbivore mites	0.0002 (0.0003)	0.0003 (0.0003)	0.0008 (0.0007)	0.329
Herbofungivore mites	0.0001 (0.0001)	0.002 (0.001)	0.008 (0.007)	0.152
Fungivore mites	0 (0)	0.003 (0.004)	0.02 (0.02)	0.174
Nematovore mites	0 (0)	0.002 (0.001)	0 (0)	0.112
Omnivore mites	0.05 (0.03)	0.17 (0.24)	0.31 (0.22)	0.374
Predaceous mites	0.0005 ^{a} (0.0008)	0.003 ^{a} (0.004)	0.08 ^{b} (0.04)	0.016 *
Herbivore collembola	0.001 (0.002)	0 (0)	0.001 (0.002)	0.689
Herbofungivore collembola	0.002 (0.003)	0.006 (0.008)	0.05 (0.07)	0.426
Fungivore collembola	0.0006 (0.001)	0.01 (0.02)	0.14 (0.10)	0.057
Symphyla	0 (0)	0.03 (0.06)	0.03 (0.03)	0.418

[1]bacterial activity: Thymidine incorporation rate; [2]bacterial activity: Leucine incorporation rate.

Table 5.3 Continued

Total biomass	137.0 [b] (16.99)	46.20 [a] (6.01)	82.98 [ab] (10.63)	0.024 *
Total microarthropod biomass	0.06 (0.02)	0.23 (0.21)	0.62 (0.47)	0.133
Microarthropod taxa richness	6.67 [a] (2.52)	15.33 [a] (7.51)	27.67 [b] (1.53)	0.037 *
Shannon H index	1.25 (0.50)	1.35 (0.77)	2.44 (0.18)	0.281
Pielou evenness J	1.52 (0.34)	1.12 (0.51)	1.69 (0.12)	0.322

used orchard (p = 0.026 and p=0.014, respectively).The ratio of fungal to bacterial biomass is indicative for C sequestration and disturbance, where a higher ratio indicates a higher C sequestration and lower disturbance. The ratio did not differ statistically significantly between the sites, although the data indicated a greater ratio in the extensively used orchard compared to the intensively cultivated orchard. Soil pH is known to influence microbial activity, but it was not significantly correlated with Thy and Leu (p = 0.518 and p = 0.546, respectively). To identify the role of microbial biomass in soil structure formation, we tested the correlations of WSA with bacterial and fungal biomass. WSA was negatively correlated with fungal biomass (r = —0.93, p = 0.239) and bacterial biomass (r = —0.87, p = 0.335).

Total nematode biomass did not differ between the sites. We only found a smaller biomass of herbivorous nematodes in the extensively used olive orchard, while we found no differences in the other groups of nematodes, nor in biomass of protozoa (amoebae and flagellates), between the sites (Table 5.3).

Microarthropod abundance varied from 7000 m^{-2} in the intensively cultivated orchard to over 200 000 individuals m^{-2} in the pasture, but did not differ statistically significantly among the three sites. The relative dominance of omnivorous mites in the intensively cultivated olive orchard (95 ± 5% of total microarthropod abundance were omnivorous mites) was remarkable, compared to both extensively used olive orchard (68 ± 32%) and pasture (67 ± 3%). Although total microarthropod biomass did not differ significantly between sites, many trophic groups within the microarthropods showed a similar pattern towards greater biomass in the pasture compared to the olive orchards. For predaceous mites this difference was significant (p = 0.016), while for fungivorous Collembola this was nearly the case (p = 0.057). When looking at the microarthropod taxa present in the three sites, the data showed a nested pattern, with a progressive species loss from the pasture towards the intensively cultivated olive orchard, and not so much of a species turnover (Appendix 5.1). That is, the taxa present in the intensively cultivated olive orchard

are mostly also present in the extensively used orchard and in the pasture, with additional taxa present at the latter sites.

In total, 49 taxa of microarthropods were found in the study sites (Appendix 5.1). Only one taxon (omnivorous prostigmatid mites from the family Alicorhagiidae) was found exclusively in the intensively cultivated olive orchard. Seven taxa were exclusively found in the extensively used olive orchards, whereas 13 taxa were only found in the pasture sites. As a result, soil microarthropods had a greater taxa richness (on average 28 taxa present) in the pasture compared to the intensively cultivated (7 taxa) and the extensively used (15 taxa) olive orchards ($p = 0.037$). When including evenness in the diversity measures, no statistically significant differences were found however, not in the Shannon diversity index nor the Pielou evenness index.

5.4 Discussion

5.4.1 Soil aggregate formation, soil organic matter, and soil nutrient cycling

Soil aggregate formation is an important index for soil quality. The intensively cultivated olive orchard had a much lower WSA than the extensively used olive orchard and the pasture. This is consistent with literature, which shows that tillage negatively affects soil aggregate stability (Beare et al., 1994), although Novara et al. (2012) found little differences between arable fields, orchards and garrigue in Sicily. Soil structure (WSA) was strongly positively correlated to C content and clay content in our study, which is also consistent with literature (Six et al., 2006; Wright et al., 2007). In contrast to our expectation, we found a negative correlation between fungal biomass and WSA. Several studies have shown that fungal biomass and activity enhance WSA (Beare et al., 1997; Bossuyt et al., 2001). Both hyphae and exudates produced by fungi (polysaccharides) are assumed to serve as bonding material (De Gryze et al., 2005). Fungal products, compared to bacterially derived products, are more chemically resistant to decay and preferentially protected from decomposition through interactions with clay and soil aggregates (Simpson et al., 2004). The negative correlation resulted from the extensively used orchard, which had the highest WSA, and the lowest bacterial and fungal biomass and activity. We think that the low water availability limited microbial activity mostly at the extensively used orchard. The limited water availability simultaneously caused physical changes such as swelling and shrinking of the clay-rich soils. Physical factors therefore might have been more important than microbial factors in the build-up and stability of soil aggregates in this system.

All parameters related to soil organic matter contents, such as TOC, TN and HWC showed highest values at the extensively used olive orchard, while C and N mineralisation rates were both highest at the pasture. The TOC and TN contents in our study were in the same order of magnitude as contents reported from less harsh environments (Culman et al., 2010; Holtkamp et al., 2011), while also higher SOC contents were found in extensive compared to intensive agriculture by Muñoz-Rojas et al. (2012). The lower C and N contents in the intensively cultivated orchard might have been due to leaf litter removal and soil tillage in this site, in combination with the lower clay content. The absence of these activities in the extensively used orchard may have led to an accumulation of plant and olive residues in a relatively undisturbed upper soil horizon, resulting in relatively high amounts of organic C and N. Tillage has been shown to decrease the C content in the topsoil in olive orchards (Aranda et al., 2011), whereas the addition of olive leaves and pruning residues has been shown to increase the TOC and HWC content in the topsoil (Fernández-Romero et al., 2014).The litter of olive trees is lignin-rich (30.4%), with a high C : N ratio (33.0) and is therefore thought to be difficult to decompose (Canali and Benedetti, 2006; Gallardo and Merino, 1993). This substrate generally favours slow fungal over fast bacterial activity, because fungi are assumed to be better able to degrade lignin-rich substances (Bossuyt et al., 2001). We found indications for a higher fungal to bacterial biomass ratio in the extensively used olive orchard, although differences were not statistically significant. In addition to substrate quality, soil pH is known to affect microbial activity; higher pH is thought to enhance bacterial activity (Bååth and Anderson, 2003) and to decrease the ratio of fungal to bacterial activity (Blagodatskaya and Anderson, 1998). We did indeed find the lowest bacterial activity in the extensively used orchard, which also had the lowest pH (5.4, in comparison with 5.9 at the pasture and 6.9 at the intensively cultivated orchard). In addition to the effect of land use type and soil microbes, also climatic conditions have been shown to affect soil C contents. In Italy, C sequestration has been found in dry areas with an annual precipitation of less than 900 mm, whereas in wetter areas a decrease in soil C content was found (Alberti et al., 2011). In our study area this would confound the differences between the sites based on land management type, as a precipitation gradient existed from the intensively cultivated olive orchard (567 mm) to the pasture (1335 mm).

5.4.2 Soil as habitat for soil organisms

All microbial parameters, i.e. the biomasses of bacteria and fungi and the two indicators for microbial activity, showed statistically significant minimum values at the extensively used olive orchard. The microbial biomass in the extensively used olive orchard was with 37 kg C ha^{-1} much lower than reported from less harsh environments (de Ruiter et al., 1993;

Holtkamp et al., 2008), while the pasture (72 kg C ha^{-1}), and especially the intensively cultivated olive orchard (111 kg C ha^{-1}), reached values that are closer to values reported in literature. For example, Holtkamp et al. (2008) report 60—100 kg microbial C ha^{-1} for fields in transition from arable field to heathland in the Netherlands, while de Ruiter et al. (1993) report similar biomasses from arable fields in the Netherlands (90—100 kg C ha^{-1}) and prairie soil in the USA (150 kg C ha^{-1}), but much higher values for arable fields in USA (400—550 kg C ha^{-1}) and Sweden (900—1300 kg C ha^{-1}) (all values correspond to 0—10 cm soil depth). Other studies provide higher microbial biomass levels ranging from 300 to 1300 kg C ha^{-1} based on chloroform fumigation methods (e.g. (Culman et al., 2010; Schnürer and Rosswall, 1987; Schröter et al., 2003)), but values found using this method are not directly comparable to microscopic counting (Martens, 1995). The intensity of agricultural management at the intensively cultivated olive orchard including tillage, as well as soil pH and leaf litter composition, led to the expectation that we would find a lower fungal to bacterial biomass ratio, as indicator for C sequestration and disturbance, compared with the extensively used orchard and pasture. A lower ratio indicates a lower C sequestration and higher disturbance. We indeed found indications for a lower ratio in the intensively cultivated olive orchard, but the differences were not significant.

5.4.3 Microarthropod biomass and diversity

Total soil microarthropod biomass and taxonomic richness within the soil microarthropods have been proposed as biological soil quality indicators (Gardi et al., 2009; Parisi et al., 2005), but the suitability of these parameters has not yet been tested on soils under harsh conditions. Total microarthropod biomass in our systems, especially in the intensively cultivated olive orchard, was with 0.06—0.72 kg C ha^{-1} lower than biomasses of 0.5—3.8 kg C ha^{-1} reported from less harsh arable systems (de Ruiter et al., 1993; Holtkamp et al., 2011). In our sites, microarthropod taxonomic richness strongly increased along with microarthropod biomass from the intensively cultivated olive orchard, to the extensively used olive orchard, to the pasture. Microarthropod taxa richness was higher in our study than reported from semi-arid croplands in central Spain (Kautz et al., 2006), comparable to the values found by Tsiafouli et al. (2005) in pine forests in Greece, and in the lower range of the values found on farms in Iceland and Austria (chapter 2). The higher richness we found in the pasture, compared to the olive orchards, confirms findings in Mediterranean Spain showing the highest richness in Oribatid mite communities in pastures and forests in comparison with cropland (Arroyo and Iturrondobeitia, 2006). This pattern of increasing microarthropod biomass and taxonomic richness could be related to a lower disturbance of the topsoil in the pasture, for which the microarthropods are known to be very sensitive

(Wardle, 1995), but could also be related to soil moisture availability. Soil moisture availability in our sites increased with elevation. This was caused by the increasing average precipitation and decreasing average temperature (Table 1), hence decreasing evaporation, leading to a high soil moisture content in the pasture as compared to the olive orchards. Also Tsiafouli et al. (2005) reports an increasing species richness and diversity of soil microarthropods with an increase in water availability in an experimental setup in pine forests in Greece.

We found statistically significant differences in taxa richness, but not in the Shannon diversity index (SDI), nor in Pielou evenness. Kautz et al. (2006) finds comparably low SDI values in croplands in central Spain, despite a lower taxa richness and microarthropod abundance. It appears that taxonomic richness of microarthropods is able to differentiate between land management practices, hence is useful as an indicator of ecological soil quality, whereas the SDI may separate harsh sites from other sites, but is not sensitive enough to detect differences between different land management practices under harsh conditions.

5.4.4 Limitations

The sites discussed in this study are part of a coherent set of CZOs throughout Europe, ranging from a soil formation cycle to soils at risk of degradation as presented in this paper. The aim of the present study was to investigate soil biological properties under different land management types in southern European soils that are at risk of potential soil degradation and desertification, via an integral approach including physical, chemical and biological soil processes. The presently chosen design did not allow to pronounce upon land management effects in a generalised way, however, as the study included information from only one example per land management type. As these single examples of land management types were measured at various plots, we could statistically test differences between sites, but we could not generalise our results to an interpretation in terms of land management. Generalisation over land management type was also constrained by other potentially important factors that may have played a role in the observed differences between the sites, such as temperature, moisture availability, and clay content. A second reason to treat our results with caution is that the measurements were carried out at one particular moment, i.e. May 2010. Therefore we lack information regarding variability in time and/or effects of seasons. For future research aiming at improved generalisation of the results of these study sites, accounting for temporal and spatial variability by extending the sampling design is recommended.

5.5 Conclusions

The novelty of the present study is the investigation of a large range of soil biological properties under different land management types under relatively harsh conditions on semi-arid Crete. The soil biological parameter most sensitive to land management seemed to be the microarthropod richness: taxa richness increased from the intensively cultivated olive orchard to the extensively used orchard to the pasture. This confirmed the use of this parameter as indicator for ecological soil quality under semi-arid conditions. Microbial biomass and especially microarthropod biomass showed lower values in our harsh study sites than reported from less affected areas. In this way they may also be suitable as ecological indicators for soil degradation. The ratio of fungal to bacterial biomass, which is frequently proposed as indicator for C sequestration and disturbance, did not show a clear pattern in our study, probably because at our sites many factors may have affected this ratio, such as tillage, pH and leaf litter composition, and might therefore be less suitable as indicator for soil quality under harsh conditions.

Appendix 5.1 Biomasses (kg C ha^{-1}) of the microarthropod taxa in the soil food web at three different sites on Crete, Greece (I = intensively cultivated olive orchard, E = extensively used olive orchard, P = pasture). Trophic groups: omnivorous mites (Ommi), fungivorous mites (Fumi), nematovorous mites (Nemi), predatory mites (Prmi), herbivorous mites (Hemi), herbofungivorous mites (HFmi), herbofungivorous collembolans (HFco), fungivorous collembolans (Fuco) and Symphylans (Symp). Numbers represent mean and standard deviation (between brackets), measured in the topsoil (0—10 cm).

Site		I	E	P
Acari				
Astigmata	Ommi	0.0003 (0.0005)	0.0025 (0.0044)	0.0021 (0.0037)
Tyrophagus	Ommi	0.0018 (0.0031)	0.2417 (0.3952)	
Mesostigmata	Ommi			0.0007 (0.0012)
Epicriopsis	Fumi		0.0001 (0.0002)	
Hypoaspis	Prmi	0.0005 (0.0008)	0.0005 (0.0008)	0.0087 (0.0076)
Leioseius	Prmi			0.0069 (0.0088)
Macrocheles	Prmi			0.0045 (0.0079)
Pachylaelaps	Prmi			0.0045 (0.0079)
Rhodacaridae	Prmi			0.0110 (0.0129)
Zercon	Nemi		0.0009 (0.0008)	
Oribatida	Ommi		0.0213 (0.0097)	0.0400 (0.0542)
Aphelacarus acarinus	HFmi			
Brachychthoniidae	HFmi	0.0001 (0.0001)	0.0005 (0.0008)	0.0014 (0.0016)
Ceratozetidae	Ommi		0.0008 (0.0014)	0.0103 (0.0104)
Cosmochthonius	Hemi	0.0002 (0.0003)	0.0003 (0.0002)	0.0008 (0.0007)
Damaeidae	Fumi		0.0003 (0.0003)	0.0005 (0.0009)
Hermanniella	HFmi			0.0005 (0.0009)
Licnodamaeus pulcherrimus	HFmi		0.0011 (0.0008)	0.0002 (0.0003)
Mycobatidae	HFmi			0.0024 (0.0034)
Nanhermanniidae	HFmi		0.0005 (0.0009)	
Oppiidae	Ommi		0.0005 (0.0009)	0.0200 (0.0271)
Oribatellidae	HFmi			0.0008 (0.0006)
Pelopsidae	Fumi			0.0003 (0.0006)
Rhinoppia	Fumi		0.0021 (0.0030)	
Tectocepheus	Ommi			0.0275 (0.0110)
Prostigmata				
Alicorhagiidae	Ommi	0.0136 (0.0058)		
Erythraeidae	Prmi		0.0005 (0.0009)	
Eupodes	Ommi		0.0013 (0.0023)	0.0080 (0.0121)
Microtydeus	Ommi	0.0090 (0.0020)	0.0125 (0.0084)	0.0942 (0.0837)
Nanorchestes	Ommi			0.0119 (0.0154)
Paratydeidae	Prmi		0.0015 (0.0026)	0.0287 (0.0151)

Pyemotes	Prmi			0.0072 (0.0068)
Pygmephorus	Fumi		0.0010 (0.0010)	0.0177 (0.0188)
Rhagidia	Prmi		0.0010 (0.0009)	0.0041 (0.0042)
Scutacarus	Ommi		0.0041 (0.0064)	0.0212 (0.0200)
Speleorchestes	Ommi	0.0013 (0.0012)		0.0182 (0.0225)
Stigmaeidae	Prmi			0.0014 (0.0024)
Tarsonemus	Ommi	0.0006 (0.001)	0.0018 (0.0030)	0.2529 (0.1300)
Tydeidae	Ommi	0.0516 (0.0439)	0.0003 (0.0004)	0.0032 (0.0038)
Collembola				
Entomobryomorpha				
Lepidocyrtus	HFco		0.0011 (0.0087)	0.0188 (0.0214)
Lepidocyrtus lignorum	HFco		0.0050 (0.0087)	
Poduromorpha				
Friesea	Fuco			0.0096 (0.0167)
Hypogastrura	Fuco		0.0046 (0.0030)	
Mesaphorura	Fuco			
Onychiuridae	HFco	0.0017 (0.0030)		0.0289 (0.0501)
Onychiurus	Fuco	0.0006 (0.001)		0.0967 (0.0670)
Paratullbergia	Fuco		0.0079 (0.0136)	0.0084 (0.0110)
Symphypleona				
Sminthuridae	Heco	0.0011 (0.0020)		0.0014 (0.0024)
Protura	Fuco		0.0005 (0.0009)	0.0251 (0.0287)
Symphyla	Symp		0.0336 (0.0582)	0.0250 (0.0284)

Chapter 6

General Discussion

Jeroen P. van Leeuwen

6.1 Introduction

Soil is one of the most important natural resources for life on Earth and provides important ecosystem services, such as carbon (C) sequestration, nutrient cycling and hydrological regulation. Soil quality, here defined as the soil's ability to deliver these ecosystem services, is being drastically reduced in many locations and regions worldwide due to human activities such as intensive agriculture, commercial forestry and urban development. This loss in soil quality ultimately leads to soil degradation, erosion and desertification (Milgroom et al., 2007). Losses in soil quality impose a severe and increasing risk for the growing human population. It is therefore essential that we are aware of the importance of protecting soil, and at the same time understand processes that build up and regenerate soil.

The key objective of the present study is to obtain a better understanding of soil food web structure and functioning, and how structure and functioning develop during processes of soil formation and degradation under various land use types. Soil formation, soil quality, and soil degradation have been extensively approached from an abiotic perspective, with emphasis on soil structure, water erosion and nutrient cycling (Celik, 2005; Kosmas et al., 1997; Solomon et al., 2000). Soil organisms have received less attention, and the studies that have been performed have often focused on single groups of organisms, such as microbes (Bending et al., 2004), nematodes (Ferris et al., 2001), or microarthropods (Hodkinson et al., 2003; Kaufmann, 2001), instead of on the level of the structure and composition of the soil food web as a whole. Although the importance of soil organisms in soil quality and fertility is more and more acknowledged (Brussaard et al., 2007; Cole et al., 2006; De Deyn et al., 2003; Lavelle et al., 2006), soil organisms have mostly been studied in productive agricultural fields (Birkhofer et al., 2008; Carpenter-Boggs et al., 2000; Doran and Zeiss, 2000) or in well-developed natural ecosystems (De Deyn et al., 2003; Schröter et al., 2003), but not during the first stages in soil formation or in degraded soils. As described in chapter 1, the main research question of this thesis is therefore: "How do soil food webs, and the ecosystem services they provide, respond to changes in land use at various stages of the soil life cycle?" To answer this question, some more detailed objectives are formulated, each focusing on a particular stage in the soil life cycle:

1. To investigate soil food web assembly during early soil formation and vegetation succession.
2. To investigate soil food web structure and functioning under different agricultural practices in productive soils.

3. To investigate the soil microbial community structure and activity in different land uses and at different soil depths.
4. To investigate soil food web structure and functioning in soils that are considered to be at risk of degradation and desertification.

In this final chapter, firstly I recapture and discuss the results obtained in the field surveys as described in chapter 2-5. Secondly, I combine these results in order to answer the main overarching research question. Finally, I end this chapter with a short conclusive synthesis.

6.2 Specific objectives

6.2.1 Soil food web assembly during soil formation and vegetation succession

Studying natural soil ecosystem development and the assembly of the soil food web in particular, in the early stage of the soil life cycle is fundamental for understanding processes that build up and regenerate soil. In chapter 2, I investigated how soil food webs develop along soil formation in chronosequences in two glacier forefields in Iceland (forefield of the Skaftafelljökull) and Switzerland (forefield of the Damma Glacier). Along both chronosequences the soil ecosystem developed as expected, i.e. an increase in soil C and nitrogen (N), vegetation cover and plant species richness, soil total biomass (total and per trophic groups), number of trophic groups, and trophic chain length. The results showed how all components of terrestrial ecosystems (soil formation, vegetation development, soil food web assembly) occured in concert, and along stable pathways of ecosystem development.

6.2.2 Soil food web structure under different agricultural practices

Intensive agricultural production is seen as an important driver for the loss of soil quality. Studying the influence of different agricultural practices is necessary in order to better understand how to protect and regenerate the soil's ability to deliver ecosystem services. In chapter 3 I investigated the effects of differing agricultural management (conventional or organic) on soil biological quality in farms in Iceland (grasslands) and Austria (croplands). The results showed that farming type did not systematically influence organic matter contents or composition, nor soil C and N contents. Also soil food web structure, in terms of presence of trophic groups of soil organisms, was highly similar among all farms, indicating a low sensitivity of trophic structure to land use or climate. However, overall soil organism biomass and soil microarthropod diversity were higher on organic farms than on conventional farms. This difference was found

across countries, and farm, crop and soil types. These results confirmed that organic farming can enhance soil biomass and organism diversity. Additionally, microarthropod diversity was found to be a sensitive and consistent indicator for land management.

6.2.3 Soil microbial community structure in different land uses and soil depths

As shown in chapter 3, agricultural land management can affect soil biological properties. In chapter 4 I investigated whether such effects of land use can also be found in deeper soil layers. Soil microbial biomass, activity and community structure were therefore investigated at different soil depths (A, AC and C horizons) in forests, grasslands and arable fields. All microbial biomass estimates decreased with soil depth, irrespective of land use type. Both the absolute microbial biomass, and the community structure differed between topsoil and subsurface layers. When comparing the soil microbial community under different land use types, a differentiation from a similar community structure in the subsurface of each land use type to distinct populations in the topsoil was revealed. These results indicated that the effect of land use was mostly limited to the topsoil, while pedological conditions were mostly determining the microbial community below the rooting depth.

6.2.4 Soil food web structure and functioning in soils under risk of degradation

As shown in chapters 3 and 4, land management can strongly affect soil biological properties in productive soils. On the long-term, e.g. after millennia of intensive agricultural use, soils can become at risk of degradation and desertification, especially under harsh climatic conditions. In chapter 5 I investigated how soil food web structure and functioning are affected by land management (intensive and extensive olive orchard versus pasture) in semi-arid Crete. Soil organism biomasses in all sites were relatively low compared to literature values reported for less harsh systems. A comparison between the Crete sites showed that microarthropod richness was higher in the pasture compared to both olive orchards. These results confirmed that microarthropod taxonomic richness is a very useful indicator for ecological soil quality, also because in the present study it appeared to be sensitive enough to show differences between land management practices under harsh conditions.

6.3 Soil organism biomass, activity and diversity during the soil life cycle

Biological measures such as biomasses, activity and diversity of soil organisms have shown their indicative value in individual sites (Gardi et al., 2009; Rutgers et al., 2009; Sharma et al., 2011). However, generalisability is an important characteristic of a useful indicator. Here, I investigated a set of soil biological parameters in all European Critical Zone Observatories (CZOs). This analysis included the data presented in chapters 2-5, together with the data from semi-natural forests in Austria, and productive coniferous forests in Czech Republic (Banwart et al., 2012) (Figure 1.1). The different CZOs do not represent solely a difference in their place along the soil life cycle. They also provide a large range of conditions, including different land use types and land management histories, climatic conditions and soil types.

The biological parameters that were identified as potential soil quality indicators in the individual chapters can in this way be evaluated in the context of the soil life cycle. In general, these biological soil parameters were expected to show an optimum curve, when plotted on the formation-productive-degradation axis. Along this axis, the soil food web was expected to build up in biomass and structure to become highest in productive intermediate soils, followed by a decrease in soil under risk of degradation. Soil organism biomass and food web structure indeed built up in the youngest soils and decreased in the oldest soils. However, intermediate sites did not have higher biomass or a more complete food web structure, because land use type turned out to be much more important than the stage of the soil on the life cycle. Ideally, land use types would have been divided equally over all different stages of the cycle for the detection of patterns due to soil formation, but this is not the case in the CZO dataset. In the CZO set, land use was related to soil age, i.e. natural vegetation was present mostly on the youngest soils, where arable land was mostly present on the oldest soils.

6.3.1 Soil microbial biomass and activity

Soil microbial biomass and activity are often used as indicator for soil quality (Rutgers et al., 2009; Sharma et al., 2011), as microbes play important roles in biochemical processes and are closely linked to soil fertility via decomposition of organic matter and nutrient cycling (Brussaard et al., 2007). Microbial biomass and activity have indeed been shown to reveal differences between land use types over a large geographical range (Creamer et al., 2014). In the present study, I also found that the microbial community structure strongly differed with land use types in terms of functional group abundances as assessed by a PLFA analysis (chapter 4). But the studied sites were from intermediate stages in the soil

life cycle and lacked differences in processes related to soil formation and degradation. When sites along the soil life cycle were compared, microbial biomass was found to be low in young, developing soils, especially in the chronosequence in Iceland, following the expectations (chapter 2). Biomass values in intermediate, productive sites differed mostly between different land use types. Forests contained a higher microbial biomass than grasslands and arable fields. This confirmed the results of other studies (Joergensen, 1996; Nsabimana et al., 2004). Within land use types, differences were found as a result of specific land management practices. For example, organic farms tended to have a higher microbial biomass than conventional farms (chapter 3) and also differences were found between olive orchards and pasture in semi-arid Crete (chapter 5). The main driver for differences in the microbial biomass seemed to be the C input in the soils through litter deposition and root turnover (chapter 4), confirming the results of Griffiths et al. (1999). Soil organic C correlated also to microbial biomass in the natural chronosequences in Iceland and Switzerland and agricultural fields in Austria and Iceland (chapter 2, 3). These results confirmed therefore the predictive value of microbial biomass and activity for C sequestration potential that has been found for a large geographic region (Creamer et al., 2015). The input, both quantity and especially quality, of organic matter also influenced the ratio of bacterial to fungal biomass. In Crete for example, the ratio of bacterial to fungal biomass was lowest in the extensive orchard, were quality of organic matter was lowest. The differences in bacterial to fungal biomass ratio probably were caused by management practices such as the frequency of disturbance and amount and type of organic amendments added to the soils.

6.3.2 Soil nematode biomass

Nematodes have received a lot of attention for their role as important microbivores and as indicator for soil quality, e.g. related to life-history traits (Bongers and Bongers, 1998), functional group biomass and diversity (Ferris et al., 2001). In the CZOs covering the soil life cycle, total nematode biomass was highest in the young soils of the Damma glacial forefield. This was an unexpected result, as the soil in the Damma forefield are early successional soils without a full vegetation cover. In contrast, total nematode biomass in the similar aged glacial forefield in Skaftafell was much lower (chapter 2). In comparisons within land use types, differences were found as the result of land management practices. For example organic farming showed higher nematode biomass compared to conventional farming (chapter 3) and intensively used orchards showed higher nematode biomass than extensively cultivated orchards (chapter 5). However, when sites were aggregated into land use types, no significant differences were found. All land use types and soil ages

revealed comparable estimates for total nematode biomass, in the range of nematode biomasses from other studies in agricultural fields (de Ruiter et al., 1993), forests (Schröter et al., 2003) and grasslands (Holtkamp et al., 2008). Also when biomasses were compared for the different trophic groups (e.g. fungivores, bacterivores, omnivores), no differences were found between land use types when all soils along the soil life cycle were compared. Hence, nematode biomass is less suitable as soil quality indicator on a larger spatial scale, but can be used for testing the effects of specific agricultural practices.

6.3.3 Soil microarthropod biomass

Microarthropod biomass was highly variable over all sites along the soil life cycle, and over all land use types (Figure 6.1A). The range of biomass values found in the CZOs falls within the range of microarthropod biomasses from other studies (de Ruiter et al., 1993; Holtkamp et al., 2011). Microarthropod biomass however showed a decrease from the natural developing sites in the Damma glacier field via forest and grasslands to the arable fields in Austria and Crete (Figure 6.1D). These sites can be seen as a soil disturbance gradient, with the glacial forefield showing lowest disturbance, and arable fields showing highest disturbance. These results are in line with previous studies showing higher biomass in forests (Minor and Cianciolo, 2007), and in grasslands (Vreeken-Buijs et al., 1998) compared to arable fields. Microarthropod biomass can therefore be used as indicator for soil disturbance when different land uses are compared, but due to the high variability within sites is less suitable as indicator at a lower spatial level, e.g. when comparing different agricultural practices.

6.3.4 Soil microarthropod richness and diversity

Taxonomic richness and diversity of soil microarthropods have earlier been proposed as biological soil quality indicators, as microarthropods are sensitive to environmental change and disturbances (Bispo et al., 2009; Cluzeau et al., 2012; Gardi et al., 2009; Gulvik, 2007; Parisi et al., 2005). Within particular areas and land use types, I also found that microarthropod richness and diversity can be useful indicators for soil quality, as these measures were able to show differences as a result of land use and management (chapters 2, 3). This was also shown for sites under conditions for which these indicators have not been tested so far, such as under the semi-arid, harsh conditions in Crete (chapter 5). First we saw that microarthropod richness and diversity increased with soil productivity in the glacial forefield in Damma (chapter 2). Also, the farmlands under different management in Iceland and Austria, showed a higher microarthropod diversity in the organic farms than in the conventional farms (chapter 3) confirming results from other agricultural areas throughout Europe (Doles et al., 2001; Macfadyen et al., 2009).

In Crete, microarthropod taxonomic richness strongly decreased with increasing soil disturbance from the pasture to the intensively cultivated olive orchard, showing that microarthropod diversity is able to indicate differences in land management under harsh conditions (chapter 5). The question remains however whether microarthropod richness and diversity can also indicate different stages along the soil life cycle. Soil microarthropod taxonomic richness and diversity tended to decrease in sites along the soil life cycle from the young glacial sites in Switzerland to the old arable sites in Crete (Figure 6.1B,C). However, differences in richness and diversity were stronger between land use types than between stages of the soil life cycle, with higher microarthropod richness in natural areas and forest than in grasslands and arable fields (Figure 6.1E,F).

In the natural chronosequences presented in chapter 2, soil microarthropods have only been sampled in part of the successional stages. Also in other studies, datasets of microarthropod diversity are incomplete (Creamer et al., 2015). This indicates potential problems with the use of these organisms as indicator. First of all, sampling these organisms can be challenging in certain soil types, and extraction of the microarthropods from the soil is labour intensive and time consuming. Secondly, identification of microarthropods to a low taxonomic level relies on a rare expertise and can be very difficult. With progressive technological developments, for example the use of environmental DNA extraction and barcoding of the extracted microarthropods (Taberlet et al., 2012), the analyses of these species might become faster and more cost-effective, and will therefore become more prominent in soil surveys and valuable indicators in biological monitoring studies (Gardi et al., 2009).

6.3.5 Earthworms

Finally I must note that one important group of soil organisms is missing in this thesis: earthworms. In the field surveys carried out for this thesis, earthworms were left out of the sampling for practical reasons. Earthworms are relatively difficult to sample representatively. Either, they have to be extracted from soil cores in the field or large volumes of soils have to be transported to the lab, which was hardly possible given the various locations. I realize that earthworms do play an important role in decomposition of organic matter (Lavelle and Martin, 1992), C and N mineralisation (Lubbers et al., 2013) and soil structure formation as ecosystem engineers (Pérès et al., 2010).

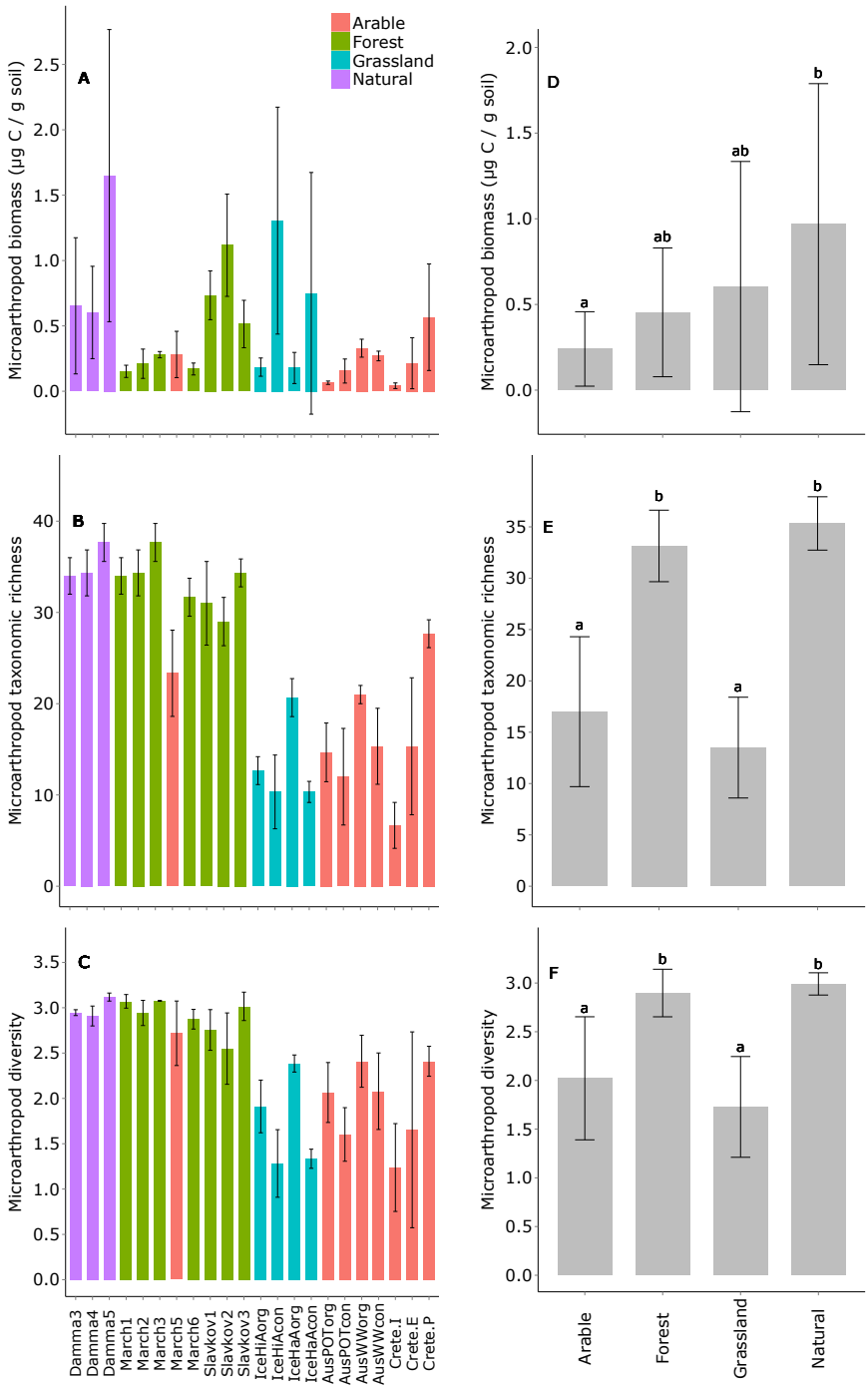

Figure 6.1 Microarthropod biomass (A,D), taxonomic richness (B,E) and Shannon diversity index (C,F) for all sites (left pane), and per land use (right pane). The small letters indicate significant differences based on Anova with Tuckey HSD post-hoc tests.

6.4 Soil food web metrics during the soil life cycle

Based on biomasses of functionally defined groups of soil organisms, I constructed soil food web diagrams for all sites along the soil life cycle. The topological structure of the food webs was found to be very similar in most sites (Figures 2.1, 3.1 and 5.1). Also other food web metrics such as link density, connectance, mean and maximum trophic level, and maximum chain length were very similar. Strong topological similarity of food webs from different land use types has also been shown in other studies (de Ruiter et al., 1993; Holtkamp et al., 2008; Schröter et al., 2003). This insensitivity to land use and land management strongly indicates that the qualitative topological structure of the soil food webs is not a useful metric to relate soil biodiversity to ecosystem functioning, which requires a more quantitative description, e.g. in terms of biomass values, feeding rates and interaction strengths.

6.5 Soil food web functioning and the provision of ecosystem services

Above, I discussed how in the present study the soil biological community was affected by land use and land management. Next step is how to translate the effects on soil biological communities to effects on soil ecosystem functioning and the provision of soil ecosystem services, such as soil structure formation, C sequestration and nutrient cycling (Brussaard et al., 1997; Brussaard et al., 2007; Lavelle et al., 2006).

Regarding soil formation, soil microbes are known to enhance soil structure formation via the production of exudates that enhance aggregation of soil particles (De Gryze et al., 2005; Tisdall and Oades, 1982; Wright et al., 2007) and via physically binding of soil particles by fungal hyphae (Bossuyt et al., 2001). Also soil fauna can play a role in creating a stable soil pore structure through moving in the soil and the formation of faecal pellets (Jastrow and Miller, 1991; Lavelle et al., 2006; Lee and Foster, 1991; Oades, 1993). However, in the present study, carried out in the CZO sites, soil organisms did not seem to be the main driver for soil aggregate formation, as soil organism biomass and activity did not correlate consistently with soil aggregate stability, showing opposite patterns in different sites. Microbial biomass was positively correlated to soil porosity, closely linked to aggregate stability (Regelink et al., 2015), in the three compared land use types in Austria (chapter 4), but was negatively correlated to aggregate stability in Crete (chapter 5), whereas no correlation was found in the investigated farms in Iceland and Austria (chapter 3). Measured soil physical and chemical parameters, such as iron-(hydro)oxide, clay content and soil pH, were found to provide a better explanation for aggregate stability, which was also found by Regelink and colleagues (Regelink et al., 2015).

Regarding soil ecosystem functioning, a lot is already known about the role soil organisms play in C sequestration and nutrient cycling (Brussaard et al., 2007; Lavelle et al., 2006). Microbial organisms determine the dynamics of soil organic C and N, through the decomposition of SOM (Six et al., 2006). Recently, microbial biomass and activity have been found to be the best predictors for C storage potential (Creamer et al., 2015). Soil fauna facilitates the decomposition of organic matter by microbes through shredding of larger particles, increasing the availability and reactive surface of soil organic matter (SOM) (Lavelle et al., 1993). In addition, grazing by microbivores such as nematodes can stimulate microbial activity (Brussaard et al., 1997; Buchan et al., 2013). Apart from influencing microbial activity and hence decomposition of SOM, soil fauna can also contribute directly to soil ecosystem functioning through trophic activities in the soil food web (de Ruiter et al., 1993; Verhoef and Brussaard, 1990). Also in the present study on the CZO sites soil microbial biomass and activity showed a strong correlation with soil C and N mineralisation rates and soil C content. This correlation was found over all sites along the soil life cycle. Also biomass of the faunal trophic groups correlated with C and N mineralisation and C content, but these correlations were less strong. This indicated that soil microbes form the most important soil organisms in relation to the provision of ecosystem services such as C sequestration and nutrient cycling, which was expected given the dominance of the soil microbes in the soil biomass as was also found in the studies CZO sites.

6.5 Concluding remarks

This study investigated structure and functioning of the soil food web and ecosystem services during processes of soil formation and degradation under various land use types. In the first stages of the soil life cycle, soil food webs were found to assemble with increases in biomass and activity of the soil organisms and an increase in vegetation cover and diversity. In the intermediate productive soils, land use type and land management were the main drivers affecting soil food web structure and functioning, although this effect was primarily found in the topsoil, where plant roots have their spheres of influence. At the later stages of the soil life cycle, organism biomass and activity were strongly negatively affected by the long-term intensive agriculture, also due to the harsh environmental conditions of the sites we studied, i.e. the CZO at the island of Crete. Soil food web topological structure was very similar at all sites, independent of climate, land use and management. In contrast, quantitative measures such as trophic group biomass were found to be sensitive to land use, management, and climatic conditions. Therefore these measures can

be used as indicators for soil quality, also because these quantitative measures can be related to soil ecosystem services. A special interesting indicator of soil quality was the soil microarthropod taxonomic richness and diversity. The usefulness of this indicator was already suggested by several studies, and was also confirmed by the present findings. In addition, the present results showed that this indicator is also sensitive enough to indicate differences in soils under risk of degradation.

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Summary

Soil is one of the most important natural resource for life on Earth and provides important ecosystem services, such as production of food, feed and fibre, climate regulation through sequestration of carbon, purification and regulation of water and attenuation of contaminants. Soil quality, here defined as the soil's ability to deliver these ecosystem services, is drastically reduced in many locations and regions worldwide due to human activities such as intensive agriculture, commercial forestry and urban development. This loss in soil quality ultimately leads to soil degradation, erosion and desertification, imposing a severe and increasing risk for the growing human population. It is therefore essential that we are aware of the importance of protecting soil, and at the same time understand processes that build up and regenerate soil. The key objective of the present study is to obtain a better understanding of soil food web structure and functioning, and how structure and functioning develop during processes of soil formation and degradation under various land use types. As described in chapter 1, the main research question of this thesis is therefore: "How do soil food webs, and the ecosystem services they provide, react to changes in land use at various stages of the soil life cycle?" This research question is answered through investigating soils and soil food webs along particular stages of the soil life cycle.

This thesis forms part of the SoilTrEC project (Soil Transformations in European Catchments), in which the network of European Critical Zone Observatories (CZOs) is studied with the aim to describe and quantify the physical, chemical, and biological processes that are critical to soil ecosystem functioning. Included in the network are sites along the soil life cycle: natural sites along soil formation gradients (Switzerland, Iceland), productive agricultural and forested sites differing in soil management (Austria, Iceland, Czech Republic), and agricultural sites under risk of degradation (Greece).

I investigated in chapter 2 how soil food webs develop along soil formation in chronosequences in two glacier forefields in Iceland and Switzerland. Along both chronosequences the soil ecosystem developed as expected, i.e. an increase in soil C and N, plant cover and species richness, soil organism biomass (total and per trophic group), number of trophic groups, and trophic chain length. The results show how all components of terrestrial ecosystems (soil formation, vegetation development and soil food web assembly) occur in concert, and along stable pathways of ecosystem development.

Intensive agricultural production can be an important driver for the loss

of soil quality. Studying the influence of different agricultural practices is necessary for understanding how to protect and regenerate the soil's ability to deliver ecosystem services. I investigated the effects of differing agricultural management (conventional or organic) on soil biological quality in farms in Iceland (grasslands) and Austria (croplands) in chapter 3. The results showed that organic farming does not systematically influence organic matter contents or composition, nor soil C and N contents. Also soil food web structures, in terms of presence of trophic groups of soil organisms, were highly similar among all farms, indicating a low sensitivity of trophic structure to land use or climate (i.e. Iceland vs. Austria). However, soil organism biomass and diversity were higher on organic farms than on conventional farms. This difference has been found in both countries, and across farms, crops and soil types. These results confirm that organic farming can enhance soil biomass and organism diversity. Additionally, microarthropod diversity was found as a sensitive and consistent indicator for soil quality under different land management.

As follow-up on the results described in chapter 3, i.e. the effects of agricultural land management on biological soil properties, I studied whether the effects of land use are also found in deeper soil horizons in chapter 4. I investigated soil microbial biomass, activity and community structure in different soil depths (i.e. A, AC and C horizons) in forests, grasslands and arable fields. Results showed that all microbial biomass estimates decreased with soil depth, irrespective of land use. Both the absolute microbial biomass and the community structure differed between topsoil and subsurface layers. The strong effects of land use in the topsoil decreased with soil depth. This indicates that the effect of land use is mostly limited to the topsoil, and pedological conditions are mostly determining the microbial community structure below the rooting depth.

As next step I looked at soils at the end of the soil life cycle, that are currently under risk of degradation after millennia of intensive agriculture under harsh climatic conditions. Soil food web structure and functioning in agricultural sites under different land management (intensive and extensive orchard versus pasture) in semi-arid Crete have been investigated in chapter 5. Soil organism biomasses in all studied sites were low compared to literature values reported from soils under less harsh conditions. Microarthropod richness was higher in the pasture than in both olive orchards, again confirming that microarthropod taxonomic richness is an useful indicator for ecological soil quality. The results in this chapter show that the microarthropod species richness indicator is able to separate harsh sites from other systems, and is also sensitive enough to show differences between land management practices under harsh conditions. Therefore this indicator also has potential as biological indicator for soil degradation.

Biological measures such as biomasses, activity and diversity of soil organisms were found to have an indicative value in our studied sites (chapter 2-5). To evaluate the generality of these results, I also studied these biological parameters in soils from all CZOs along the soil life cycle in chapter 6. This analysis includes the data presented in chapters 2-5, together with data from semi-natural forests in Austria and productive coniferous forest in Czech Republic. Along the formation-productive-degradation axis, the soil food web was expected to build up in biomass and structure, be highest in the intermediate soils, and decrease in soils at or nearby degradation. However, this expectation was not confirmed by the overall analysis, as land use type turned out to be much more important than the stage of the soil on the life cycle.

In the analysis of all CZO sites in chapter 6, the possible role of soil organisms in the soil ecosystem functioning was also investigated, in terms of soil structure formation and C and N mineralisation. Although soil organisms are known to have an important role on soil structure formation, I did not find clear indications of such a role in the studied CZOs. Soil physical and chemical parameters, such as iron-(hydro)oxide, soil organic C and soil pH, were found to have a stronger impact on water stability of aggregates. Soil microbial biomass and activity were much stronger correlated with ecosystem process rates such as C and N mineralisation rates. Also biomass of other trophic groups correlated, although less strongly than soil microbes, with C and N mineralisation and C content.

In conclusion, I have found that soil food webs assemble in a directive manner: organism biomass and activity increase with soil productivity. In productive soils, land use type and land management are the main drivers affecting soil food web structure and functioning, although this effect is limited to the topsoil. Pedological conditions mostly determine the microbial community structure below the rooting depth. Under harsh conditions, soil organisms are negatively affected by duration of intensive agricultural land use. Additionally, I have found that soil microarthropod taxonomic richness and diversity consistently qualify as indicator for soil biological quality.

Samenvatting

De bodem is een van de belangrijkste natuurlijke hulpbronnen voor het leven op aarde en voorziet in belangrijke ecosysteemdiensten, zoals voedsel- en brandstofproductie, klimaatregulatie door het vastleggen van koolstof, het zuiveren en reguleren van grondwater en het afbreken van verontreinigende stoffen. Bodemkwaliteit, gedefinieerd als het vermogen van de bodem voor het leveren van de genoemde ecosysteem diensten, is sterk gereduceerd op veel plekken in de wereld door toedoen van menselijke activiteiten, zoals intensieve landbouw, commerciële bosbouw en stedelijke uitbreiding. Dit verlies in bodemkwaliteit kan uiteindelijk leiden tot bodem degradatie, erosie en verwoestijning, wat een toenemend probleem vormt voor de groeiende wereldbevolking. Daarom is het belangrijk dat we ons bewust zijn van het belang van bodembescherming, en tegelijkertijd dat we de processen begrijpen die een belangrijke rol spelen in bodemvorming en regeneratie van de bodem. Het belangrijkste doel van deze studie was om de structuur en het functioneren van bodem voedselwebben beter te begrijpen. Daarnaast heb ik ook gekeken naar hoe de structuur en functioneren van bodemvoedselwebben worden beïnvloed door processen tijdens bodemvorming en -degradatie, en in verschillende landschapstypen. Zoals beschreven in hoofdstuk 1 was de onderzoeksvraag van deze thesis dan ook: "Hoe reageren bodemvoedselwebben, en de ecosysteemdiensten die door de bodemorganismen geleverd worden, op veranderingen in landgebruik in de verschillende stadia van de levenscyclus van de bodem?" Deze onderzoeksvraag heb ik geprobeerd te beantwoorden door bodems and bodemvoedselwebben in verschillende levensstadia van de bodem te onderzoeken.

Deze thesis vormt een onderdeel van het EU project SoilTrEC (Soil Transformations in European Catchments). In dit project werd een netwerk van Europese locaties bestudeerd om de belangrijke fysische, chemische en biologische bodemprocessen te beschrijven en te kwantificeren. In dit netwerk zijn locaties opgenomen uit verschillende stadia van de levenscyclus van de bodem: plaatsen waar het proces van natuurlijke bodemvorming plaatsvindt (in Zwitserland en IJsland), productieve bodems waarop landbouw en bosbouw met verschillend beheer wordt uitgevoerd (in Oostenrijk, IJsland en Tsjechië) en landbouwgebieden onder druk van degradatie (in Griekenland).

Ik heb in hoofdstuk 2 onderzocht hoe voedselwebben zich ontwikkelen tijdens het proces van bodemvorming, aan de voet van twee gletsjers in IJsland en Zwitserland. In beide chronosequenties ontwikkelde het bodemecosysteem zich zoals verwacht, d.w.z. er waren toenames in

koolstof en stikstof in de bodem, in bedekking en soortenrijkdom van de vegetatie, in biomassa van bodemorganismen (zowel totaal als per trofische groep), in het aantal trofische groepen in het voedselweb en in lengte van de voedselketen. De resultaten lieten zien dat alle onderdelen van terrestrische ecosystemen zich gelijktijdig en stabiel ontwikkelen.

Intensieve landbouw kan een belangrijke factor zijn in het verlies van bodemkwaliteit. Het bestuderen van het effect van de verschillende typen agrarisch beheer is noodzakelijk om te begrijpen hoe het vermogen van de bodem om ecosysteemdiensten te leveren beschermd en verbeterd kan worden. In hoofdstuk 3 heb ik onderzocht wat het effect van type agrarisch beheer (biologisch of conventioneel) op bodemkwaliteit is, in boerderijen op IJsland (graslanden) en in Oostenrijk (akkers). De resultaten lieten zien dat organisch beheer geen systematisch effect heeft op de hoeveelheid of samenstelling van organisch materiaal in de bodem, of op gehalten van koolstof en stikstof in de bodem. Ook de topologische structuur van het voedselweb, de aanwezigheid van trofische groepen, verschilde niet tussen biologische en conventionele boerderijen, wat aantoonde dat de gevoeligheid van de trofische structuur voor veschillen in landgebruik of klimaat laag is. Echter, de biomassa en biodiversiteit van bodemorganismen waren wel hoger in biologisch beheerde boerderijen. Dit verschil vond ik in beide landen, en bij de verschillende boerderijen, gewassen en bodemtypen. Deze resultaten bevestigen dat biologische landbouw de biomassa en diversiteit van bodemorganismen kan verhogen. Daarbij bleek de diversiteit van microarthropoden een gevoelige en consistente indicator te zijn voor bodemkwaliteit onder verschillend agrarisch beheer.

Voortbouwend op de resultaten beschreven in hoofdstuk 3, d.w.z. de effecten van agrarisch beheer op bodembiologische eigenschappen, heb ik onderzocht of de effecten van landgebruik ook in de diepere bodemlagen terug te vinden zijn. Ik heb daarbij gekeken naar biomassa, activiteit en structuur van de microbiële gemeenschap, in drie bodemlagen (dwz. A, AC en C horizons) in bos, grasland en akker. De resultaten toonden aan dat alle bepalingen van microbiële biomassa afnemen met bodemdiepte, onafhankelijk van het type landschap. Zowel de absolute biomassa, als de structuur van de gemeenschap verschilden tussen de bovenste en de diepere bodemlagen. De sterke effecten van het type landschap op de bodemorganismen in de toplaag namen af met bodemdiepte. Dit gaf aan dat de effecten van landgebruik vooral beperkt zijn tot de toplaag in de bodem, en de pedologische omstandigheden de structuur van de microbiële gemeenschap in de diepere bodemlagen onder de wortelzone bepalen.

De volgende stap in het onderzoek was om te kijken naar bodems die zich aan het eind van de levenscyclus van de bodem bevinden, die onder druk staan van degradatie na duizenden jaren intensieve landbouw onder extreme klimatologische omstandigheden. Ik heb daarvoor in hoofdstuk 5 gekeken naar de structuur en het functioneren van bodemvoedselwebben in bodems onder verschillend agrarisch beheer (intensieve en extensieve olijfboomgaarden en pastoraal beheer) op het semi-aride Kreta. Biomassa van bodemorganismen op alle onderzochte locaties bleek laag te zijn in vergelijking met literatuurwaarden voor bodems onder minder extreme klimatologische omstandigheden. De soortenrijkdom van microarthropoden was hoger onder pastoraal beheer dan in de olijfboomgaarden, wederom bevestigend dat de taxonomische rijkdom van microarthropoden een bruikbare indicator voor ecologische bodemkwaliteit is. De resultaten in dit hoofdstuk toonden aan dat deze indicator in staat is om extreme locaties te onderscheiden van gematigde gebieden, en daarnaast gevoelig genoeg is om verschillen in agrarische beheer binnen de extreme gebieden te laten zien. Daarmee heeft deze indicator ook potentie als biologische indicator voor bodemdegradatie.

Biologische metingen zoals biomassa, activiteit en diversiteit van bodemorganismen bleken een indicatieve waarde te hebben voor bodemkwaliteit in de onderzochte gebieden (hoofdstukken 2-5). Om deze resultaten te kunnen generaliseren heb ik deze biologische parameters vergeleken in bodems van alle CZOs in de levenscyclus van de bodem in hoofdstuk 6. Deze analyse omvatte de data gepresenteerd in hoofdstukken 2-5, samen met aanvullende data van half-natuurlijke bossen in Oostenrijk en productieve naaldbossen in Tsjechië. Over de bodemvorming-productieve bodem-bodemdegradatie gradiënt was verwacht dat het bodemvoedselweb toeneemt in biomassa en structuur tijdens bodemvorming, een optimum bereikt in de intermediaire bodems, en afneemt naarmate bodems degraderen. Maar deze verwachting werd niet bevestigd door de complete analyse, omdat landschapstype veel belangrijker bleek te zijn dan de het stadium van de bodem in de levenscyclus.

In de analyse van alle CZO locaties in hoofdstuk 6 heb ik ook de mogelijke rol van organismen in het functioneren van het bodemecosysteem bekeken, in termen van structuurvorming in de bodem en het mineraliseren van koolstof en stikstof. Hoewel bodemorganismen bekend staan om het spelen van een rol in de vorming van bodemstructuur, heb ik dit niet gevonden in de onderzochte locaties. Bodemfysische en chemische parameters, zoals ijzer-(hydro)oxide, gehalte van organische koolstof en pH van de bodem, bleken een belangrijkere rol te spelen in stabiliteit van bodemaggregaten. Biomass en activiteit van microbiële organismen waren sterker gecorreleerd aan ecosysteem processen zoals

mineralisatie snelheden van koolstof en stikstof. Ook de biomassa van andere bodemfauna liet een (minder sterke) correlatie met koolstof en stikstof mineralisatie en het koolstofgehalte zien.

Concluderend heb ik gevonden dat bodemvoedselwebben zich stabiel ontwikkelen: biomass en activiteit van bodemorganismen nam toe met bodemproductiviteit. In productieve bodems bleken landschapstypen en bodembeheer de belangrijkste factoren voor de structuur en het functioneren van het bodemvoedselweb, hoewel dit effect beperkt was tot de toplaag van de bodem. Onder extreme omstandigheden worden bodemorganismen negatief beïnvloed door langdurige intensieve landbouw. Daarnaast vond ik dat de soortenrijkdom en diversiteit van microarthropoden in de bodem geschikt zijn als indicator voor ecologische bodemkwaliteit.

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About the author

Jeroen P. van Leeuwen was born on April 12, 1985 in St. Michielsgestel, The Netherlands. After completing secondary school in 2003, he started his Bsc-study Forest and Nature Conservation at Wageningen University. Specialising in ecology, he focused for his Bsc-thesis on the role of tannin in acorn predation. In 2006, he continued with a Msc Forest and Nature Conservation with a major in ecology at Wageningen University. For his first Msc-thesis, he conducted field experiments in NW Peru to investigate the effect of water availability, shade and herbivory on *Prosopis* seedling growth and survival, and simultanuously identified the diet of the lizard *Dicrodon guttulatum* as important native herbivore. In his second thesis, in cooperation with the Alterra research institute in Wageningen, he investigated the genetic diversity of adder-populations (*Vipera berus*) in the Netherlands. In November 2010 he started his PhD research, as part of the EU project SoilTrEC, culminating in this thesis. During his PhD research, also his scientific interest in herpetology remained active. Currently he is employed as postdoctoral researcher in the EU-H2020 project Landmark.

List of publications

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Van Leeuwen, J.P., G.J. Lair, G. Gísladóttir, S.M. Bernasconi , J. Bloem, L. Hemerik, P.C. de Ruiter. Development of the soil food web in glacial chronosequences in Switzerland and Iceland. *(submitted)*

Van Leeuwen, J.P., G.J. Lair, J. Bloem, I. Djukic, L. Hemerik, P.C. de Ruiter. Land use differences affect soil microbial communities at different soil depths in the Danube floodplain. *(submitted)*

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PE&RC Training and Education Statement

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

Review of literature (4.5 ECTS)

- The soil life cycle

Writing of project proposal (4.5 ECTS)

- Soil food webs and ecosystem services during soil transformations (2011)

Post-graduate courses (7.6 ECTS)

- Dynamics of organic matter in soil; Copenhagen university (2011)
- Introduction to R for statistical analysis; PE&RC (2011)
- Workshop navigating in socio-environmental; PE&RC (2011)
- Life history theory; RSEE (2013)
- Basics of parameter estimation tutorial; Systems Biology (2013)
- Multivariate analysis; PE&RC, SENSE (2014)

Deficiency, refresh, brush-up courses (6 ECTS)

- Models of biological processes and environmental quality (2011)
- Ecological models and data in R (2011)

Competence strengthening / skills courses (3.3 ECTS)

- Competence assessment; WGS (2011)
- Project and time management; WGS (2012)
- Voice and Presentation skills Training (V&PT); WGS (2013)
- Techniques for writing and presenting a scientific paper; WGS (2013)

PE&RC annual meetings, seminars and weekends (4.7 ECTS)

- PE&RC Weekend (2011, 2014)
- PE&RC Day (2012)
- NERN Symposium biodiversity (2014)
- Netherlands Annual Ecology Meeting (NAEM); * (2014)
- Netherlands Annual Ecology Meeting (NAEM); ** (2011, 2013, 2015)
- Netherlands Annual Ecology Meeting (NAEM) (2012)

Discussion groups / local seminars / scientific meetings (6.7 ECTS)

- R Discussion group (2011)
- Ecological theory and application (2011-2013)
- Food web ecology (2011-2014)
- Wageningen ecology and evolutionary seminary (2011-2014)
- Systems biology group (2012-2014)
- Wageningen centre for soil science (2012-2014)
- Modelling and statistics network (2013)

International symposia, workshops and conferences (6.6 ECTS)

- Geochemistry of the earth's surface symposium; *,**; Boulder, USA (2011)
- Food webs; **; Giessen, Germany (2013)
- First global soil biodiversity conference; **; Dijon, France (2014)

Lecturing / supervision of practical's / tutorials (18.2 ECTS)

- Wiskunde (2011-2015)
- Food web ecology (2012-2013)
- Webs of terrestrial diversity (2014-2015)

*Oral, **Poster

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