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SoilEffects – start characterization of the experimental soil

soil effects

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Front page: The experimental field on May 11, 2011 during manure application on the arable system plots. Higher up in the slope, the grass system plots. Photo by Sissel Hansen.

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SoilEffects – start characterization of the experimental soil

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Sammendrag:

Denne rapporten er en sammenstilling av startundersøkelser av jordfysikk, jordkjemi og jordbiologi fra et forsøksfelt der vi skal sammenlikne vanlig blautgjødsel fra melkekyr med råtnerest etter biogassproduksjon. Råtneresten består av blautgjødsel som er utråtnet i biogassanlegg slik at en del organisk materiale er omdannet til biogass. Forsøket er hovedaktiviteten i prosjektet «SoilEffects» (2010-2014). Forsøksfeltet ligger på Tingvoll gard på Nordmøre. Siden gjødsel fra biogassanlegg inneholder mindre lett tilgjengelig karbon enn ubehandlet blautgjødsel, kan det tenkes at det vil påvirke humusinnholdet eller andre forhold i jorda. Hensikten med feltforsøket er å undersøke om vi kan finne noen slike tendenser. Tre vekstsesonger er imidlertid for kort tid til at man kan si noe sikkert om slike forhold, så en viktig hensikt med prosjektet er å etablere et langvarig feltforsøk. Formålet med denne rapporten er å presentere forsøksplanen, dokumentere resultatene av startkarakteriseringen av forsøksfeltet, og fortelle litt om prosjektets utvikling. Dette vil understøtte senere vitenskapelig publisering av resultatene. Med unntak av noen få resultat for vannløselig karbon og mikrobiologi i 2011, er alle resultater i denne rapporten basert på undersøkelser fra høsten 2010 eller våren 2011, i og rundt forsøksfeltet FØR det ble tilført noe gjødsel. Rapporten vil være av interesse for dem som skal gjennomføre tilsvarende forsøk andre steder. Effekter av de ulike gjødselbehandlingene ble undersøkt for ulike jordegenskaper i 2011, 2012 og 2013, og vil bli dokumentert i egne publikasjoner.

Feltforsøket er inndelt i to plantesystem, varig eng (20 forsøksruter), og åkervekster (20 forsøksruter). Hver forsøksrute måler 8 m x 3 m. Enga ble etablert i 2009 med korn som dekkvekst. Åkerdelen ble etablert våren 2011 ved å pløye av en del av enga fra 2009. Åkerdelen vil ha årlig pløying eller annen jordarbeiding, fjerning av alt plantemateriale over vanlig stubbehøyde, og ingen dyrking av belgvekster. Hensikten med disse tiltakene er å legge til rette for nedbrytning av organisk materiale i jorda, slik at effekt av gjødselbehandling kan komme tydeligere fram. Innen hver del av forsøket (eng og åkervekster) er det fem forsøksledd. Forsøksleddene består av to gjødslingsnivå, høy og lav gjødsling med råtnerest eller vanlig bløtgjødsel, og en kontrollbehandling uten gjødsling. Det er fire gjentak av hver behandling, tilfeldig fordelt innen fire blokker i hver del. Jordvariasjonen på feltet er betydelig, men startkarakteriseringen viste at for de fleste egenskapene vi undersøkte, var det ikke statistisk sikre forskjeller mellom forsøksleddene.

Det er høyere moldinnhold i engdelen enn i åkervekst-delen av forsøket. I øvre lag av jorda (0-20 cm) var innholdet av organisk materiale målt som glødetap i gjennomsnitt 11,3 % i engdelen og 6,6 % i åkerdelen av forsøket. Kalkulert ved hjelp av målinger av innholdet av total karbon (C) var de tilsvarende verdiene 11,03 % og 5,97 %. I norsk jord regnes jord med moldinnhold 3-6 % som moldrik, og moldinnhold 6-12 % for svært moldrik. Innholdet av total karbon var 6,41 og 3,47 % i gjennomsnitt, og innholdet av total nitrogen var 0,39 og 0,21 %. Det var godt samsvar mellom målingene av moldinnhold ved hjelp av glødetap, total C og total N.

Innholdet av vannløselig organisk karbon var også høyere i engdelen av forsøket. I gjennomsnitt for de 20 forsøksrutene i engdelen inneholdt øvre jordlag 0,32 mg organisk C per g lufttørket jord når den ble ekstrahert med kaldt vann (cold water extractable C, CWEC). Verdien økte til 1,7 mg når man ekstraherte med varmt vann (hot water extractable C, HWEC). Tilsvarende verdier i åkerdelen var 0,23 og 1,1 mg.

Moldinnholdet varierte mer mellom forsøksrutene i engdelen enn i åkerdelen av forsøket, men i øvre jordlag var det ikke statistisk sikre forskjeller mellom forsøksleddene i noen del av forsøket. Likevel er startvariasjonene viktige å ta hensyn til når man skal diskutere eventuelle endringer i moldinnhold over tid.

Mekanisk sammensetning av jorda, målt som andel sand, silt og leire viste at jordtypen er siltig mellomsand på engdelen av forsøksfeltet både i øvre jordlag og i laget under (20-40 cm). I åkerdelen var jorda noe tyngre, i gjennomsnitt med 16 % mindre sand, 11 % mer silt og 4 % mer leire enn i engdelen. I en blokk fikk øvre jordlag betegnelsen sandig lettleire, mens resten var siltig mellomsand. I tre av blokkene var det lettleire i 20-40 cm dyp, i den fjerde var det siltig mellomsand. Tyngre jord i åkerdelen, som ligger lavere i terrenget, kan forklares ved at avsetningene er blitt vasket ut og omlagret under landhevingen. Innholdet av grus, det vil si partikler > 2 mm i diameter, var gjennomgående lavt, og utgjorde mindre enn 10 % av totalvekta av jordprøvene.

Engdelen hadde bedre vannlagringsevne, luftpermeabilitet og totalt poreinnhold enn åkerdelen. Både vannlagringsevnen og luftvekslingsevnen ble imidlertid vurdert som tilstrekkelig gode i begge systemene. Det var en nær sammenheng mellom jordas evne til å holde på fuktighet, og innholdet av organisk materiale. Aggregatsammensetningen ble målt i åkerdelen. Jorda hadde for det meste en enkeltkornstruktur. Dette er ikke uventet for sandjord. Det ble funnet 16 % aggregater med diameter 2 - 6 mm, og 16 % > 6 mm. Disse aggregatene var stabile, i det 85 % beholdt formen etter simulert nedbør.

Det var små forskjeller på pH og næringsinnhold i øvre jordlag i de to delene av forsøket da vi startet opp. Gjennomsnittsverdiene for eng/åkerdelen var pH 5,82/5,87; P-AL 2,87/2,31; K-AL 5,25/5,24; Mg-AL 4,34/3,53; Ca-AL 107,6/80,7 og K-HNO_3 122,4/175. Næringsinnholdet er målt som mg næringsstoff per 100 g tørr jord.

Akkumulert jordrespirasjon (CO_2) og mikrobiell sammensetning (microbial community structure) ble målt før og etter gjødsling. I åkerdelen ble det også gjennomført pløying og harving i dette tidsrommet. Respirasjonen var ulik i engdelen og åkerdelen av forsøksfeltet. Respirasjonen ble

påvirket av både gjødsling og plantesystem (inkludert jordarbeiding). I 2011 var det ingen sikre effekter av gjødsling (målt etter 5 dager) på mikrobiell sammensetning, men dette kan endre seg over tid når effekten av flere gjødslinger akkumuleres.

Vi fant til sammen fem arter av meitemark i jorda på forsøksfeltet. *Apporrectodea caliginosa* (gråmeitemark) var mest vanlig, men *Lumbricus terrestris* (stor-meitemark) var også vanlig forekommende. *Octolasion cyaneum* (blåmeitemark) forekom stort sett i åkerdelen av forsøket. I gjennomsnitt fant vi 133 meitemark m^{-2} i engdelen og 117 i det som skulle bli åkerdelen av forsøket. Gjennomsnittlig biomasse var noe høyere i åkerdelen (63,5 g m^{-2}) enn i engdelen (42,1 g m^{-2}).

Spretthaler ble samlet i engdelen av forsøket i forsøksledd uten gjødsel og med sterk gjødsling, til sammen 12 forsøksruter. Det ble ikke samlet spretthaler i åkerdelen av forsøket. Vi fant 17 arter av spretthaler (collembolans), i gjennomsnitt var det 7950 individer m^{-2}. Det var stor variasjon i både tetthet og artssammensetning, og forskjellen var større mellom behandlinger enn mellom blokker. 11 arter ble funnet i alle forsøksleddene. De vanligste artene på forsøksfeltet var *Mesaphorura macrochaeta, Protaphorura armata og Isotomurus graminis*. De to første er hvite og lever i jorda, mens den sistnevnte er grønnlig og lever i strølaget.

Summary:

This report describes the establishment, experimental plan and initial soil characteristics of the field experiment linked to the project "Effects of anaerobically digested manure on soil fertility - establishment of a long-term study under Norwegian conditions" (SoilEffects, 2010-14). The aim of the SoilEffects project is to identify potential risks and benefits for soil fertility when animal manure is anaerobically digested for biogas production.

The field experiment was established on Tingvoll research farm in 2011. A biogas plant was built at this farm in 2010, to digest the manure from a herd of about 25 organically managed dairy cows. This report describes the initial characterization of the soil biology, chemistry and physics, along with the background of the project, the selection process of the research field and the project design. Effects of the manure treatment and application will be studied during 2011-14. The aim of this report is to function as a reference for later publications, and to inform other scientists establishing medium long-term field trials. Except from a few results on water-soluble C and soil microbiology from 2011, all results presented here are based on studies conducted in autumn 2010 or spring 2011, before any manure was applied. Effects of the experimental treatments were studied for different soil characteristics in 2011, 2012 and 2013 and will be presented in separate publications.

The field experiment has two cropping systems; grass (perennial grass-clover ley) with 20 experimental plots, and arable with 20 plots. Each experimental plot measures 3 m x 8 m. The ley was established in 2009 with cereals as a cover crop. The arable system was established in 2011, by ploughing a part of this ley. In the arable system, the soil is ploughed annually in spring, no legumes are grown, and aboveground plant material is removed at harvest. This practice is intended to stress the maintenance of soil organic matter in the arable system, to possibly reveal clearer effects of the experimental treatments. Within each cropping system, five experimental treatments are compared. They comprise two fertilization levels for each type of manure, plus a control treatment with no manure application. Each treatment has four replicates, randomly distributed within four blocks in each system.

There is a significant soil variation on the experimental field. However, for most of the studied characteristics, no statistically valid differences were found between average values across blocks within each cropping system.

The content of soil organic matter (SOM) is higher in the grass system than the arable system.

In the upper soil layer (0-20 cm) the average SOM content measured by ignition loss was 11.3 % in the grass and 6.6 % in the arable system. Analyzed by total-C measurements, the corresponding SOM values were 11.03 % and 5.97 %. In Norwegian soil, SOM values between 3 and 6 % are regarded as high humus contents ("moldrik"), whereas values between 6 and 12 % are regarded as very high. The average values for total C (0-20 cm) were 6.41 in the grass and 3.47 % in the arable system, and for total-N 0.39 and 0.21 %.

On average for all treatments in the grass system (n= 20), the upper soil layer contained 0.32 mg organic C per g soil (air dried) by extraction in cold water (CWEC), increasing to 1.7 mg by hot water extraction (HWEC). In the arable system, the corresponding values were 0.23 and 1.1 mg.

The SOM content of the grass system was higher and more variable than that of the arable system, and differences between blocks were greater and more statistically significant in the grass than in the arable system. Differences in the initial SOM between the means of plots that have been assigned to different subsequent treatments of manure applications were on the whole much smaller than those between blocks within the same crop system. Nevertheless, significant differences were found in some cases, and thus the initial SOM status of the soil should be taken into account when interpreting differences that may arise after the treatments have been carried out for a number of years.

The soil texture, loamy sand ('siltig mellomsand') was similar in all replicate blocks and both depths in the grass system. It was slightly heavier and somewhat more variable in the arable system, with on average 16 % less sand, 11 % more silt and 4 % more clay. Somewhat heavier soil in the deeper parts of the terrain may be explained by washing out the soil layer during post-glacial land elevation. The gravel contents were fairly low (< 10 %) in all cases.

Soil moisture retention and aeration properties of the upper soil layer were measured on each plot. Total porosity, aeration properties and moisture retention at low tension were all clearly greater in the grass system than in the arable system. Satisfactorily high levels of aeration and plant-available water-holding capacity were found in both systems. Close relationships were seen between the moisture retention and the soil organic matter content. This accounts for many of the differences in such properties that were found between blocks.

Soil aggregate size distribution was measured in the seedbed of the arable system plots. This confirmed that the predominant structure of the soil may be described as 'single-grain', with only 16 % aggregates of 2-6 mm and 16 % aggregates > 6 mm. There was little variation between blocks in the aggregate size distribution. The stability of soil aggregates (2-6 and 6-10 mm) to simulated rainfall was high (>85 %) in all cases, with little variation between blocks or treatment means.

The soil nutrient content was comparable in the two cropping systems. The nutrient concentrations in the upper soil layer (0-20 cm) were P-AL 2.87/2.31; K-AL 5.25/5.24; Mg-AL 4.34/3.53; Ca-AL 107.6/80.7; K-HNO_3 122.4/175 mg of nutrient 100 g^{-1} dry soil in the grass/arable system. The pH value (H_2O) was 5.82/5.87.

The accumulated soil respiration and the microbial community structure differed between the grass and the arable system. Soil respiration seemed to be influenced both by manure application and cropping system. In 2011, no significant change in the soil microbial community structure was found five days after manure application. This, however, may change with repeated manure applications over several years.

Five earthworm species were identified in the field experiment. *Apporrectodea caliginosa* was the most common, but also *Lumbricus terrestris* was abundant. *Octolasion cyaneum* was found mainly in the arable system. The average density was 133 earthworms m^{-2} in the grass system and 117 in the arable system. The average biomass was somewhat higher in the arable system (63.5 g m^{-2}) than in the grass system (42.1 g m^{-2}).

Collembolans were sampled from the grass system, in treatments with no or high manure application (but before manure application), from totally 12 plots. 17 species of collembolans were found, with an average density of and 7950 individuals m^{-2}. The variation in species composition and density was high, and larger between treatments than between blocks. 11 species were found in all treatments. The most numerous collembolan species were the soil dwelling, white *Mesaphorura macrochaeta* and *Protaphorura armata*, and the litter dwelling greenish *Isotomurus graminis*.

Land/Country:	Norway
County: Møre and Romsdal	
Municipality:	Tingvoll
Location:	Tingvoll farm

Approved

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1. The project: SoilEffects

1.1 General background

Anaerobic digestion of organic wastes to produce methane (CH_4) for energy purpose is a well-established technology. In Europe, some countries supporting renewable energy, e.g. Germany, have established many farm-level biogas plants in recent years. Some are found on organic farms or institutions (e.g. vocational schools), but many organic farmers are still reluctant to adopt this technology. Soil fertility is the primary goal of any organic farmer, aiming for self-sufficient production systems with minimal purchases of nutrients and organic matter. Soil organic matter, humus, is the key stone in formation of soil structure and crop nutrition (Elmholt et al. 2008). Humus is essential for soil aggregate formation and stability, which affect water and nutrient behavior in the topsoil. Soil humus content is influenced by the on-farm recycling of organic matter, and a principal argument against digesting animal manure for biogas production is that this may reduce the quality and quantity of humus in the soil. During digestion, organic matter will be transformed to methane, CH_4 and carbon dioxide, CO_2 and thereby be lost from the farm cycle of carbon (C), instead of being available for natural degradation processes in the soil.

Animal manures increase and maintain soil fertility, partly due to their positive effect on the soil humus content. Based on more than 30 years of research, the DOK-experiment in Switzerland (Mäder et al. 2002) has demonstrated that organic farming systems, when compared with systems using only mineral fertilizers, contribute to establish soil fertility. The organically managed soils contained more humus, had a more desirable structure with a higher capacity of water infiltration, and a more active microbial community to sustain the processes of plant nutrient turnover. In Norway, a higher level of humus was maintained in soil amended with animal manure since 1922, than if mineral fertilizer was applied (Riley 2007). Positive effects of animal manure, combined with ley in the crop rotation, have also been found on earthworm activity and soil structure (Riley et al. 2008). Animal manure provides food and increase the biomass of earthworms (Curry 1976; Andersen 1979; Hansen and Engelstad 1999), but may be toxic in the short term (Curry 1976). The effects on earthworms of anaerobically digested slurry are less studied, especially for slurry based on plant material. Ernst et al. (2008) tested the effects of conventionally cattle slurry and a digested mixture of cattle slurry, grass silage and maize (ratio 10:1:16) digested for 200 days, on earthworms in microcosms. The biomass of the litter eating species (*Lumbricus terrestris* and *Apporectodea longa*) increased in both slurry treatments, whereas the biomass of *A. caliginosa,* which to a larger extent is soil-eating, decreased. The biomass decline of *A. caliginosa* was significantly stronger with application of digested slurry. Geophagous (soil eating) species such as *A.caliginosa* and *A.rosea* are the all over dominating species in arable soils in Norway (Pommeresche and Løes 2009), and hence the results of Ernst et al. (2008) are of special interest for Norwegian conditions. Can we expect a negative impact on *A. caliginosa* and other earthworms when the slurry at Tingvoll farm is anaerobically digested?

Agronomic studies of application of anaerobically digested manure (digestate) have mostly focused on crop yield (e.g. Möller et al., 2008). Digestate impact on soil nutrient content and enzyme activity has been studied, e.g. by Vago et al. (2009), although with other organic wastes added to the manure during digestion. Replacing mineral fertilizers with biogas residues (digestate) in a crop production system with low access to animal manure will probably increase the soil organic matter content. In an animal husbandry system, the

digestate is recycled to the soil as fertilizer and it is an open question whether the anaerobic digestion impacts the soil organic matter content and quality, or possibly other soil characteristics.

1.2 SoilEffects, aims and structure

In 2010, a project to study the questions described above was initiated at Tingvoll farm by Bioforsk Organic Food and Farming in cooperation with Bioforsk Arable Crops and the National Environmental Research Institute (NERI) at Aarhus University, Denmark. Our aim is to evaluate, in an organically managed dairy cow system, whether the soil fertility can be maintained with anaerobically digested manure as good as with untreated manure. The complete title of the project is "Effects of anaerobically digested manure on soil fertility - establishment of a long-term study under Norwegian conditions", and the short name (acronym) is SoilEffects. The project period is from October 1, 2010 to September 30, 2014.

The main aim of SoilEffects is to establish a field experiment to compare long-term effects of anaerobically digested versus non-digested manure (slurry) on crucial soil physical, chemical and biological characteristics, and report the results achieved in the early transition period. Secondary aims are to:
-Localize appropriate sites for a long-term field experiment within Tingvoll research farm, and conduct the initial site characterization
-Observe effects of the early transition period (3 years) on soil fauna (earthworms and other key fauna organisms)
-Observe effects of the early transition period on soil physical, chemical and microbiological conditions (soil density; soil pH, nutrients, organic matter content and quality; accumulated respiration, microbial community diversity)
-Measure the effect of digested manure on the local Tingvoll earthworm population by pot experiments under controlled environmental conditions
-Characterize the activity of microorganisms and important members of soil fauna (springtails)

The available funding is 3.2 mill NOK, granted from the Research Council of Norway and the Agricultural Agreement Fund. The project team (core team), which is composed of the four authors of this report, is supported by a relevance team with representatives from the Norwegian Farmers' Union (Anne Katrine Jensen), The Norwegian Farmers and Smallholders' Union (Øystein Ormbostad), the Norwegian Agricultural Extension Service (Maud Grøtta) and Norwegian Centre for Ecological Agriculture (Ketil Valde/Martha Ebbesvik). It is also supported by a research team with representatives from FiBL Switzerland (Dr. Paul Mäder), the Biodynamic Research Association in Sweden (Dr. Artur Granstedt), and Bioforsk Organic Food and Farming (Dr. Sissel Hansen).

1.3 Scientific background and hypotheses

Anaerobic digestion changes the chemical composition of the slurry. Digested slurry has an increased pH value, and a higher share of the total N content as ammonium (NH_4^+). The viscosity is reduced, meaning that it flows and infiltrates the soil more readily (Möller and Müller 2012). Further, digested slurry has a reduced content of organic matter (OM), total nitrogen (N) and carbon (C) and a reduced biological oxygen (O_2) demand. The C: N ratio is reduced as compared to non-digested slurry. Hence, the amount of C applied to the soil

may be significantly less when manure is digested before application. On the other hand, organic matter in non-digested slurry is rapidly mineralized in the soil (Johansen et al., 2013). Thus, non-digested slurry, with more rapidly decomposing organic matter, may not contribute any more to the formation of stable humus than the digested slurry, in which the applied amount of C will be lower, but probably more stable. We may also hypothesize that the negative effects of less available organic matter for soil life may be balanced by a better plant availability of applied N, beneficial for plant growth. This in turn may give more plant debris production, contributing to increase the humus in the soil. At least in ruminant production systems, where much of the soil will be used for grass-clover ley and not annually ploughed, the effects on soil fertility may be minor. These questions have not been sufficiently studied under Nordic climatic conditions, and need also to be evaluated under long-term field conditions.

The application of less easily degradable organic C may impact the earthworm population negatively, because the most common species in Norway, the field worm (*Aporrectodea caliginosa Savigny*) (Pommeresche & Løes, 2009) is endogeic (soil eating) and probably unable to compensate reduced input of organic C by increased availability of plant material. Applying digested slurry, endogeic earthworms may lose the competition with soil microorganisms for available C as shown by Ernst et al. (2008). We also expect earthworms to be negatively affected by slurry digestion, due to sensitivity to ammonium (Edwards, 1988), which may be found in relatively high concentrations in the digestate. However, as NH_4^+ is usually rapidly transformed to nitrate in cultivated soil, this risk may be small in practice. Possible reductions in earthworm activity may reduce the soil content of OM and impact negatively on soil physical characteristics, and hence reduce soil quality in general.

In the project proposal, nine hypotheses were formulated, but no.4 is not relevant (see chapter 2.3).

1) The digested manure will enhance plant N uptake and hence increase crop yield levels as compared to non-digested manure, because N will be more readily available in the digested manure.
2) Increased yield levels in treatments with digested manure will result in more root biomass and other plant residues that will maintain the humus content and quality of the soil. Hence, we do not expect to reveal significant negative effects of anaerobic digestion on the ability of the manure to support soil quality and fertility.
3) The nutrient content of the soil will decrease in treatments with digested manure because higher crop yields will remove more plant nutrients.
(4) In treatments where the topsoil is removed, soil quality and fertility will increase more rapidly in the treatments with non-digested manure as compared to digested manure and mineral fertilizer.)
5) We expect the earthworm fauna to be negatively affected by the anaerobic digestion of the manure, because these animals are sensitive to ammonium which may be found in relatively high concentrations in anaerobically digested manure. However, as ammonium is usually rapidly transformed to nitrate in cultivated soil, we propose that this risk will be small in practice.
6) We propose that the application of diminished amounts of easily degradable organic C will impact the earthworm population negatively, because the most common earthworm species in Norwegian cultivated soil, the field worm (*A. caliginosa*) is endogeic and hence may not be able to compensate the reduced application of organic C by an increased availability of plant material. By application of digested manure, endogeic earthworms may lose the competition with soil microorganisms for available C.
7) If hyp. 6 is confirmed, we propose that reduced earthworm activity will impact negatively on the soil organic matter content and soil physical characteristics that indicate a satisfactory soil quality.
8) We expect that carbon mineralization from soils amended with digested manure will be less as compared to when raw manure is applied. This will be the case both on a short- and long-term timeline.
9) Differences in the soil microbial community will be induced by manure application treatments and verifiable by using phospholipid fatty acids (PLFA) profiling.

2. Field selection and changes in project design

2.1 Background: Tingvoll farm, an organically managed research site

The organic experimental farm at Tingvoll (62°54'N, 8°11'E) belongs to the foundation Norwegian Centre for Ecological Agriculture (NORSØK), which also hosts a division of the Norwegian Institute for Agricultural and Environmental Research, Bioforsk Organic Food and Farming with about 40 employees. Organic management of the farm, with dairy cows, was established in 1988. During 2010, a new house was built for the herd of about 25 dairy cows. A small biogas plant was established alongside, to digest the manure from the herd. Equipment has been installed to compare digested and non-digested slurry.

Figure 2.1. The new building for the dairy herd at Tingvoll Farm (to the left), with manure storage in an open concrete tank (right). To the right of this tank, the biogas digesters may be seen. Later, a house was built over the digesters. Photo by Anita Land, November 2010.

The fields on Tingvoll farm are located along a fjord, well below the upper marine limit which is about 120 m a.s.l. at this site (Follestad, 1989). Hence, especially the deeper soil layers contain marine deposits with high silt and clay contents. During land elevation, the surface layer was washed out and re-distributed and hence are comprised of coarser soil. The bedrock is precambric gneiss.

2.2 Selection of a field for the SoilEffects experiment

To avoid masking of manure treatment effects due to fertile soil conditions, the experimental site was placed on a field with relatively low productivity, "Sagmyra" (translation: the moorland ("myr") next to the sawmill ("sag")). The field was cultivated from a poorly drained area with deciduous forest about 1970. It still requires large applications of manure to increase the level of plant nutrients, especially phosphorus (P). Field studies by the Norwegian institute of Land Inventory in 1992 showed that the soil is an imperfectly drained marine deposit. The soil type on Sagmyra was named "Saltkjelen silty medium-coarse sand". The name "Saltkjelen" refers to a nearby site where a soil profile was dug, and samples were analyzed for soil organic matter and texture. The total C-content in the plough layer (0-20 cm) at the Saltkjelen site was 5.7 %, and the soil texture was 67 % sand, 25 % silt and 8 % clay. The layers below the plough layer were classified as loam (Fig. 4.1) in the B-horizon, and silt loam in the C-horizon. The soil was

defined as Brunisolic according to the Canadian soil classification system, which was used as an international reference in Norway at that time.

The decision to use Sagmyra for the field experiment was taken in the first project meeting, which was arranged on September 9-10, 2010. Hugh Riley, Anders Johansen, Reidun Pommeresche, Anne-Kristin Løes, Sissel Hansen (Bioforsk Organic Food and Farming) and Artur Gransted (the Biodynamic Research Institute, Sweden) participated in this meeting; Paul Mäder (FiBL) was absent.

2.3 Project design and changes

Initially, the project was planned to study effects of manure treatment on two sites, with fertile and less productive, recently cultivated soil, to better reveal the effect of the manure as a soil conditioner. To further stress the effect of manure application on soil fertility, we planned to remove the fertile topsoil on half of the plots. It was planned to conduct the study only on grassland, due to restricted funding.

During the review of the project proposal, several useful comments were received; e.g. that the legumes in the grassland would interfere with the effects of manure application. However, none of the referees complimented the idea of removing the topsoil. Due to the soil formation process (see chapter 2.1), the soils at Tingvoll are already quite variable. Removing the topsoil, which is more homogenous than the subsoil due to soil tillage and fertilization, would have left us with a highly variable research field. It would also cause problems with surface runoff being drained into the research plots. Hence, we decided to change our approach, to use only one, less productive site and leave the topsoil intact. This made it possible to include a cropping system of arable crops, with annual ploughing and soil tillage, in addition to perennial ley. In the arable system, all above-ground plant material above normal cutting height is removed, to stress the system with respect to organic matter and possibly reveal clearer effects of the slurry treatment.

2.4 Experimental crops

The grass-clover ley used in the experiment was established by the farmer at Tingvoll farm in 2009, with oats (cv. Belinda) for green fodder as a cover crop. The green fodder was harvested on July 5, and again on September 3, 2009. In 2009, 25 tonnes ha^{-1} of solid farmyard manure were applied. In 2010, no manure was applied. The first year ley was harvested on June 22 and August 16, 2010. All harvest occurred as round bales of fodder. The production on the whole field, which is about 0.74 ha, was 7 + 6 bales in 2009 and 9 + 5 bales in 2010.

By the establishment of the project, the area to become the arable system was ploughed and cultivated in spring 2011. Oats were grown in the arable system in 2011. Later crops will be annual green fodder crops and cereals. To avoid interference with manure application effects, no legumes will be undersown in the arable system.

The ley established in 2009 surrounds the experimental field, and the farmer plans to plough this ley in 2014 or 2015.

2.5 Manure application: Equilibration method and amounts

As initially planned, we compare two levels of manure application. In the arable system, the high manure level corresponds to about 170 kg total N ha^{-1} yr^{-1}. This is equal to the amount of N that EU regulations allow organic farmers to purchase. The low level is 50 % of the high level, and comprises 85 kg total N ha^{-1} yr^{-1}. In the grass-clover ley, further called grass system, the low level mimics an organically managed system purchasing about 30 % of the energy intake for the cows as concentrates, amounting to 110 kg total N ha^{-1} yr^{-1}. The high level is two times the low amount, 220 kg total N ha^{-1} yr^{-1}. This level mimics a conventional farm where mineral fertilizers are purchased in addition to the concentrates, contributing to generally higher yield levels and larger amounts of manure available per hectare. Control treatments without manure application are also included.

Within each cropping system, four blocks were defined and the five treatments were randomly distributed within each block (Fig. 2.2). Two cropping systems, five manure treatments and four replicates of each treatment give totally 40 experimental plots.

T	1 UH	2 DH	3 UL	4 DL	5 N	6 DH	7 UL	8 UH	9 DL	10 N
T	11 DL	12 N	13 DH	14 UH	15 UL	16 UL	17 N	18 UH	19 DL	20 DH
21 DL	22 UL	23 UH	24 DH	25 N	26 DL	27 UH	28 N	29 UL	30 DH	T
31 DL	32 UH	33 N	34 UL	35 DH	36 DL	37 UH	38 UL	39 DH	40 N	T

Figure 2.2. Distribution of treatments within the grass system (green, plots 1-20) and the arable system (yellow, plots 21-40). U= Undigested slurry, D= Digested slurry. L = Low amount, H = high amount. N = no manure (control). T = Test plots for training (pink). Plot size = 3 m x 8 m, each row of plots separated by a border of grassland 2 m width (lilac).

The manure treatments are abbreviated as follows:

Control treatment with no manure	**= Control**
Undigested slurry, low level	**= UL**
Undigested slurry, high level	**= UH**
Digested slurry, low level	**= DL**
Digested slurry, high level	**= DH**

Because anaerobic digestion may impact the N content of the manure, it was planned to equilibrate the volumes of manure to be applied in each treatment by the total P content, which is not assumed to be influenced by the digestion process (Möller and Müller 2012). Chemical analyses of manure were carried out by Eurofins, Sweden. The concentrations of P and other minerals varied considerably, even for parallel samples from the same batch of manure. Hence, we had to use other characteristics to determine the exact amounts of manure to be added to each experimental plot. The content of total N in the manure is expected to be the manure characteristic with the largest impact on plant growth. Hence, we chose to keep this factor as equal as possible in treatments with digested and undigested slurry. Sticking to the idea of equilibrating the P applications might have caused differences in the amount of applied N not being related to the digestion process. The levels of total N that we sought to apply in each treatment are shown in Table 2.1.

Table 2.1. Applied amounts of N in the manure treatments, kg N ha^{-1} yr^{-1}

Treatment	Arable system	Grass system
No manure (Control)	0	0
Low (DL, UL)	85	110
High (DH, UH)	170	220

The digested slurry in 2011 was produced in an experimental batch digester at UMB, Ås. In a batch process, it is possible to determine the dry matter content and other characteristics in the input manure and the output digested manure. Still, the uncertainty of the chemical extraction and detection methods used for manure analyses may be large compared with the possible effects of the digestion. In a continuous digestion process, such as in the Tingvoll farm biogas plant where about 500 liters of slurry are fed into and let out from the digester twice per day, the conditions to measure changes in chemical composition due to anaerobic digestion are even more difficult. The variations in the dry matter content of the input manure, which is closely linked to the concentrations of nutrients, will likely be so large that it is necessary to analyze a very high number of samples to estimate true changes in chemical composition linked to anaerobic digestion.

2.6 Location of the experimental field by mapping the soil organic matter (SOM) content

In mid-October 2010, an area was marked out on the field “Sagmyra” where an experimental field with straight angles could possibly be placed, and the farmer could still easily drive around it with machinery. 84 plots sized 8 m x 3 m were marked by sticks in each corner, and defined in a system of four rows A, B, C, D, and 21 columns (Fig. 2.5). Between each row of plots, a strip of 2 m width was set aside to avoid surface runoff from one plot to another.

On October 20-21, a Geonor soil rig (Figs. 2.3, 2.4) was used to sample the soil in two depths, 0-20 cm (upper soil layer) and 20-40 cm (lower soil layer) by 5 augerings per plot along the center of each plot. These samples are further called the **autumn 2010 auger samples**. The samples (n= 168) were dried, sieved and analyzed for ignition loss at Bioforsk Arable crops, Apelsvoll (see chapter 3.1). Bulked samples were used for measuring the soil texture (chapter 4.1), and samples from the selected 40 experimental plots were analyzed for total N and C (chapter 3.2).

Figure 2.3.

Erling Berentsen sampling soil from the experimental plots through a small snow layer. October 21, 2010. Borghild Gjørsvik driving the tractor.

Photo by Olaf Østbø.

Figure 2.4.
Detail of the soil sampling equipment. Tip of the auger, diameter 4 cm. The 0-40 cm soil cylinder was divided into 0-20 and 20-40 cm subsamples.

Photo by Olaf Østbø.

Based on the SOM values of the upper soil layer from the 2010 auger samples, we chose the most even part of the field for the experiment. The most even plots were not in the same "columns" (1-21, se Fig. 2.5) in the grass system as in the arable system. To ensure that the experimental field was formed as a straight rectangle, we included two extra plots in the upper left (A7, B7) and bottom right (C17, D17) corner. The extra plots makes it possible e.g. to test manure application, seed planting etc. in a realistic way before we start the real work.

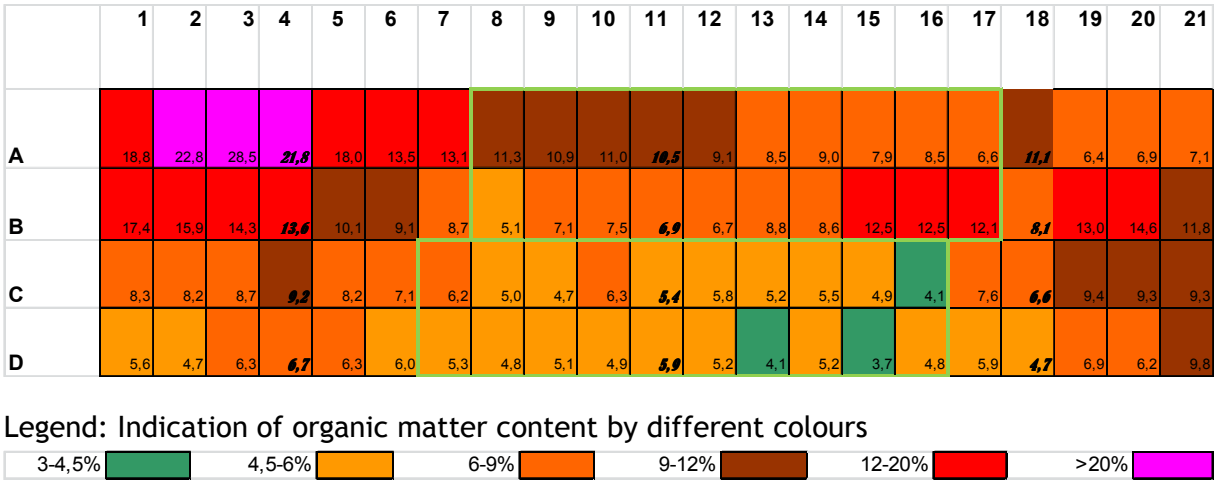

Figure 2.5. Soil organic matter content (%) in the upper soil layer of 84 possible experimental plots, calculated as ignition loss x 0.96 -0.85 (see chapter 3.3). Columns 8-17, rows A and B are used for the grass system, and columns 7-16, rows C and D for the arable system. A7, B7, C17 and D17 are extra plots for training and storage.

The selected 40 plots were divided into two parts (Fig. 2.6), one for the grass system (G) and one for the arable system (A). Each system consists of four replicate blocks, G1-4 and A1-4. The arable system was placed on the part of land with lowest content of organic matter (Fig 2.5), to increase the probability of finding effects of manure treatment on SOM characteristics. The average content of OM in the upper soil layer at the start of the experiment, measured in October 2010 was 3.9 % in the arable and 7 % in the grass system.

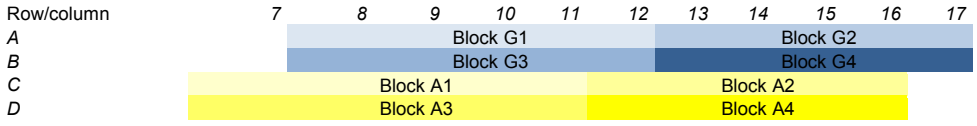

Figure 2.6. Location of system and replicate blocks within the field experiment. Grass system blocks (with perennial grass-clover ley) G1-G4 in blue colours, arable system blocks A1-A4 in yellow colours.

2.7 Soil sampling for initial soil characterization

In addition to the 2010 auger samples, a further soil sampling was conducted in on April 28, 2011, on the 40 selected experimental plots. Samples from the upper soil layer (0-20 cm) were taken by hand-augering as composite samples from five locations from each side of the plots, in total 10 augerings per plot, inner diameter 1.8 cm. Each sub-sample comprised ca. 45 g soil (fresh weight). The exact location of each sub-sample was measured by a rule. For each location, the distance to the "long wall" of the plot (length 8 m) was 65 cm. The distance from the "short wall" of the plot (length 3 m) to the utmost locations was 2 m, and the distance between each location in between the utmost locations was 1 m (2+1+1+1+1+2=8 m). These samples are further called the **spring 2011**

auger samples, and were used to analyze ignition loss (chapter 3), total C, total N (chapter 3), pH and soil nutrient contents (chapter 5), water-soluble C (chapter 3) and various soil microbiological characteristics (chapter 6). To study the effect of manure application on water-soluble C and microbiolological characteristics, 6 augerings were made by the same system as the spring 2011 auger samples. In this case, the distance to the "long wall" was 65 cm instead of 60, and the distances between the augerings were 2 m (2+2+2+2=8 m; 3 augerings on each side of the plot).

In addition, soil cylinders (7-11 cm depth) were sampled for physical studies. 3 cylinders (100 cm^3) were taken at 2 m intervals about 80 cm from the left hand border of each plot. These are further called the **spring 2011 cylinder samples**. Ignition loss was measured in these samples as well (chapter 3.1).

3. Initial soil characteristics: Soil organic matter

Soil organic matter (SOM) is a fundamental soil constituent, which affects soil fertility in many ways. It is important in relation to nutrient availability, soil structure and numerous physical, chemical and biological processes in soil. As the field experiment aims at assessing the effects of organic amendments (digested and undigested slurry), it was essential to obtain a clear picture of possible variations in the initial SOM contents of the soil. This was obtained by analyzing the total amount of SOM, indirectly by ignition loss and directly by total C and total N analyses, and additionally by analyzing the amount of water-soluble C as a measure of the C readily available for soil (micro-) organisms.

Ignition loss was measured in the 2010 auger samples (upper + lower soil layer), in the 2011 auger samples (upper soil layer, 0-20 cm) and in the 2011 cylinder samples (upper soil layer, 7-11 cm). Total C and total N were measured in the 2010 auger samples (both layers) and the 2011 auger samples (upper soil layer). Nitrogen (N) is an important constituent of SOM, and the C : N ratio in soil is a governing factor in relation to SOM turnover and nutrient release.

Ignition-loss results are presented in section 3.1, and total C and N in section 3.2, where relationships between all three parameters are also discussed.

It should be noted that all these measurements were made before any slurry application, and represent thus the initial SOM status of the soil, not that of subsequent treatments.

For all characteristics described in this chapter, mean values per replicate block in each cropping system, and per treatment in each system are shown in the tables. For simplicity and to save some space, in each table mean values for all samples (n = 20) in each cropping system and soil depth are shown to the right of the mean block values, whereas standard deviations (n = 20) are shown to the right of the treatment values. Least significant differences (LSD 5%) and the levels of significance (P-values, shown by abbreviations) are included and shown in one column in the tables. These values were calculated by Minitab software, Version 15. P-values <0.05 may be regarded as statistically significant. Those between 0.05 and 0.1 are described in this report as tendencies and those >0.1 are not significant. The level of significance is shown by *** = P < 0.001; ** = P <0.01; * = P < 0.05; + = P < 0.1; ns = P > 0.1.

In the initial executive summary, values from the 2011 auger samples are presented in those cases where more than one set of data are available.

3.1 Ignition-loss

Method
Ignition-losses of the 2010 auger samples and the 2011 cylinder samples were measured at Bioforsk Arable crops Apelsvoll as the percentage weight loss of oven-dry subsamples (~10 g) of gravel-free fine earth (< 2 mm) after ignition in steel crucibles at 550° C for 4 hours in a laboratory furnace (see Fig. 4.3). The 2011 auger samples were analyzed similarly by Eurofins Food & Agro Testing Sweden AB, Kristianstad, Sweden, with a temperature of 500

+/- 10° C for 3 hours. For their analyses of ignition loss, this laboratory reports an uncertainty level of +/- 10 %.

Mean values of ignition loss per replicate block in each system, and per treatment in each system of the three sets of samples are given in tables 3.1-3.3.

Results

Table 3.1. ***Autumn 2010 auger samples.*** *Mean values of ignition-loss (%) per replicate block and treatment within each plant system, measured at two depths in autumn 2010. Mean and Std.dev. columns show values for all plots in the respective plant system and soil depth (n=20). Treatments: Control with no manure; DL = digested slurry, low level; DH =digested slurry, high level; UL = undigested slurry, low level; UH= undigested slurry, high level of manure application. Soil depths in cm.*

System	Depth	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
Grass	0-20	11.9	9.3	7.8	12.3	1.7 ***	10.3
	20-40	4.6	4.4	5.5	5.0	1.3 ns	4.9
Arable	0-20	6.6	6.2	6.3	5.7	0.9 ns	6.2
	20-40	3.8	4.0	4.0	3.7	0.8 ns	3.9

System	Depth	Control	DL	DH	UL	UH	LSD5% &P	Std.dev.
Grass	0-20	9.1	10.4	11.0	10.1	10.9	1.9 ns	2.3
	20-40	3.9	4.8	5.3	5.0	5.4	1.5 ns	1.0
Arable	0-20	6.3	6.8	6.1	6.1	5.8	1.0 ns	0.7
	20-40	4.3	4.3	3.4	3.8	3.7	0.9 ns	0.6

Table 3.2. ***Spring 2011 cylinder samples.*** *Mean values of ignition-loss (%) per replicate block and treatment within each plant system, measured in spring 2011. Mean and Std.dev. columns show values for all plots in the respective plant system (n=20). Treatments explained in Table 3.1. Soil depths in cm.*

System	Depth	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
Grass	7-11	12.4	11.9	7.9	13.6	3.5 *	11.4
Arable	7-11	5.9	6.1	6.4	5.8	0.9 ns	6.8

System	Depth	Control	DL	DH	UL	UH	LSD5% &P	Std.dev.
Grass	7-11	10.0	11.0	12.0	11.7	12.5	3.9 ns	3.1
Arable	7-11	6.6	6.4	5.7	6	5.7	1.0 ns	0.7

Table 3.3. ***Spring 2011 auger samples.*** *Mean values of ignition-loss (%) per replicate block and treatment within each plant system, measured in the upper soil layer in spring 2011. Mean and Std.dev. columns show values for all plots in the respective plant system (n=20). Treatments explained in Table 3.1. Soil depths in cm.*

System	Depth	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
Grass	0-20	13.5	10.5	8.6	12.7	2.0 ***	11.3
Arable	0-20	7.1	6.8	6.4	6.0	0.8 +	6.6

System	Depth	Control	DL	DH	UL	UH	LSD5% &P	Std.dev.
Grass	0-20	10.8	11.2	12.1	10.7	11.9	2.3 ns	2.4
Arable	0-20	6.7	7.1	6.4	6.3	6.5	0.9 ns	0.6

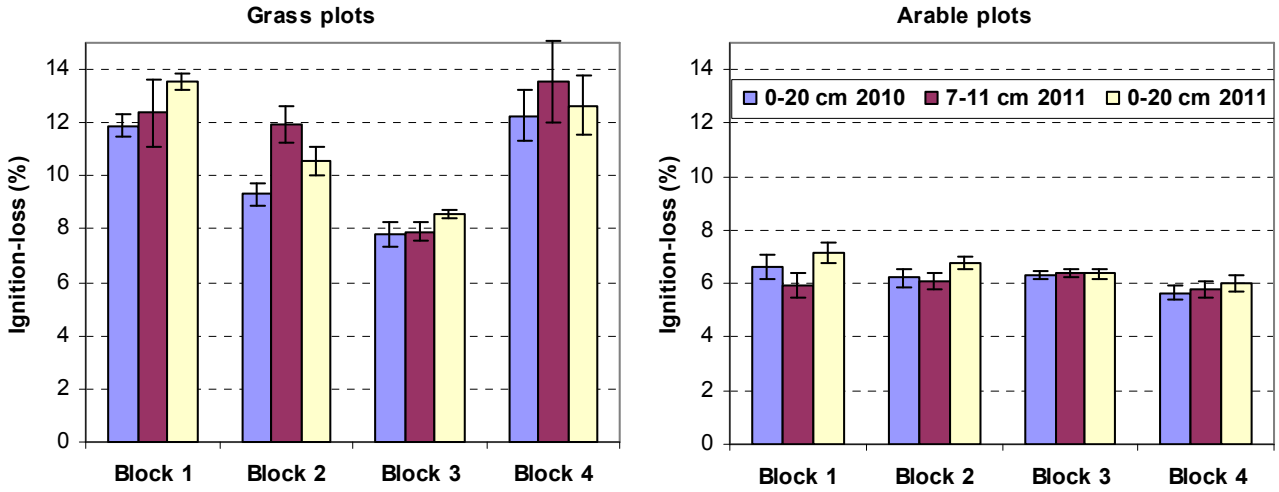

Figure 3.1. Comparison of block means (+/- se) of ignition-losses measured in the upper soil layer on three occasions (0 - 20 cm in Oct. 2010, 7-11 cm in April 2011 and 0-20 cm in April 2011).

Analyses of variance (ANOVAR) were performed for each plant system and soil depth to establish whether there were systematic differences between replicate blocks and treatments in the initial measurements of ignition-losses.

In the upper soil layer of the grass system, there were significant differences in mean ignition-loss between the replicate blocks in all three sample sets, but not in the lower soil layer. No significant difference was found between the means of the plots to be used for different treatments.

The coefficients of variation in this system were however high, around 25 %. In the arable system, on the other hand, the analyses showed little difference between the means of plots which were to receive different treatments, with P-values approaching significance in only one case. The coefficients of variation were here much lower, around 10-15 %. As expected, differences between the upper and lower soil layer (Table 3.1) were highly significant in both plant systems.

The ignition-losses measured on different blocks in the three sets of samples from the upper soil layer are compared with each other in Figure 3.1. In the grassland system, the values of the 2010 auger samples were somewhat lower than those measured for the 2011 auger samples. This is most likely a result of variation due to soil sampling, but a systematic difference between the two laboratories cannot be excluded. The differences were nevertheless in most cases within the range of the standard errors of their respective means. In the arable system, there was no consistent difference between sample sets.

Overall, the grass system soil block had markedly higher ignition-loss values than the arable system. The differences were greatest on blocks 1 and 4 of the grass system, and least on block 3. The absolute difference between the two systems was on average 4.7 % in the upper soil layer and 1 % in the lower layer. In relative terms, the ignition-loss was 75 % higher in the upper soil layer of grass plots than that in the upper soil layer of arable plots. In the lower soil layer, the corresponding difference was 25 %.

3.2 Total Carbon and Nitrogen

Methods
Analyses of total carbon (Tot-C) and nitrogen (Tot-N) were made on the 2010 auger samples and the 2011 auger samples at the Department of Plant and Environmental sciences, University of Life Sciences (UMB), Ås. Prior to the analyses, sub-samples of sieved soil (< 2 mm) were grounded in an agate mortar.

Analysis of total C was performed by the "*dry combustion*" method proposed by Allison, and described in Nelson & Sommers (1982). Ca. 200 mg of crushed soil was weighed in for analysis in a Leco CHN 1000 instrument. This instrument oxidizes carbon to CO_2 at 1050°C, and measures this gas by means of an infra-red light cell.

Analysis of total nitrogen (N) was performed by the *Dumas* method, described in Bremner & Mulvaney (1982). The principle is the same as for total carbon, but in this case nitrogen oxide compounds (NOx) are reduced to N_2 using a copper catalyst, and the concentration of this gas is measured in a thermal conductivity cell on the same instrument as above.

Results
Both results are expressed on a dry matter basis. Mean values of Tot-C and tot-N are given per block and per treatment in table 3.4 for the 2010 auger samples, and in table 3.5 for the 2011 auger samples.

The carbon data varied between the grass and arable system in much the same way as did the ignition-losses. Block effects were significant in the grass system, but much less so in the arable system. In the nitrogen data, similar differences between blocks were found in the grass system. In the arable system, block 4 had significantly lower total N values than the other blocks. There was no significant difference in total C between plots that were to receive different treatments, but there were tendencies to differences in total N in the lower soil layer of both systems. The latter total N levels were very low, and are thought unlikely to affect crop growth.

Table 3.4. Mean values per block and treatment of total C and total N (%) measured at two depths in autumn 2010. Mean and Std.dev. columns show values for all plots in the respective plant system and soil depth (n=20). Treatments explained in Table 3.1. Soil depths in cm.

	Depth	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
Total C							
Grass	0-20	6.03	4.56	3.76	6.68	1.14 ***	5.26
	20-40	1.96	2.34	2.52	2.51	0.64 ns	2.33
Arable	0-20	3.12	2.97	2.90	2.41	0.54 +	2.85
	20-40	1.48	1.78	1.56	1.88	0.68 ns	1.67

	Depth	Control	DL	DH	UL	UH	LSD5% &P	Std.dev.
Grass	0-20	4.61	5.40	5.68	5.09	5.51	1.28 ns	1.41
	20-40	1.85	2.21	2.58	2.44	2.58	0.71 ns	0.52
Arable	0-20	2.93	2.94	2.81	2.86	2.72	0.60 ns	0.42
	20-40	1.84	2.19	1.30	1.63	1.41	0.75 ns	0.54

Total N							
	Depth	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
Grass	0-20	0.39	0.26	0.24	0.40	0.08 ***	0.32
	20-40	0.11	0.10	0.14	0.12	0.04 ns	0.12
Arable	0-20	0.17	0.17	0.17	0.13	0.04 *	0.16
	20-40	0.06	0.09	0.08	0.09	0.04 ns	0.08

	Depth	Control	DL	DH	UL	UH	LSD5% &P	Std.dev.
Grass	0-20	0.28	0.32	0.35	0.32	0.34	0.09 ns	0.09
	20-40	0.08	0.10	0.14	0.13	0.14	0.04 *	0.04
Arable	0-20	0.17	0.16	0.17	0.16	0.16	0.04 ns	0.03
	20-40	0.08	0.12	0.06	0.08	0.06	0.04 +	0.03

Table 3.5. Mean values per block and treatment of total C and N (%) measured in the upper soil layer (0-20 cm) in April 2011. . Mean and Std.dev. columns show values for all plots in the respective plant system and soil depth (n=20). Treatments explained in Table 3.1. Soil depths in cm.

	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
Total C						
Grass	8.11	5.64	4.54	7.34	1.10 ***	6.41
Arable	3.84	3.70	3.28	3.06	0.46 **	3.47
Total N						
Grass	0.51	0.35	0.28	0.44	0.06 ***	0.39
Arable	0.22	0.22	0.20	0.18	0.03 *	0.21

	Control	DL	DH	UL	UH	LSD5%&P	Std.dev.
Total C							
Grass	5.93	6.19	7.05	5.97	6.91	1.23 ns	1.65
Arable	3.51	3.64	3.39	3.38	3.43	0.52 ns	0.43
Total N							
Grass	0.36	0.38	0.43	0.38	0.43	0.07 ns	0.10
Arable	0.21	0.21	0.20	0.20	0.20	0.03 ns	0.02

The nitrogen values showed very strong linear relationships with the carbon values in both sample sets. For the 2010 auger samples the relation was:

Tot.N = -0.033 + 0.067 * tot.C (r^2=0.98, n=80)

For the 2011 auger samples it was:

Tot.N = -0.01 + 0.063 * tot.C (r^2=0.99, n=40).

Measured values of both total C and total N were systematically somewhat higher in the spring 2011 auger samples than in the samples taken at the same depth in autumn 2010 (Fig. 3.6). The reason for this is unclear, but it may have been caused by differences in the methods of sampling, sample pretreatment or temperature difference at the laboratory (see chapter 3.1). The autumn 2010 auger samples were taken by means of a mechanized soil auger (see chapter 2.5), with which 3 cm wide soil cores were taken to 40 cm depth, and split into two samples. After drying, these samples were sieved in a rotating 2 mm sieve machine, ensuring thorough aggregate crushing. The spring 2011 auger samples were taken to 20 cm using a hand auger with an inner diameter of 1.8 cm, and subsamples were sieved by hand for analysis. It is conceivable that the autumn 2010 auger samples contained slightly more sand and/or less organic matter than the spring 2011 auger samples, thus accounting for the differences in the C and N levels measured.

Despite the difference in the levels of C and N in these two sample sets, the C: N ratios in the upper soil layer were in both cases similar, and mostly within the range 15-18 (Fig. 3.7), which is in agreement with previous findings for humose soils under cultivation in Norway (Riley 2000). At carbon contents < 3 %, however, the C : N ratio increased exponentially. The samples concerned were mostly from the lower soil layer (20-40 cm). Such a result was unexpected, and may possibly indicate low efficiency of N-recovery when the analytical method is applied at low N levels. Alternatively, it may have been caused by the presence of woody plant residues, or the lower soil layer may have contained some carbonate or other mineral carbon (e.g. graphite from shale). Since the soil has a peat character due to imperfect drainage, the mineral soil is of marine origin and shell sand has been applied for liming the soil, both explanations are possible.

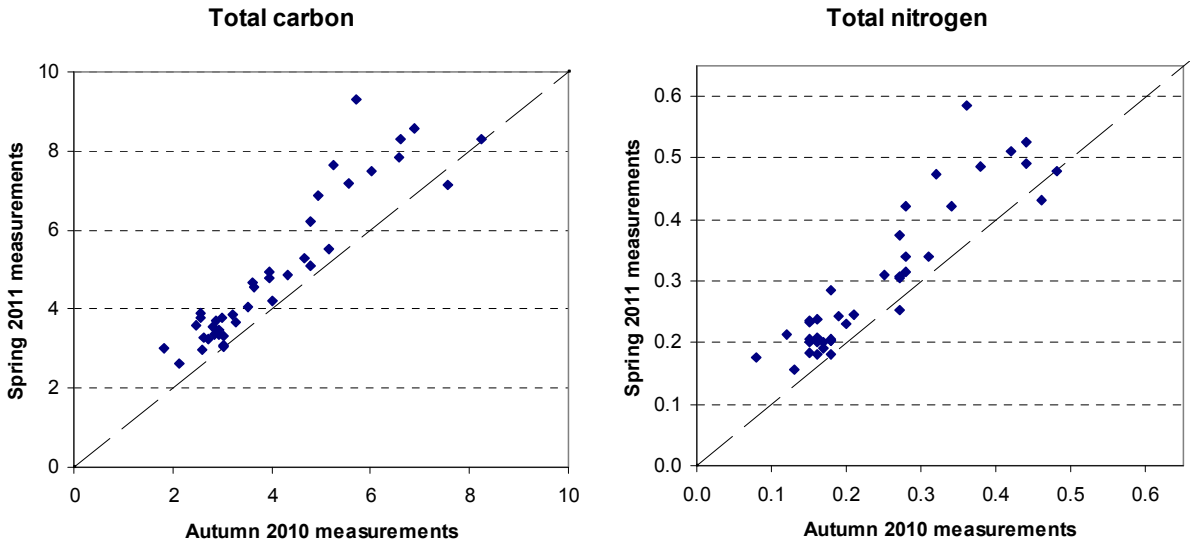

Figure 3.6. Plots of total C and N (% of dry soil) measured in the upper soil layer (0-20 cm) in two sets of samples (spring 2011 versus autumn 2010).

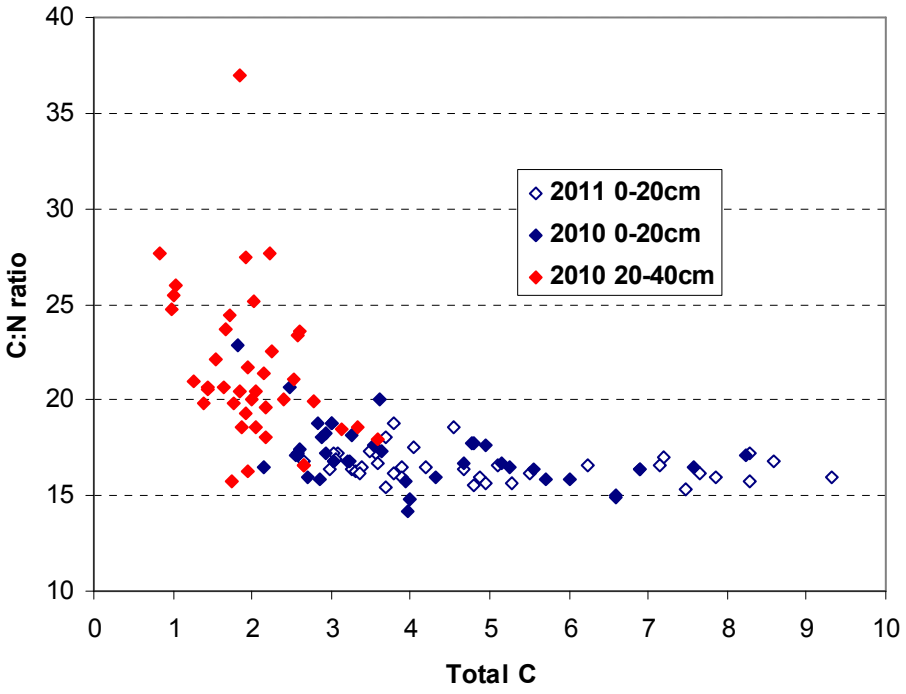

Figure 3.7. Soil total C : N ratios at plotted against total C concentration

Mean values of C : N ratios measured in each block and in plots assigned different treatments are shown in table 3.6. In the grass system, analyses of variance revealed significant differences in C : N ratios between blocks in both soil layers, but differences between means of plots assigned to different treatments were only significant in the lower soil layer. In the latter case, the control treatment had the highest C : N ratio, and the plots designated for undigested slurry had the lowest. The difference was attributable more to the level of C than to that of N.

In the arable system, there were no significant effects within the upper soil layer, neither between block means nor the means of plots assigned to different treatments. There were, however, some significant differences in C : N ratios within the lower soil layer. In this case, block 2 had the lowest ratio, as did the plots designated for a low level of digested slurry. The results for C : N ratios in the lower soil layer should, however, be interpreted with caution, due to the doubts expressed earlier about the reason for their being higher than in the upper soil layer.

In order to be able to estimate the organic matter content of the soil samples for which only ignition-loss is measured (the 2011 cylinder samples), it was of interest to compare the carbon data with that of ignition-loss for the sample sets where both parameters were measured (the autumn 2010 and spring 2011 auger samples).

Table 3.6. Mean values per block and treatment of C : N ratios measured at 1) 0-20 cm in autumn 2010, 2) at 0-20 cm in April 2011 and 3) 20-40 cm in autumn 2010. . Mean and Std.dev. columns show values for all plots in the respective plant system and soil depth (n=20). Treatments explained in Table 3.1. Soil depths in cm.

Grass plots	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
1) 0-20	15.6	18.0	15.6	16.6	1.3 **	16.4
2) 0-20	15.8	16.3	16.0	16.8	0.4 **	16.2
3) 20-40	17.9	26.1	18.5	21.2	4.0 **	20.9

	Control	DL	DH	UL	UH	LSD5%&P	Std.dev.
1) 0-20	16.9	17.0	16.3	16.0	16.1	1.4 ns	1.3
2) 0-20	16.5	16.4	16.3	15.8	16.2	0.5 ns	0.5
3) 20-40	24.8	22.9	19.4	19.0	18.6	4.5 *	4.8

Arable plots	Block 1	Block 2	Block 3	Block 4	LSD5% &P	Mean
1) 0-20	18.0	17.5	16.7	19.2	2.1 ns	17.8
2) 0-20	17.7	16.6	16.6	16.8	1.1 ns	16.9
3) 20-40	24.1	19.9	21.3	23.5	2.9 *	22.2

	Control	DL	DH	UL	UH	LSD5%&P	Std.dev.
1) 0-20	17.8	18.8	17.0	17.9	17.8	2.4 ns	1.6
2) 0-20	16.8	17.5	16.9	16.6	16.7	1.2 ns	0.8
3) 20-40	24.6	19.2	23.2	21.1	22.8	3.2 *	3.1

Total C is normally converted to SOM by multiplying with a factor of 1.72 (Howard 1965). Ignition-loss values are normally higher than organic matter calculated this way (Riley 1996), due both to the fact that ignition causes the destruction of part of the clay lattice, and to the fact that the ignition-loss also includes carbonate and other non-organic carbon sources, if these are present. As the soil on the experimental site contains relatively little clay, the differences between ignition loss and total-C calculated SOM values were in this case not very great; about 0.5% to 1% at ignition-losses below about 10% (Fig. 3.8.). Correlations were highly significant in both datasets, but differences between ignition-loss and SOM calculated from carbon measurements were slightly greater for the 2010 auger samples than for the 2011 auger samples. In the former, ignition-loss was measured at Bioforsk Øst, Apelsvoll, whilst in the latter it was measured by Eurofins in Sweden, at a somewhat lower temperature (500 as compared with 550 °C). In order to calculate the SOM of the soil cores used for soil physical analysis (see section 4.2), where total-C analyses were not available, the relationship in the left-hand part of Fig. 3.8 was chosen because both analyses (ignition loss and soil physics) were then conducted by the same laboratory.

Calculating the SOM values for the cylinder samples does not mean that we want to present SOM values based on other factors than the established value of 1.72 for samples where total C is available. For a general characterization of the experimental field, the initial SOM content was 1.72 * 6.41 % = 11.03 % in the grass system, and 1.72 * 3.47 % = 5.97 % in the arable system. These values compare well with the values received by the ignition loss measurements; 11.3 and 6.6 %.

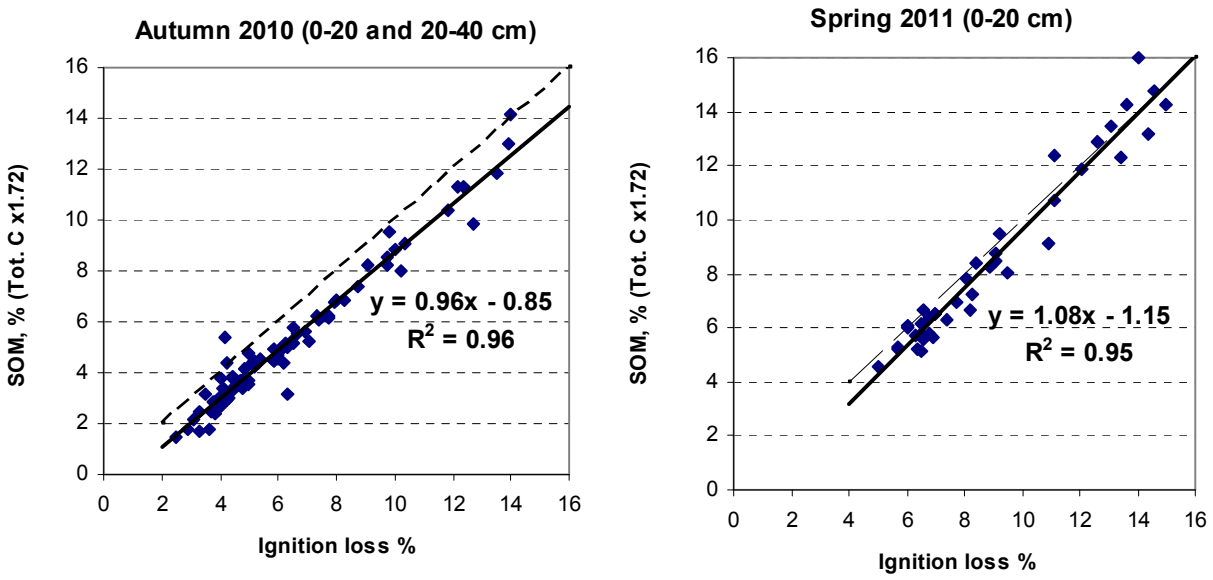

Figure 3.8. Comparisons of soil organic matter (calculated as tot.C x 1.72) with ignition-losses measured at 0-20 cm in autumn 2010 (left) and with those measured at 0-20 cm in spring 2011 (right).

3.3 Water-soluble C

A share of the 2011 auger samples were frozen and sent to the Department of Environmental Science at AU (Denmark) to analyze the soil for content of readily available organic carbon (C), which in this context is defined as the fraction of the soil organic pool that can be extracted in water (Sparling et al. 1998). Previous studies have shown that this fraction is governing the short-term behavior of microbial communities regarding structure, growth dynamics and turnover of C and N. At high concentrations of available organic C, the turnover may be so fast that oxygen is depleted and result in a burst of N_2O emission due to respiratory denitrification (Parkin 1987; Johansen et al. 2013). Because undigested and digested slurry may differ greatly in content and quality of organic C (Arthurson 2009), we expect the two types of slurry to affect differently on the soil microbial community and the processes it performs. These measurements were intended to give information about the conditions for microbial communities and C and N turnover early in the experiment.

Method

Measurement of the soil pool of readily available organic C was adapted from Sparling et al. (1998). Five g of air-dried soil was extracted in 25 ml of UV-Milli-Q water (agitated 30 min, 30 rpm, 22°C) followed by centrifugation (20 min, 3500 rpm, 20°C) and filtration (0.45 µm, 2.5 cm, PTFE membrane, Frisinette, DK) of the uppermost 7 ml of the supernatant. This cold-water-extractable (CWEC) fraction is expected to be the most readily available part of the soil organic C pool. The remaining soil pellet was suspended by vortexing (25 s) and re-extracted overnight (80°C) in 25 ml UV-Milli-Q water followed by similar centrifugation and filtration steps. This hot-water-extractable (HWEC) fraction is supposedly less easy to break down by the soil microorganisms. The concentration of organic C in the extracts was measured using a Shimadzu TOC-5000 analyzer (Shimadzu Corp., Kyoto, JP).

Results

There is reason to believe that the amount of water-extractable C (WEC) will be influenced by application of manure. In 2011, application of undigested slurry occurred on May 4 and application of digested slurry on May 5 in the grass system. In the arable system, all manure was applied on May 11. The upper soil layer was sampled for a second measurement of WEC five days after manure application. In the grass system, this sampling occurred on May 9 and in the arable system on May 16.

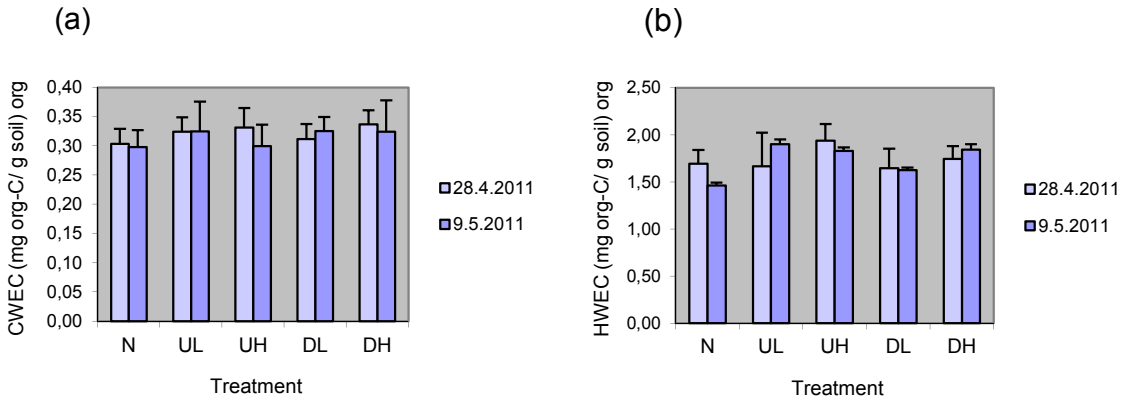

Figure 3.9. Cold-water extractable organic carbon, CWEC, a) and hot-water extractable organic carbon, HWEC, b) in grass system plots sampled in 2011, at April 28 and May 9 (five days after manure application). Bars represent SEM (n=4). N: Control, no manure; UL: Undigested slurry,Low level; UH: Undigested slurry, high level; DL: Digested slurry, low level; DH: Digested slurry, high level. NB: The x-axis differs between figures a and b.

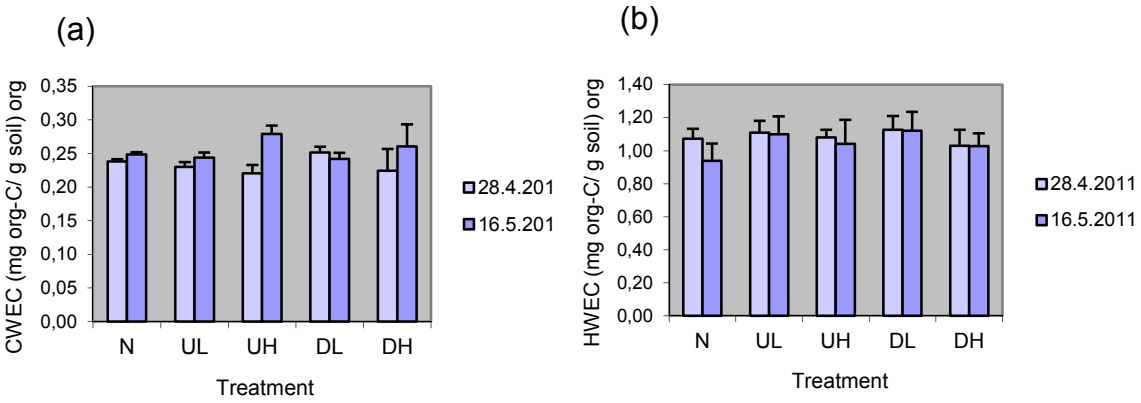

Figure 6.2. Cold-water extractable organic carbon, CWEC, a) and hot-water extractable organic carbon, HWEC, b) in arable system plots sampled in 2011 on April 28 and May 16 (five days after manure application). Bars represent SEM (n=4). N: Control, no manure; UL: Undigested slurry,Low level; UH: Undigested slurry, high level; DL: Digested slurry, low level; DH: Digested slurry, high level. NB: The x-axis differs between figures a and b.

As expected, more C is extracted by increasing the extraction temperature, so the HWEC values are generally about 5 times as high as the CWEC values (Figs. 6.1, 6.2). On average for all treatments in the grass system (n= 20), the upper soil layer (0-20 cm) contained

0.32 mg organic C per g soil (air dried) by extraction in cold water (CWEC), increasing to 1.7 mg by hot water extraction (HWEC). In the arable system, the corresponding values were 0.23 and 1.1 mg. Hence, the upper soil layer in the grass system contained 20-30% more CWEC and 50-60% more HWEC than in the arable system. This corresponds well to the total SOM levels, which were significantly higher in the grass system.

In the grass system, no indications of increase in cold or hot WEC were found as a response to application of fertilizer materials. In the arable system, CWEC seemed to increase after fertilizer application, at least in the treatments with high levels of applied. Lack of a similar trend in the grass system may be due to the generally higher SOM content, or that the applied amounts of manure were lower.

It is noteworthy that in both systems, there seems to be a drop in HWEC in the control treatment, after fertilizing, which is not found in any of the manure treatments. This may reflect a decomposition of C in the soil that may have been masked by the addition of manure.

It is possible that clearer effects on the WEC of manure application could have been found if the time span between manure application and soil sampling had been shorter than 5 days, like shown by Johansen et al. (2013).

The data can be associated with PLFA data to reveal eventual linkage between soil content of available organic C and presence of microorganisms and microbial community structure.

3.4 Soil Organic Matter, summary and conclusions

- The initial soil organic matter (SOM) content of all plots in the trial was charted by measuring ignition-loss, total carbon (C) and nitrogen (N) contents. The results of all three methods were in agreement with each other.
- The SOM of the grass system was higher and more variable than that of the arable system, and differences between blocks were greater and more statistically significant in the grass than in the arable system.
- Differences in the initial SOM between the means of plots that have been assigned to different subsequent treatments of manure applications were on the whole much smaller than those between blocks within the same crop system.
- Nevertheless, significant differences were found in some cases, and thus the initial SOM status of the soil should be taken into account when interpreting differences that may arise after the treatments have been carried out for a number of years.
- The C:N ratios found in the upper soil layer (ca. 16-18) were in the normal range for a soil with a relatively high level of SOM, but the ratios found in the lower soil layer were higher than expected (> 20) and should be taken with precaution. Likely causes are discussed.
- SOM calculated from carbon data were compared with ignition-loss measurements, and a formula for calculating SOM from ignition-loss is given for cases when data for total C are not available. This formula is used for the 2011 cylinder samples in section 4.2.

4. Initial soil characteristics: Physical conditions

Soil physical conditions are important for crop growth and may be affected by manure application through direct effects of the organic matter that it contains, or indirectly though the stimulation of microbiological activity, resulting in improved soil structure. In order to assess soil physical conditions at the start of the trial, a number of basic soil characteristics were studied. As for results given in section 3, the results presented here all relate to the soil conditions that were present before any treatment had been applied.

Soil texture is a basic soil property that affects nearly all soil physical parameters. Pore size distribution affects moisture retention and aeration, as well as transport properties. Soil aggregation is a parameter that is important for seedbed quality and soil aggregate stability is important for the resistance of soil to structural damage due to the effects of external factors such as rainfall.

Some of the equipment used for soil physical analyses is illustrated in Figures 4.2-4.9.

4.1 Soil texture

Mechanical analysis was performed to find the percentage size distribution (by weight) of mineral particles in the soil, in order to establish its soil textural class. The latter refers to the placing of the soil within a soil textural triangle, such as that proposed by Sveistrup & Njøs (1984) for Norwegian soils, or the commonly used USDA triangle (see Fig. 4.1).

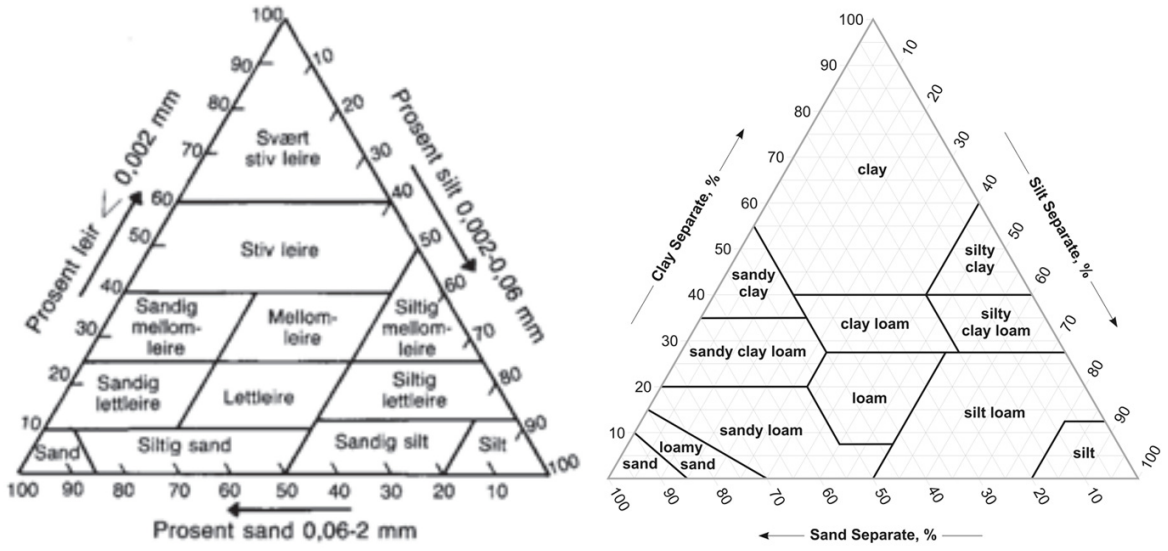

Figure 4.1. Soil textural triangles with textural classes proposed by Sveistrup and Njøs (1984) for Norwegian soils (left) and equivalent class names in the USDA triangle (right).

Method
Mechanical analysis was performed on 16 bulked soil samples taken at two depths (0-20 cm and 20-40 cm) from 5 plots on each block, using the 2010 auger samples (see section 2.5). Gravel content (> 2 mm) was measured separately for each plot before bulking (Fig. 4.2), and is expressed as the weight percentage of the complete soil sample. The term 'gravelly' is not used for soils with < 20 % gravel content.

The remaining fine earth (< 2 mm) was fractionated into sand, silt and clay (Fig. 4.4). These fractions are reported as weight percentages of the fine earth, after organic matter was first removed by oxidation with hydrogen peroxide. The analysis was performed by pipetting and drying silt and clay fractions after suitable sedimentation times, and by wet sieving and drying of the sand fractions, as described by Elonen (1975).

All samples belonged to one of the following textural classes: 'siltig sand', 'sandig lettleire' or 'lettleire', approximating the classes loamy sand, sandy loam and loam according to the USDA classification that is used in many countries. In the further text the USDA terms will be used, with the fractions of clay, silt and sand relevant for the Norwegian soil types. Loamy sand ('siltig sand') contains <10 % clay, <50 % silt and 40-85% sand. The term 'mellomsand' (medium sand) denotes that the total sand fraction contains < one third coarse sand and < two thirds fine sand. Sandy loam ('sandig lettleire') contains 10-25 % clay, <25 % silt and 50-90 % sand. Loam ('lettleire') contains 10-25 % clay and 25-50 % silt. These soil textures are very typical of soils formed on morainic parent material in western Norway. Loamy sand is the dominating soil type in the upper soil layer of the experimental field.

Results
The soil texture, loamy sand ('siltig mellomsand') was similar in all replicate blocks and both depths in the grass system part of the experiment (Table 4.1). It was slightly heavier and somewhat more variable in the arable system part, with overall ca. 16 % less sand, 11 % more silt and 4 % more clay than in the grass system part when both soil layers are considered together. Blocks 1-3 of the arable system had clearly heavier soil in the lower layer than did block 4 in this section. Somewhat heavier soil in the deeper parts of the terrain may be explained by washing out the soil layer during post-glacial land elevation. The gravel contents were fairly low (< 10 %) in all cases.

Table 4.1. Particle size distribution (after Atterberg grain size scale) and soil textural class (as proposed by Sveistrup & Njøs 1984) at two depths in each block (Bl.) of the two plant systems in the SoilEffects field experiment. Means and standard deviations (St.d) shown for each block (comprising 5 plots) in each system.

	Grass system						Arable system					
0-20 cm:	**Bl. 1**	**Bl. 2**	**Bl. 3**	**Bl. 4**	***Mean***	***St.d.***	**Bl. 1**	**Bl. 2**	**Bl. 3**	**Bl. 4**	***Mean***	***St.d.***
Gravel	3	5	3	4	*4*	*1*	2	7	5	8	*5*	*3*
Coarse sand	7	9	6	6	*7*	*1*	7	9	7	6	*7*	*1*
Medium sand	20	30	21	21	*23*	*5*	21	20	21	21	*21*	*1*
Fine sand	36	40	44	42	*41*	*3*	33	36	31	38	*35*	*3*
Sum sand	***63***	***79***	***71***	***69***	***71***	***7***	***61***	***65***	***59***	***66***	***63***	***3***
Coarse silt	14	9	14	13	*13*	*2*	15	15	16	16	*16*	*1*
Medium silt	8	4	5	8	*6*	*2*	10	7	10	7	*9*	*2*
Fine silt	5	3	3	4	*4*	*1*	5	3	6	5	*5*	*1*
Sum silt	***27***	***16***	***23***	***24***	***23***	***5***	***30***	***25***	***32***	***28***	***29***	***3***
Clay	10	5	6	7	*7*	*2*	9	11	9	6	*9*	*2*
Textural class[1]	si.m .sa.	si.m .sa.	si.m .sa.	si.m .sa.			si.m .sa.	sa. ll.	si.m .sa.	si.m .sa.		
20-40 cm:	**Bl. 1**	**Bl. 2**	**Bl. 3**	**Bl. 4**	***Mean***	***St.d.***	**Bl. 1**	**Bl. 2**	**Bl. 3**	**Bl. 4**	***Mean***	***St.d.***
Gravel	4	4	4	3	*4*	*1*	2	7	3	7	*5*	*3*
Coarse sand	9	6	16	6	*9*	*5*	5	8	7	7	*7*	*1*
Medium sand	24	29	19	26	*25*	*4*	18	18	17	20	*18*	*1*
Fine sand	38	46	42	49	*44*	*5*	29	29	22	37	*29*	*6*
Sum sand	***71***	***82***	***77***	***81***	***78***	***5***	***52***	***54***	***46***	***64***	***54***	***7***
Coarse silt	12	10	12	11	*11*	*1*	18	17	18	17	*18*	*1*
Medium silt	7	3	4	3	*4*	*2*	11	11	14	6	*11*	*3*
Fine silt	4	1	2	1	*2*	*1*	7	7	8	5	*7*	*1*
Sum silt	***22***	***15***	***18***	***15***	***18***	***3***	***36***	***34***	***40***	***28***	***35***	***5***
Clay	7	4	5	3	*5*	*2*	12	12	14	8	*12*	*3*
Textural class[1]	si.m .sa.	si.m .sa.	si.m .sa.	si.m .sa.			lett-leire	lett-leire	lett-leire	si.m .sa.		

[1] Norwegian class abbreviations according to Sveistrup & Njøs 1984 (equivalent English name):
si.m.sa.= silty medium sand (loamy sand), lettleire= light clay (loam), sa.l.-leire= sandy light clay (sandy loam)

Figure 4.2. Rotating 2 mm seives used for separating gravel from fine earth (constructed at Kise Research Station).

Figure 4.3. Furnace used for ignition-loss analysis (Carbonite).

Figure 4.4. Equipment used for mechanical analysis. Left: Setup for pipetting of silt and clay sedimentation samples. Right: Wet seive shaker used for separating sand fractions (Retsch).

Figure 4.5. Equipment used for moisture retention analysis. Left: Pressure chambers, valves and manometers (Soil Moisture Inc.) Right: Ceramic suction plate for samples (3 plates per chamber).

Figure 4.6. Flow-meter (Platon) setup used for measuring air permeability in soil cores at field capacity (Kise Res. Stn.).

Figure 4.7. Seive shaker for aggregate separation (Kise).

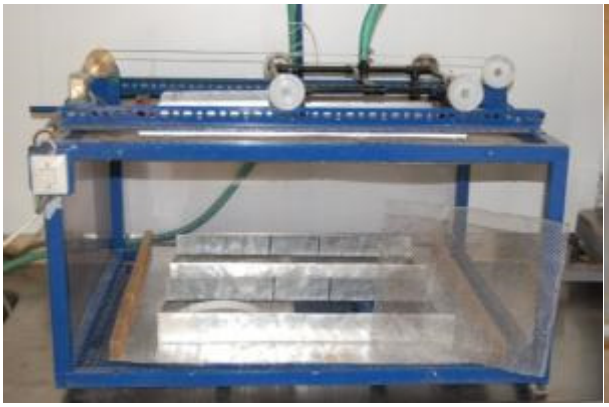

Figure 4.8.Rain simulator used for measuring soil aggregate stability (Kise Res. Stn.).

Figure 4.9. Soil aggregates from the arable plots of the SoilEffects experiment.

Figures 4.2-4.9: All photos by Hugh Riley

4.2 Soil moisture retention and aeration properties

Soil moisture retention is an expression of the soil's capability to hold water at increasing levels of soil matrix potential (matric suction or negative pressure). Pore diameter (d, in µm) is related approximately to the hydraulic head (h, in cm) by the formula: d=3000/h. For example, at a matrix suction of 100 cm hydraulic head, which is equivalent to a drain depth of 1 meter, the water will be drained from pores with a diameter larger than 30 µm. In soil physics, the matrix potential is often expressed either as kPa (100 cm head =10 kPa) or as pF (potential free energy) defined as $\log_{10}$ of the hydraulic head (100 cm head = pF 2).

From a plot of moisture retention against pF values, information may be obtained about the amount of water that the topsoil will hold when the subsoil is saturated (20 cm head, pF 1.3) and the amounts it holds at assumed field capacity (100 cm head, pF 2) and at permanent wilting point (15500 cm head, pF 4.2). The soil's capacity for plant-available water is the difference between the latter two. We differentiate at pF 3 between readily-available and more strongly-held available water. This distinguishes roughly between water held in pores that are large enough for root hairs to enter (3-30 µm), and that held in pores which are too small for root hair entry (root hair diameter is normally >5 µm).
The difference between saturation porosity and field capacity is known as the soil's air capacity, a simple indicator of its aeration status. Air permeability is a parameter related to hydraulic conductivity and gas diffusivity. It is easier to measure than either of the latter, and we do this routinely as part of the moisture retention analysis. The unit for air permeability (area) is somewhat cryptic: it is derived from conductivity (length/time) divided by air fluidity (1/(length x time)), resulting in the unit of length x length.

Methods
In April 2011, shortly after snow-melt and before any traffic on the field, 120 undisturbed cylinder samples (= the spring 2011cylinder samples) were taken within the upper soil horizon, ca. 7-11 cm depth. Within each plot, the surface sward layer was removed in three places, at the upper end, centre and lower end, 2 m apart and 60-80 cm from the left-hand side of the plots. At each place, a 100 ml cylinder (height 38 mm, internal diameter 58 mm) was pressed into the soil using a rubber mallet, and gently excavated and trimmed by hand. In the laboratory, the cylinders were saturated from below before placing them on ceramic plates (Fig. 4.5) in pressure chambers (Soil Moisture Inc.), to measure moisture retention at pressures of 2, 10 and 100 kPa. Intrinsic air permeability (Fig. 4.6) was measured after equilibrium at 10 kPa (assumed field capacity, FC), as described by Green and Fordham (1975). Moisture retention at 1500 kPa (permanent wilting point, PWP) was not measured directly, as it is very time-consuming and was not considered to be of prime interest for present purposes. Instead, it was calculated by means of a 'pedotransfer' function derived previously for sandy soils by Riley (1996):
PWP, vol % = -3.4 +1.0*SOM% +0.31*clay% -0.08*gravel% + 4.2*bulk density (R^2= 0.78, n=166)
SOM was calculated here from ignition-losses measured in each core sample, using the equation shown in Figure 3.8 (left). Clay content values used were the block means shown in table 4.1.
Mean values per plot were used for analyses of variance and for the standard deviations given here. Individual cylinder values were used in regressions. Mean values per block are shown in table 4.2 for each system (grass and arable).

Results
There were clear differences between the grass and arable systems in the soil's total porosity, moisture retention and aeration properties. The saturation porosity of the grass system soil was 6 %-units higher than that of the arable system soil. This reflects the higher organic matter content of the former soil, which in the 2011 cylinder samples was on average double that of the latter. The air permeability and air capacity (at assumed field capacity) were both higher in the grass system soil than in the arable system soil. The mean moisture retention curve of each soil is shown in figure 4.10.

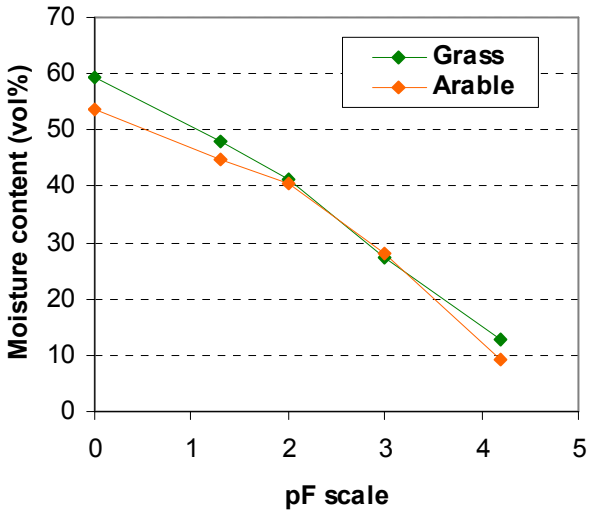

Figure 4.10. Mean moisture retention curves for the upper soil layer (7-11 cm) in the grass and arable systems.

One-way ANOVAR showed significant ($p<0.01$) or highly significant ($p<0.001$) differences between soils in all variables except for moisture contents measured at sampling, pF 2 and pF 3, and gravel contents (measured in the spring 2011 cylinder samples).

The grass system soil retained more water than the arable system soil at pF 1.3. This situation may be encountered under wet conditions with saturated subsoil. At pF 2 (most often assumed to represent the field capacity of drained soil), both soils retained similar amounts of water. The moisture content found at the time of sampling lay between these two tension levels, suggesting that the actual field capacity may be at a somewhat lower tension than pF 2 in these soils. At the boundary between readily available and less available water (pF 3), both soils held the same amount of water, whilst at permanent wilting point (pF 4.2) the arable soil appeared to retain less water than the grass soil, probably due to its lower content of organic matter (NB. the permanent wilting point was calculated indirectly).

The overall capacity for plant-available water, measured as the difference between water capacity at pF2 and pF 4.2, was thus slightly higher where the arable soil trial was planned, most of the difference being found in the less available fraction. However, neither soil is likely to be susceptible to drought, at least within the topsoil. Up to 60 mm of available water may be stored in the topsoil alone, of which almost half is readily available to plant roots. This is normally sufficient to permit crop evapotranspiration for 3-4 weeks without rain, a situation that is rare in this region.

In the grass system, the air capacity and air permeability of the soil were considerably higher than the levels that are thought to be critically low in relation to plant growth. A much-quoted critical level for the air-filled pore space at field capacity is around 10 %. This has previously been found to equate with an air permeability of 3 µm² (Riley 1988). In most cases the values measured on the arable plots were also higher than these critical levels, but critical levels were approached on block 3 of the arable soil, despite the high porosity and relatively high organic matter content of these plots. This corresponds well to the fact that this block had higher contents of clay and silt, and less of sand than the other system blocks, especially in the lower soil layer (Table 4.1).

Within each system, there were significant differences between blocks in the porosity and many of the moisture retention properties, but not in the aeration properties. Block 3 of the grass system had significantly lower porosity and moisture -holding capacity than the other blocks in that system, clearly due to its lower SOM content. The differences between blocks were much smaller in the arable system. Arable Blocks 2 and 4 had generally lower moisture-holding properties than 1 and 3. In neither system were there any statistically significant differences between the treatment means, and these means are therefore not tabulated here.

Table 4.2. Porosity, moisture retention properties, aeration and selected other soil physical properties measured in April 2011 in the upper soil layer (7-11 cm) for each block

Grass system	Bl. 1	Bl. 2	Bl. 3	Bl. 4	LSD5%[1]	Mean[2]	Std.dev.[2]
Saturation porosity	60.1	61.3	55.9	60.5	3.8 *	59.4	3.3
pF 1.3	48.4	50.4	42.4	51.1	5.0 *	48.1	4.6
pF 2.0 (field capacity)	43.1	42.7	36.4	43.3	4.8 *	41.4	4.2
pF 3.0	30.4	27.9	23.0	28.8	4.3 *	27.5	4.0
pF 4.2 (wilting point)	14.5	12.7	9.5	14.6	3.0**	12.8	2.9
Air permeability µm²	9.1	10.5	11.1	8.8	3.5ns	9.9	2.9
Air capacity at pF 2	17.0	18.6	19.5	17.2	2.6ns	18.1	1.9
Readily available water	12.7	14.8	13.4	14.5	1.0 *	13.8	1.1
Less available water	15.9	15.3	13.5	14.1	1.7 *	14.7	1.5
Total available water	28.6	30.1	26.9	28.6	2.3 +	28.6	1.8
Bulk density (kg/l)	1.02	0.98	1.16	0.97	0.12*	1.03	0.11
Gravel (% of whole)	6.6	2.7	7.0	4.6	4.1ns	5.2	3.4
SOM (% of fine earth)	11.0	10.6	6.7	12.2	3.3 *	10.1	3.0
Moisture at sampling	45.3	45.8	39.7	47.3	5.2 *	44.6	4.3
Arable system	Bl. 1	Bl. 2	Bl. 3	Bl. 4	LSD5%[1]	Mean[2]	Std.dev.[2]
Saturation porosity	53.9	52.0	54.6	53.7	1.7 *	53.6	1.5
pF 1.3	45.0	43.5	46.7	43.7	2.6 +	44.7	2.1
pF 2.0 (field capacity)	40.1	39.2	42.8	39.4	2.7 *	40.4	2.3
pF 3.0	28.6	27.1	30.0	26.0	2.7 *	27.9	2.5
pF 4.2 (wilting point)	9.4	10.3	9.8	8.2	0.8 *	9.4	1.0
Air permeability µm²	7.3	5.9	3.9	7.4	3.1 +	6.1	2.9
Air capacity at pF 2	13.8	12.8	11.8	14.3	2.6ns	13.2	2.0
Readily available water	11.6	12.1	12.8	13.4	2.2ns	12.5	1.6
Less available water	19.2	16.8	20.2	17.8	2.4 *	18.5	2.2
Total available water	30.8	28.9	33.0	31.2	2.2**	31.0	2.0
Bulk density (kg/l)	1.31	1.35	1.32	1.30	0.06ns	1.32	0.05
Gravel (% of whole)	4.6	5.1	5.6	5.8	3.9ns	5.3	2.6
SOM (% of fine earth)	4.8	5.0	5.3	4.7	0.9ns	5.0	0.7
Moisture at sampling	42.4	41.2	45.0	41.8	2.8 +	42.6	2.2

[1] *P-level: **<0.01 *<0.05 +<0.1 ns=not significant* [2] *Means and standard deviations of plot means*

As suggested above, soil organic matter is probably one of the major factors that are important for soil porosity and moisture retention properties. These relationships are therefore illustrated in Fig. 4.11. Porosity and moisture-holding at low tensions rose markedly up to a SOM level of approximately 10 %, and continued to rise slightly with further increases in SOM. The relationship was less clear in the case of moisture content at pF 3, but there was nevertheless a linear increase in moisture content with increasing SOM.

The strong relationship between SOM and moisture content at pF 4.2 (wilting point) is of course an artifact of the method with which it was calculated, but it is considered realistic. Such a dependence of soil moisture content at wilting point on SOM, as well as on the soil's clay content, has been found to be common in many Norwegian soils (Riley 1996). The fact that moisture contents increase with SOM at both pF 2 and pF 4.2, explains why we found rather little variation in these samples in the soil's total capacity for plant-available water.

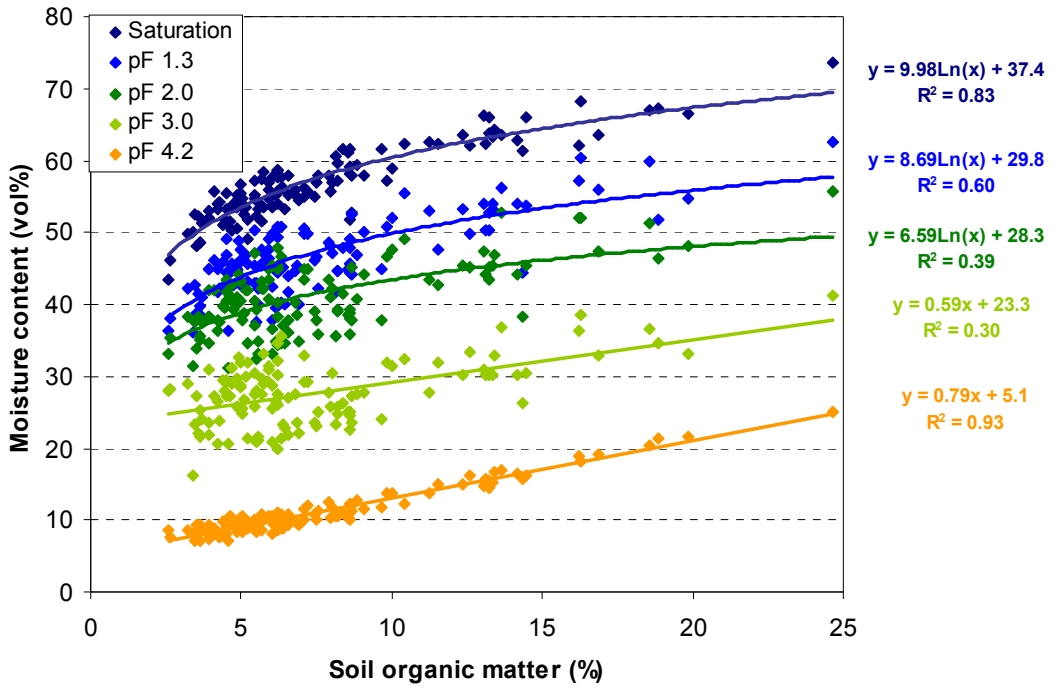

Figure 4.11. Relationships between moisture retention and soil organic matter

The soil organic matter (SOM) content of the spring 2011 cylinder samples was determined by ignition loss measurement of each soil sample, and a formula presented in chapter 3.3: SOM= 0.96 * ignition loss – 0.85. Little relationship was found between soil organic matter and either aeration properties or the total capacity for plant-available water (not shown). Nevertheless, the amount of readily available water (pF 2-3) showed a marked increase with SOM up to a level of 10 % (Fig. 4.12, left). This is probably a reflection of the soil textural differences between the samples with low SOM and those with higher SOM levels. The former had somewhat more silt and clay than the latter. The amount of readily-available water has previously been found to decrease with increasing clay content in many Norwegian soils (Riley 1996), and this seemed to some extent to be the case here

also (Fig. 4.12, right). Both SOM and clay contributed significantly in the following multiple regression equation, but its coefficient of determination (R^2) was low:

Readily available water = 12.6 – 0.32 * Clay % + 0.61 * SOM -0.02 * SOM^2 (R^2=0.25, n= 120)

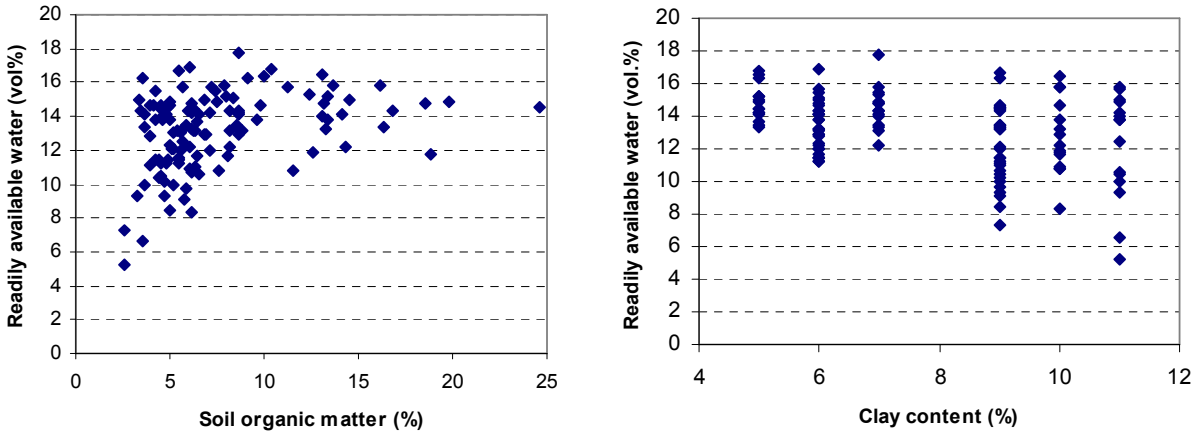

Figure 4.12. The amount of readily available water plotted against soil organic matter content (left) and clay content (right) in the soil

A further relationship of interest is that between air permeability and air capacity. The former is a very variable parameter, but one which is related to both gaseous diffusion and hydraulic conductivity. Air permeability is always measured at pF 2 (Fig. 4.13). At this suction level, pores with diameter >30 µm will be drained. At pF 1.3 only pores >160 µm will be drained. In Fig. 4.13, the relationship between air permeability and amount of air-filled pores (air capacity, vol %) is compared at two suction levels. The correlation between air permeability and air capacity was not better when only large macropores (>160 µm) were considered. Zero permeability was found in samples with 5-10 % air-filled pore space at pF 1.3 and 8-13 % air-filled pore space at pF 2 (Fig. 4.13). The permeability value of 3 µm^2 coincides with air-filled pore spaces of around 8 % and 12 %. This lends support to the view that 10 % air capacity is a critically low level.

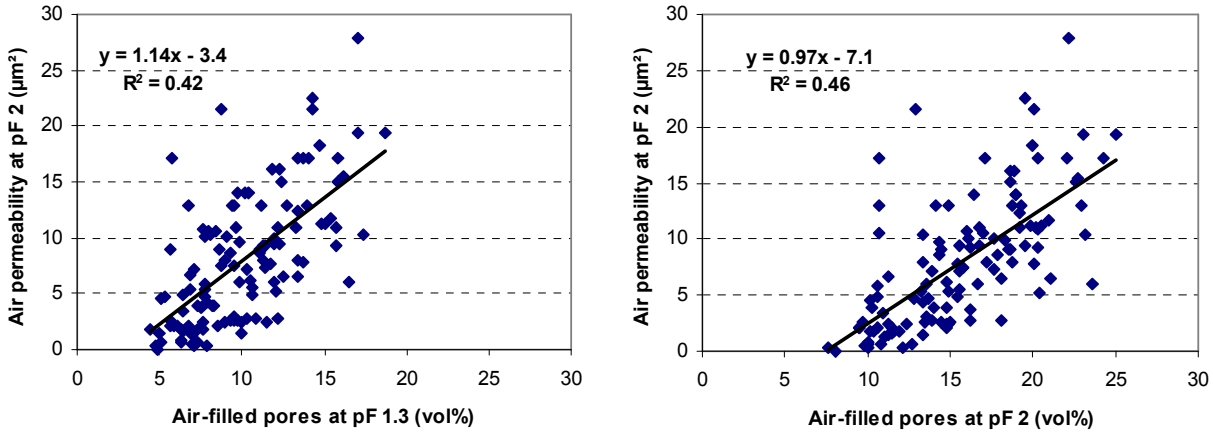

Figure 4.13.The relationship between air permeability and air-filled pore space

4.3 Aggregate distribution and stability

In clayey soils, an ideal seedbed is considered to contain a large proportion of small aggregates (< 6 mm), and a low proportion of coarse aggregates, as these cause rapid seedbed drying. Too many very fine aggregates are also undesirable, as they cause crusting after wetting and drying. Such considerations are less applicable to sandy soils, as these normally have single grain structure and little aggregation. A degree of aggregation may nevertheless be found in humus-rich sandy soils, and we wished therefore to study this. To avoid destruction of the grass system plots, this study was only conducted in the arable system.

Methods

On May 10 2011, before manure application and shortly after soil cultivation for seedbed preparation, soil samples were taken from the upper soil layer in about 5-12 cm soil depth, using a hand spade. In each plot of the arable system, four sites where the pattern from the soil cultivator was clearly visible were selected. At each site, the upper lose soil cover was carefully moved aside by hand, revealing more dense soil at about 5 cm soil depth. There, a vertical "soil profile" was made to about 12 cm soil depth by hand spade. The same spade was then used to sample 3 spades full of soil from this layer. Altogether, 12 spades of soil per plot were sampled, comprising about 2 liters of soil. The containers were stored open at outdoor (summer) temperature at Tingvoll for about 2 months, then transported by car to Apelsvoll and further stored at room temperature for several months to dry out before analysis. They were then sieved for 2 minutes on a reciprocating shaker (Fig. 4.7) containing sieves with mesh openings of 2, 6, 10 and 20 mm. Stones were removed and weighed. Aggregate size groups (Fig. 4.9) were expressed as percentages of stone-free material and their mean weight diameter was calculated using the formula of van Bavel (1949).

The stability of aggregates to simulated rainfall (Fig. 4.8) was measured for aggregate sizes of 2-6 and 6-10 mm, using similar apparatus as that described by Njøs (1967). Two samples (40 g) of each size group were placed within a diameter of 15 cm and subjected to rain-spraying for 2 minutes (pressure 1 bar, Hardi 4110-20 nozzles, nozzle height 35 cm and ca. 70 passes). Aggregate stability is given as the weight percentage of aggregates remaining on the sieve.

Results

Results of aggregate size distribution are shown in table 4.3 and Fig. 4.14.Very few statistically significant differences were found in these variables (as discussed below), and LSD values are therefore not included in the table.

The predominant soil structure type was clearly single-grain (or structureless), as the proportion of material passing the 2 mm sieve (68 %) was at least as great as the sand content of the soil reported in chapter 4.1 (63 %). Some aggregates were nevertheless found, most in the 2-6 mm (16 %) and 6- 10 mm (9 %) ranges, and very few > 20 mm. The mean weight diameter of 3-4 mm may be classed very satisfactory for seed germination.

The only statistically significant difference in aggregate size between blocks or treatments was in the fraction of 2-6 mm aggregates ($p=0.001$ and $p=0.056$, respectively). The 2-6 mm fraction was slightly higher on blocks 1 and 3 than on blocks 2 and 4. The former blocks had slightly less sand and more silt than the latter (Table 4.1). The treatment that was to receive a low level of digested slurry had slightly more 2.6 mm aggregates than the other

treatments. These differences are unlikely to have any marked effect on the crop growth on the plots concerned. The stone content of the soil was low, but stones were avoided during sampling in field and hence the stone content is not representative for the true situation in field.

Table 4.3. Aggregate size distribution in samples taken in May 2011 from the upper 5 cm of the arable system. Treatments: Control with no manure; DL = digested slurry, low level; DH =digested slurry, high level; UL = undigested slurry, low level; UH: undigested slurry, high level of manure application.

	Block 1	Block 2	Block 3	Block 4	Std.dev.[1]	Mean[2]
% < 2 mm	66.1	66.9	67.1	70.8	2.1	67.7
% 2-6 mm	17.5	15.3	16.6	15.4	1.0	16.2
% 6-10 mm	9.6	9.1	9.5	8.3	0.6	9.1
% 10-20 mm	5.8	6.1	4.9	3.9	1.0	5.2
% > 20 mm	1.0	2.6	1.9	1.6	0.7	1.8
Mean wt. diam.(mm)	3.3	3.8	3.5	3.1	0.3	3.4
Stones (% of whole)	3.9	3.5	3.1	4.7	0.7	3.8
	Control	DL	DH	UL	UH	Std.dev.[1]
% < 2 mm	67.4	67.0	68.1	68.3	67.8	0.5
% 2-6 mm	15.8	17.1	16.2	15.6	16.4	0.6
% 6-10 mm	9.1	9.8	8.6	8.5	9.6	0.6
% 10-20 mm	5.8	5.1	4.8	5.3	5.0	0.4
% > 20 mm	1.8	1.0	2.4	2.3	1.2	0.6
Mean wt. diam.(mm)	3.6	3.2	3.6	3.6	3.3	0.2
Stones (% of whole)	3.6	4.0	3.9	3.2	4.4	0.4

[1] Standard deviations are those of block and treatments means in this case

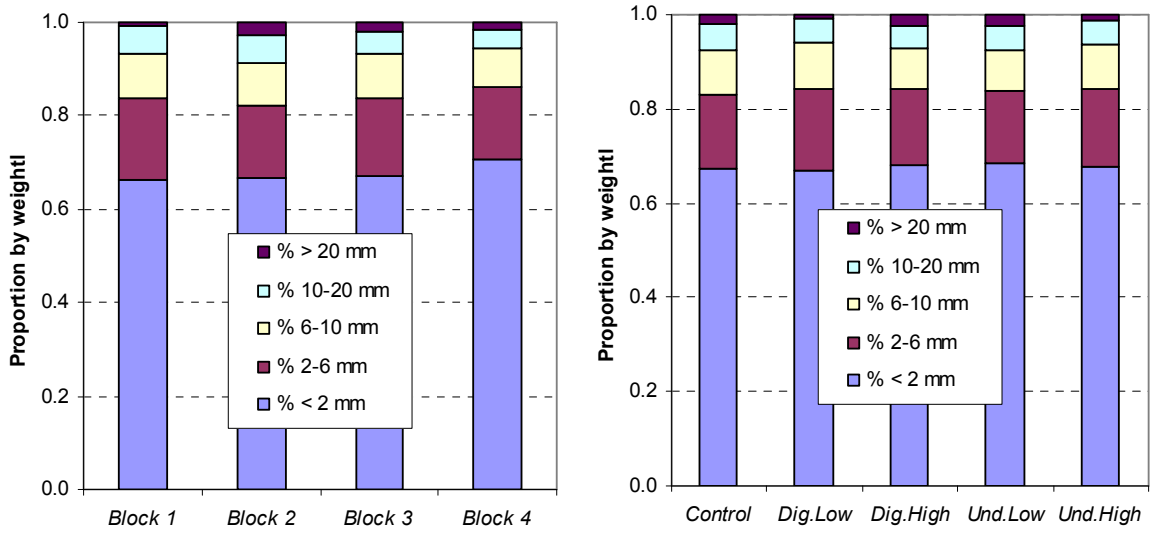

Figure 4.14. Cumulative proportions of various aggregate sizes found in the arable plots

The aggregate stability was high (85-90 %) in all cases, in both of the aggregate fractions that were measured (Table 4.4). This reflects the relatively high organic matter content of the soil and its relatively low silt and clay contents. No correlation was found, however,

between the aggregate stability of individual samples and soil organic matter content. It is possible that the samples contained some gravel, which of course is stable, but the data in chapters 4.1 and 4.2 suggest that this was unlikely to have been more than 5-10 % %.

There was a significant block effect ($p<0.001$) for both aggregate size fractions, with lower stability in block 2, and to some extent block 1, than in the other blocks. Block 2 had less silt and slightly more clay content than the other blocks. Between treatment means, there was an almost significant effect for the 2-6 mm aggregates ($p=0.055$), but not for the 6-10 mm aggregates. The former had slightly lower stability in the samples from plots that were to receive treatments with undigested slurry. In the same way as for aggregate size, we conclude that the observed differences between plots are unlikely to affect crop growth.

Table 4.4. Percentage stability of aggregates subjected to high-intensity artificial rainfall for 2 minutes. Mean values per block and per treatment

Aggregate size	Block 1	Block 2	Block 3	Block 4	Std.dev.	Tot. mean
2-6 mm	85.8	84.3	89.2	88.2	2.2	86.9
6-10 mm	88.5	85.0	90.8	90.4	2.7	88.7
	Control	Dig. Low	Dig. High	Und. Low	Und. High	Std.dev.
2-6 mm	87.2	87.3	88.0	86.1	85.7	1.0
6-10 mm	88.9	88.6	89.8	87.3	88.7	0.9

4.4 Summary and conclusions

A range of soil physical properties were measured as part of the start characterization of the experiment, in order to assess variations in initial conditions between blocks and treatments.

- Soil texture was measured blockwise at two depths. It was found to be dominantly loamy sand/sandy loam. There was slightly more silt and clay in the arable system soil than in the grass system soil. Gravel contents were low (<10 %).

- Soil moisture retention and aeration properties of the upper soil layer were measured on each plot. Total porosity, aeration properties and moisture retention at low tension were all clearly greater in the grass system than in the arable system.

- Satisfactorily high levels of aeration and plant-available water-holding capacity were found in both systems. Close relationships were seen between the moisture retention and the soil organic matter content. This accounts for many of the differences in such properties that were found between blocks.

- Soil aggregate size distribution was measured in the seedbed of the arable system plots. This confirmed that the predominant structure of the soil may be described as ‘single-grain’, with only 16 % aggregates of 2-6 mm and 16 % aggregates > 6 mm. There was little variation between blocks in the aggregate size distribution.

- The stability of soil aggregates (2-6 and 6-10 mm) to simulated rainfall was high (>85 %) in all cases, with little variation between blocks or treatment means.

5. Initial soil characteristics: Nutrients and pH

In Norway, concentrations of plant nutrients measured after soil extraction with ammonium-acetate lactate (AL) solution is used for determination of the need for fertiliser, as well as for scientific purposes (Table 5.1). Acid-soluble potassium (K-HNO_3) is used as an estimate of the K reserves that may become plant-available over time.

In the SoilEffects field experiment, the 2011 auger samples (0-20 cm soil depth, n=40) were analysed for pH, AL-extractable phosphorus (P), K, magnesium (Mg) and calcium (Ca), and acid-soluble K. The sampling date was on April 28 2011, before any manure was applied.

Table 5.1. Classification of plant nutrient availability by the content of AL-extractable nutrients and acid-soluble K (K-HNO_3) in Norwegian agricultural soil. All values in mg P, K or Mg 100 g^{-1} dry soil (Eurofins, 2009).

Class:	Low	Medium high	High	Very high
P-AL	0-4	5-7	8-14	> 14
K-AL	0-6	7-15	16-30	> 30
K-HNO_3	< 30	30-80	81-120	> 120
Mg-AL	< 2	2-4	5-9	> 9

For Ca, the concentrations must be seen in connection with pH and the content of soil organic matter (SOM). It should not be below 80-140 mg Ca 100 g^{-1} dry soil in mineral soil, or 130-210 in peat soil (Eurofins, 2009). In loamy sand with a SOM content below 12 % such as here, pH should be 6.0 for a satisfactory growth of grass and cereals. Legumes prefer a somewhat higher pH (Eurofins, 2009).

Methods

The soil used for these analyses was taken from the spring 2011 soil auger samples, where the sampling is described in chapter 2.5.

The soil analyses were carried out by Eurofins Food & Agro Testing Sweden AB, Kristianstad, Sweden. The uncertainty levels were +/- 20 %. Before analysis, the soil was air dried and crushed to pass a 2 mm sieve (ISO 11464:2006). Soil pH was measured in a soil-water suspension (1:5 v/v, ISO 10390:2005). AL-extractable P, K, Mg and Ca were determined by the Swedish standard SS028310, which follows the method of Égnér et al. (1960), where the soil was extracted with a solution containing 0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75 in the ratio of soil to solution of 1:20 (w/v). The concentrations of elements were measured with ICP-AES (Inductively coupled plasma atomic emission spectrometry). Concentrations of AL-extractable P, K, Mg and Ca in soil are abbreviated as P-AL, K-AL, Mg-AL and Ca-AL. Acid-soluble K was extracted by are extracted with 1M nitric acid, HNO_3 during heating, filtrated and analysed using ICP-AES

(Pratt 1965, slightly modified). The concentrations of plant nutrients are presented as mg 100 g^{-1} dry soil.

In the arable system, six samples had P-AL concentrations < 2 mg 100 g^{-1}. For statistical analysis, these values were set to 1.5.

Results

As expected, the average concentrations of AL-soluble P and K were low (Table 5.1 vs 5.2). However, the reserves of K seem to be significant, with high level of K-HNO_3 in the grass system and very high level in the arable system. For Ca, the level was low, and the pH value also indicates that legumes would benefit from liming.

Table 5.2. Concentrations of nutrients (mg 100 g^{-1} dry soil) and pH in the upper soil layer (0-20 cm) of experimental plots located in the grass and the arable system of the SoilEffects study, sampled on April 28, 2011 (Spring 2011 auger samples).

Grass system	Block 1	Block 2	Block 3	Block 4	LSD, 5%	Mean	Std.dev.
P-AL	2,90	2,52	3,30	2,74	0.53 *	2,87	0,47
K-AL	6,74	4,32	5,16	4,78	1.01 ***	5,25	1,15
Mg-AL	5,54	3,88	4,00	3,92	0.68 ***	4,34	0,85
Ca-AL	112,0	111,2	87,4	119,8	19.5 *	107,6	19,5
K-HNO_3	182,0	73,4	132,0	102,2	36.9 ***	122,4	47,0
pH_{H2O}	5,74	5,86	5,84	5,82	0.07 *	5,82	0,07

Arable system	Block 1	Block 2	Block 3	Block 4	LSD, 5%	Mean	Std.dev.
P-AL	2,92	2,74	1,92	1,66	0.47 ***	2,31	0,66
K-AL	5,42	6,24	4,62	4,68	0.94 **	5,24	1,02
Mg-AL	4,02	4,58	2,68	2,84	0.73 ***	3,53	0,95
Ca-AL	86,0	81,8	73,4	81,6	ns	80,7	8,3
K-HNO_3	180,0	190,0	184,0	146,0	22.5 **	175,0	22,1
pH_{H2O}	5,88	5,86	5,84	5,90	ns	5,87	0,08

For nearly all parameters, statistically significant differences were found between the four blocks in each system (Table 5.2). However, the differences between treatments were not statistically significant at the 5 % level, and hence the average values for each treatment in each system are not shown here.

6. Initial soil characteristics and first results: Soil microbiology

For soil nutrients, standard values are available for characterization of the soil with respect to the demand for manure application, liming etc. For soil microbiological characteristics, no standard values are available to characterize the practical meaning of the obtained results. Accumulated soil respiration was only carried out on soil sampled *after* manure application, since we did not have resources to characterize two parallel sets of soil samples with these analyses. The control treatment serves as the baseline for comparison of effects of manure application for these analyses. Extraction of phospholipid fatty acids (PLFA) was carried out on soil sampled *before and after* manure application.

6.1 Accumulated soil respiration

The upper layer soil samples taken after manure application were incubated in a Micro-Oxymax respirometer to measure accumulated respiration. As a response to manure application, the soil microorganisms will utilize the readily available organic C (namely CWEC) to form biomass and respire some of it to CO_2. Hence, it was expected that the soil would emit less CO_2 when amended with digested slurry as compared with undigested slurry, because the digested slurry is expected to have a lower proportion of easily degradable C (Möller et al., 2008). We also expected that this pattern would be more obvious at high level applications.

Method
Soil samples (65 g fresh weight) from both systems were placed in blue cap bottles and incubated in a Micro-Oxymax respirometer (Columbus Instruments, US) as described by Müller-Stöver et al. (2012). The bottles were automatically sampled (CO_2 concentration in headspace) every six hours (h), and flushed with filtered atmospheric air every 24 h to prevent anoxic conditions. The system was equipped with a device which ensured steady moisture conditions in the soil. This device dried the air just after leaving the bottles, and returned the water to the flasks.

Results
In both systems, at least some treatments where manure had been applied produced an emission higher than the control. This is as expected, and shows that application of manure increases the soil respiration.

In the grass system, the soil with high levels of undigested and digested slurry (UH and DH) produced more CO_2 than the control (N) and the low level treatments (UL and DL) (Fig. 6.1). Hence, the low levels of manure application apparently contributed with too little readily available organic C to induce a soil respiration higher that the control. By high level manure application, respiration rates were higher with digested slurry (5-10%) during the first 100 hours of incubation. Later, the respiration rate with undigested slurry (UH) reached the DH level. Higher respiration with digested than undigested slurry was not a result that we expected, but the difference was not very large.

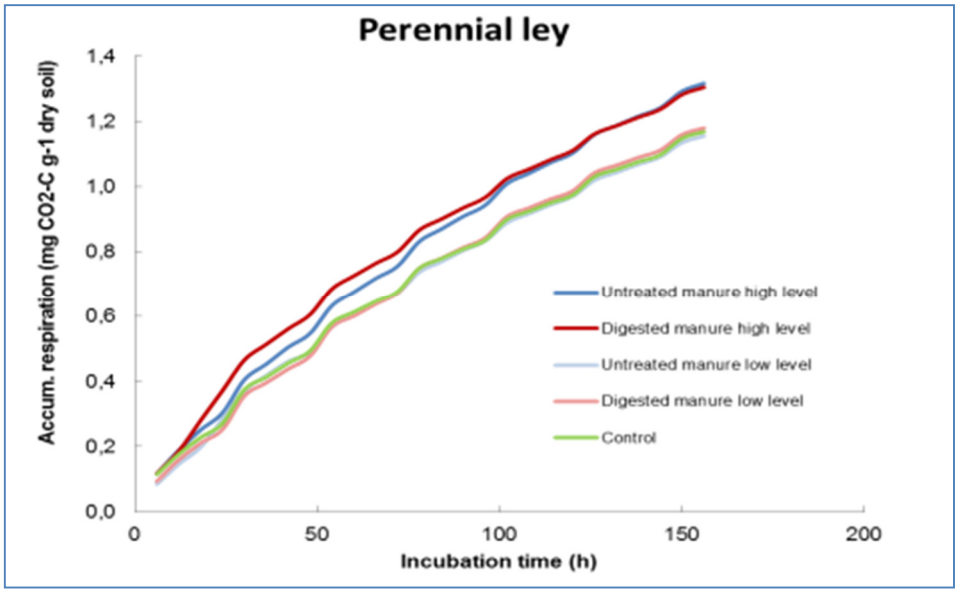

Figure 6.1. Accumulated respiration (CO_2) in grass system plots with no manure (N), Undigested slurry low level (UL), Undigested slurry high level (UH), Digested slurry low level (DL) and Digested slurry high level (DH).

In the arable system, the respiration was much higher from soil where a high level of undigested slurry had been applied than for any other treatment during the whole incubation period of about 400 hours (Fig. 6.2). The respiration from soil with other manure treatments than UH was quite similar during the first 150 hours. Thereafter, the high level of digested slurry had a slightly lower respiration than the two low-level treatments.

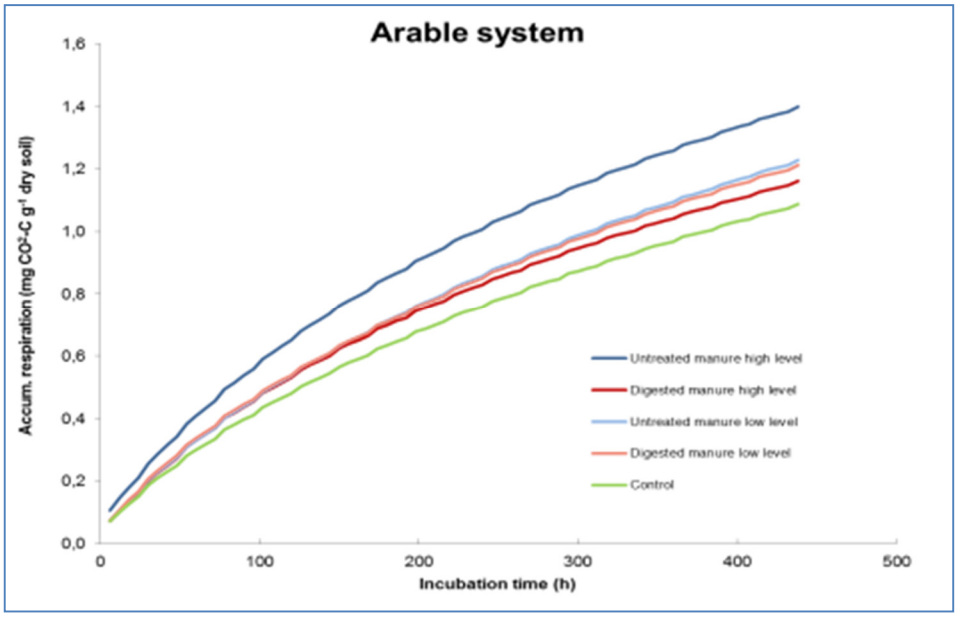

Figure 6.2. Accumulated respiration in arable system plots with no manure (N), Undigested slurry low level (UL), Undigested slurry high level (UH), Digested slurry low level (DL) and Digested slurry high level (DH).

It should be noted that the overall difference in level of accumulated respiration in the grass and arable systems were in good accordance with the content of readily available organic C (WEC) in soil. This means that the soil content of organic matter and available C seemed to govern the soil respiration more than the type and amount of applied manure. However, this pattern may change in future, following repeated applications of the different amounts and types of animal manure.

6.2 PLFA and microbial biomass

Soil microbial communities can be studied/characterized by extracting the lipids from their membranes. These polar phospholipids constitute a lipid profile which is indicating (roughly) which microorganisms are present in the soil (Zelles, 1999; Johansen et al., 2005). Moreover they can be taken as an indicator of the total microbial biomass as well as the biomass of microbial main taxonomic groups. This is possible because membranes of e.g. Gram positive, Gram negative bacteria and fungi contain lipids which are specific for these organisms.

Method
The procedure for extraction of phospholipid fatty acids (PLFA) is as described by Frostegård et al. (1993) and modified by Johansen and Olsson (2005). Five g dry weight of soil was placed in Teflon centrifuge tubes (Oak Ridge, Nalge Nunc Int., US) and extracted in 10 ml of dichloromethane/methanol/citrate buffer (0.15 M; pH 4.0; 1:2:0.8,vol:vol:vol). Supernatants from two repeated extractions were pooled and split into two phases by the addition of dichloromethane and citrate buffer. Polar lipids from the lower phase were purified and derivatized according to Joner et al. (2001). Samples were analyzed on an Agilant 7890 GC (Agilant, CA, USA) equipped with an autoinjector (splitless mode), a flame ionization detector, and a 60 m HP5 column using H_2 as carrier (2 mL min^{-1}). The initial oven temperature was 80 °C (5 min), increased at 20 °C min^{-1} to 160 °C and at 5 °C min^{-1} to 270 °C (maintained for 5 min). Inlet and detector temperatures were 230 and 270 °C, respectively. Proportions of the following main taxa of the microbial community were estimated by using PLFAs indicative of these groups: Gram-positive bacteria (PLFAs i15:0, a15:0, i16:0, i17:0, a17:0) (O'Leary and Wilkinson 1988), Gram-negative bacteria (PLFAs 18:1ω7, cy17:0, cy19:0) (Wilkinson, 1988), fungi (PLFA 18:2ω6,9) (Federle 1986) and actinomycetes (PLFAs 10me17:0; 10me18:0) (Kroppenstedt 1985). Cattle slurry and derived digested material contain only insignificant proportion of PLFAs (Johansen et al. 2013) and are, thus, not expected to disturb the microbial PLFA profile.

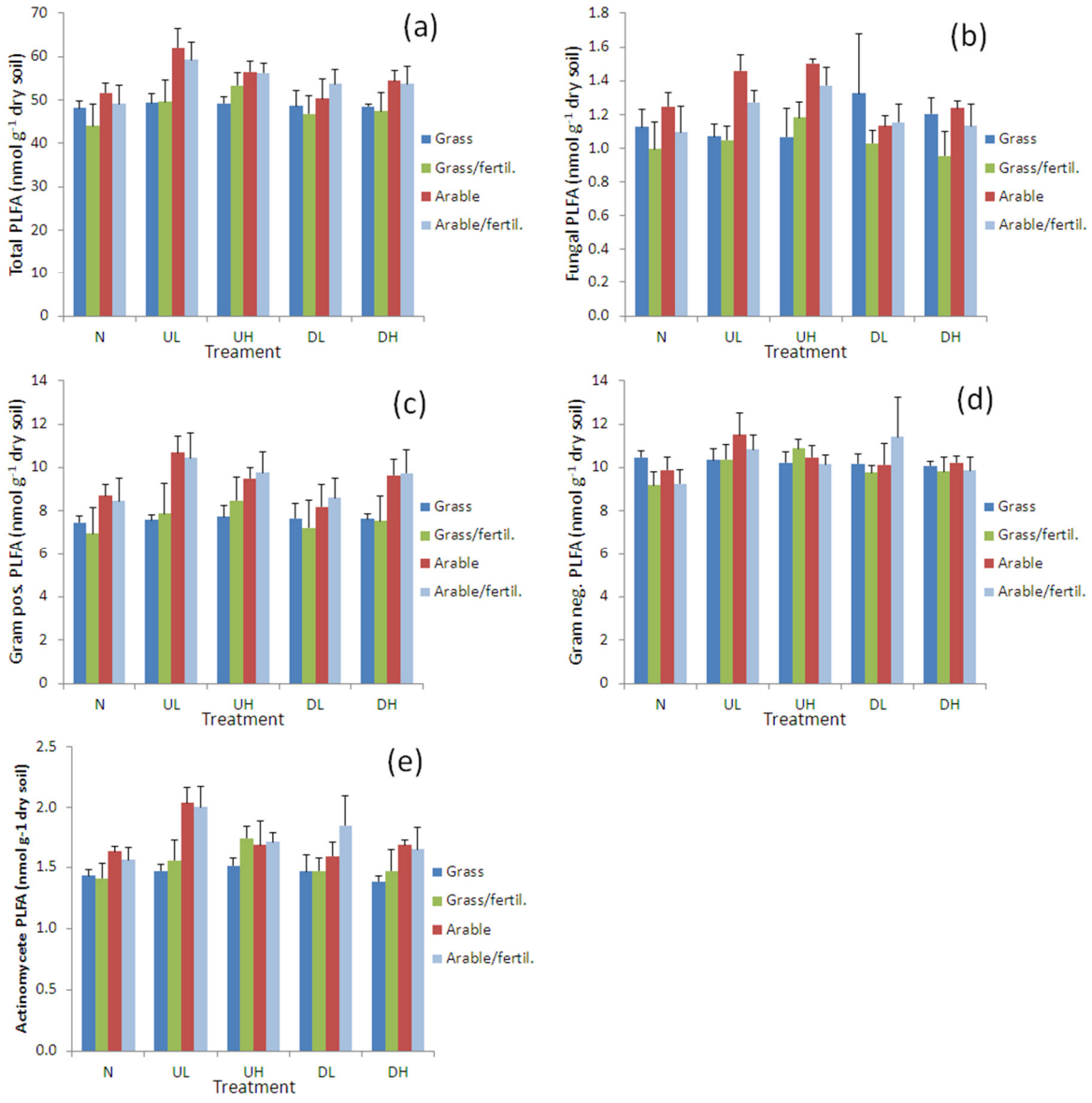

Figure 6.3. PLFA content in grass and arable system plots in spring 2011 before and after manure application. Results after manure application are shown as "Grass/fertil." or "Arable/fertil.". Treatments: Control (no manure, N), Undigested slurry low level (UL), Undigested slurry high level (UH), Digested slurry low level (DL) and Digested slurry high level (DH). PLFAs representative for total microbial community (a), fungi (b), Gram positive bacteria (c), Gram negative bacteria (d), and Actinomycetes (e) are shown. Bars represent SEM, n=4.

Results

The total microbial PLFA tended to be slightly higher in the soil of the arable system than in the grass system (Fig. 6.3a), in all fertilizer treatments. Within the individual treatments, the total PLFA content was not influenced by the manure application. This trend was also observed for fungi and actinomycetes (Fig. 6.3b, d), although blurred by the variation. PLFAs indicative of Gram positive bacteria (Fig. 6.3c) was about 20% higher in the arable system than in the grass-system. Hence, the manure treatments seemed to have no effect on the size of the total microbial population in May 2011, whereas the soil

differences between the two cropping systems seemed to impact the microbial community, especially the Gram positive bacteria.

6.3 Microbial community structure

The structure of a soil microbial community can be revealed by treating the PLFA data in a multivariate statistical approach. In praxis, the individual PLFAs (calculated as the molar-% of the entire mass of PLFAs) are submitted to principal component analysis where the principal components are a shown in a score plot (see Fig. 6.4) and represent meaningful variation in the data set.

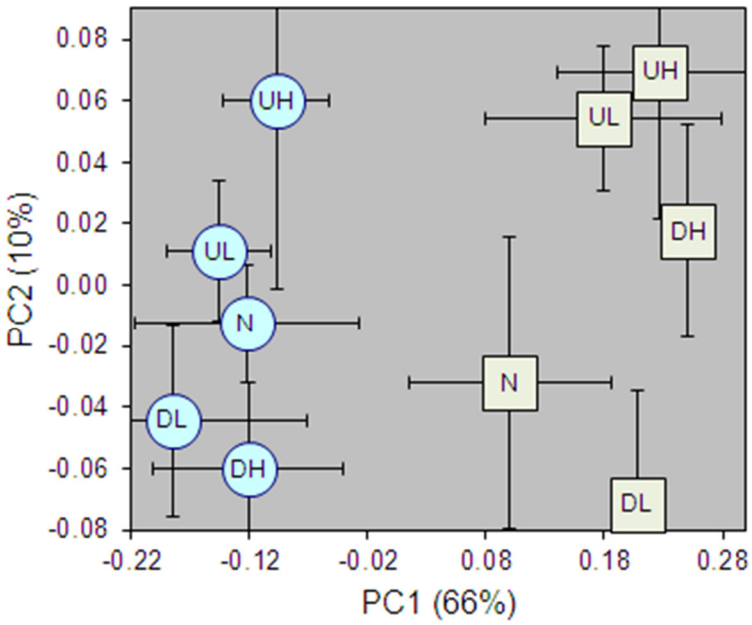

Figure 6.4. PCA score plot of PLFAs from the grass (circles) and arable (squares) system. The individual treatments are revealed inside the data symbols: no manure (N), Undigested slurry Low level (UL), Undigested slurry High level (UH), Digested slurry Low level (DL) and Digested slurry High level (DH). The data are obtained from soil sampled five days after manure application (bars represent SEM, n=4).

This means that data points located close to each other along the PC axes are alike (and vice versa) and the statistical strength increases with the value of the PC. In this case the data from the two cropping systems are grouping up separately in each side of the plot along PC1 which is representing 66% of the meaningful variation in the data set, whereas PC2 with 10% do not provide noteworthy statistical information. On the other hand, there is no separation between the data values with regard to manure treatments. Overall, this means that the microbial community structure is different in the grass and the arable system, and that the manure application, so far, has not induced any significant change in the soil microbial community structure. This, however, may change with repeated manure applications over several years.

7. Soil fauna

Earthworms and springtails (collembolans) were chosen for studies in the SoilEffects project because these groups of soil animals are common in agricultural soil. For earthworms, the common species in Norwegian soils are well known and their ecological functions reasonably well described (Pommeresche and Løes 2009). Springtails have been much less studied in agricultural land. This group is more demanding to study because of their smaller size, and much larger species diversity.

The results presented here are from the start characterization in spring 2011, BEFORE manure application. An initial study of earthworms from the autumn of 2010 has also been included.

To save some work, the effect of manure application was only studied in the treatments with high manure levels (UH, DH) in addition to the control treatments (N). Hence, *only these plots were studied by the start characterization of earthworms and springtails*. For the sampling of springtails, a grass sward is required as soil cover to avoid disintegration of the soil sample. Hence, *springtails were only studied in the grass system*.

Soil fauna characteristics are expected to express a much larger variation than soil physical and chemical characteristics, due to larger sensitivity to environmental and weather conditions.

7.1 Initial earthworm studies 2010

Earthworms constitute a key species in the soil biology, because of the important role they play in the decomposition of organic matter and structuring the soil. Crops and management significantly influence the earthworm fauna. Geophagous (soil-eating) species such as *Aporrectodea caliginosa* and *A. rosea* dominate the earthworm fauna in Norwegian arable soils (Pommeresche and Løes 2009). *Lumbricus terrestris* is also present, and has been found even in arable crop rotations with annual ploughing. In southern Norway, *L. rubellus* and *A. longa* are also common. Earthworm populations in agricultural land, recorded in autumn, may vary between 30 and 350 individuals m^{-2}, with the lowest values found in all-arable systems. The inclusion of leys in the crop rotation increases the abundance of earthworm channels, earthworm numbers and earthworm biomass.

Method
In 12 plots (marked in bold and cursive in Fig 2.5) out of the 84 plots that were initially marked up as candidates for the 40 field experiment plots, earthworms were sampled on October 25 2010 , to reveal which species were most abundant. In each of these plots, a cube of soil sized 20 cm x 20 cm x 20 cm was taken out using a metal frame (Fig. 7.2). Earthworms were sorted out by hand, and total biomass and number of individuals of earthworms were recorded and adjusted to a number per m^2 in the upper soil layer (0-20 cm) by multiplying with 25. The worms were identified to genus or species (Table 7.1), by Reidun Pommeresche. Plots belonging to the upper two rows (A, B, see Fig. 2.5, 2.6) may be seen as relevant for the grass system, and the two bottom rows (C, D) as relevant for the arable system. The SOM values in Table 7.1 are from the 2010 auger samples also presented in Figure 2.5.

Results

The number of individuals, especially of *Lumbricus* species, seemed to be higher with higher content of soil organic matter. In October 2010, the grass system part of the field contained on average 175 earthworms m^{-2}, and the arable system part contained 71 earthworms m^{-2} (Table 7.1). In the grass system part, the soil organic matter (SOM) content explained 64 % of the earthworm biomass, but in the arable system, no relation was found between SOM and biomass (analysed by linear regression in Excel). The most abundant species were the field worm, *Aporrectodea caliginosa* (53 % of the individuals), followed by the dew worm *Lumbricus terrestris* (27 %). Pink worm (*L. rubellus*) was also identified, in the arable system. In the grass system, it was impossible to differentiate between some of the individuals of the *Lumbricus*-species.

Table 7.1. Biomass, individuals of different species within age categories (juveniles (juv) and adults) and total number of earthworms (sum of individuals m^{-2}) found in plots later used in the field experiment or close to such plots. Plot numbers (A4, B4 etc.) refer to the initial 1-84 plots. Plots that were included in the field experiment are in italic, with plot numbers shown (4 and 14 = grass system; 25 and 35 = arable system). Names of species are explained in the text. Lumbr. genus are either L. terrestris or L. rubellus.

Plot	**SOM %**	**Bio-mass g m^{-2}**	L.terr. adult	L.terr. juv	A.cal adult	A.cal juv	A.rosea adult	L.rub. adult	Lumbr. genus	**Sum individ-uals m^{-2}**
A4	21,8	187		125	50	50			125	350
B4	13,6	30		75		25			75	175
A11, 4	10,5	108	25	25	125				25	200
B11, 14	6,9	46		25	25	75				125
A18	11,1	58		25	50	75				150
B18	8,1	12		25					25	50
Mean, Grass system	**12**	**73,5**								**175**
C4	9,2	23			50					50
D4	6,7	88	25		25	75				125
C11, 25	5,4	28				50		25		75
D11, 35	5,9	13				75				75
C18	6,6	0								0
D18	4,7	12		50		25	25			100
Mean, Arable system	**6,4**	**27,3**								**71**

Due to the large variation between plots, more than one soil block per plot could be tempting for later studies, but this would have been too destructive especially for the grass system plots.

Fig. 7.1. Sampling of soil cubes for earthworm studies at the SoilEffects field experimental site, October 25, 2010. From the left: Borghild Gjørsvik, Anne-Kristin Løes and Reidun Pommeresche. Reidun is standing on a sampling metal frame. Photo by Peggy Haugnes.

7.2 Start characterization: Earthworms

Systems with grass and clover in the rotations often host a higher biomass and number of earthworms compared with all-arable systems (Edwards and Lofty 1977, Schmidt et al 2003), due to less soil tillage and more plant residues left after harvesting. Animal manure provides food and increase the biomass of earthworms (Curry 1976, Andersen 1979, Hansen and Engelstad 1999), but may be toxic in the short term (Curry 1976). The effects on earthworms of anaerobically digested slurry have been less studied, especially for slurry from digested plant material. Ernst et al. (2008), tested the effects on earthworms of cattle slurry and a digested mixture of cattle slurry, grass (silage) and maize (ratio 10:1:16, 200 days) in a microcosm experiment. The biomass of the litter eating species (*Lumbricus terrestris* and *Apporectodea longa*) increased in both slurry treatments, whereas the biomass of the soil-eating species *A. caliginosa* decreased. The biomass decline was significantly stronger in treatment with digested slurry. Since geophagous (soil eating) species *A.caliginosa* and *A.rosea* dominate in arable soils in Norway (Pommeresche and Løes 2009), this result is interesting and deserves further study.

Methods
Earthworms were sampled in the arable and the grass system, in all replicate plots of the control treatment (N) and treatments with high amounts of digested (DH) or undigested (UH) slurry, on April 13th 2011 (Fig. 7.2) in due time before manure application (on May 4). One soil block was excavated per plot as described in section 7.1. The metal frame for excavating the soil cubes was placed with its upper left corner on a point that was 1 m distant from the bottom line of the plot, and 1 m distant from the left-hand side. Earthworms were hand-sorted, and biomass (dead, wet weight) and density (individuals m^{-2}) recorded. Juvenile and adult worms were identified to species by Reidun Pommeresche, according to Sims and Gerard (1999). Juvenile *Lumbricus*-individuals which could not be identify to species are named *Lumbricus sp*. The soil water content was measured in each soil cube (n= 24) by weighing samples of soil (ca. 200 g moist weight) before and after drying at 105ºC until weight was constant.

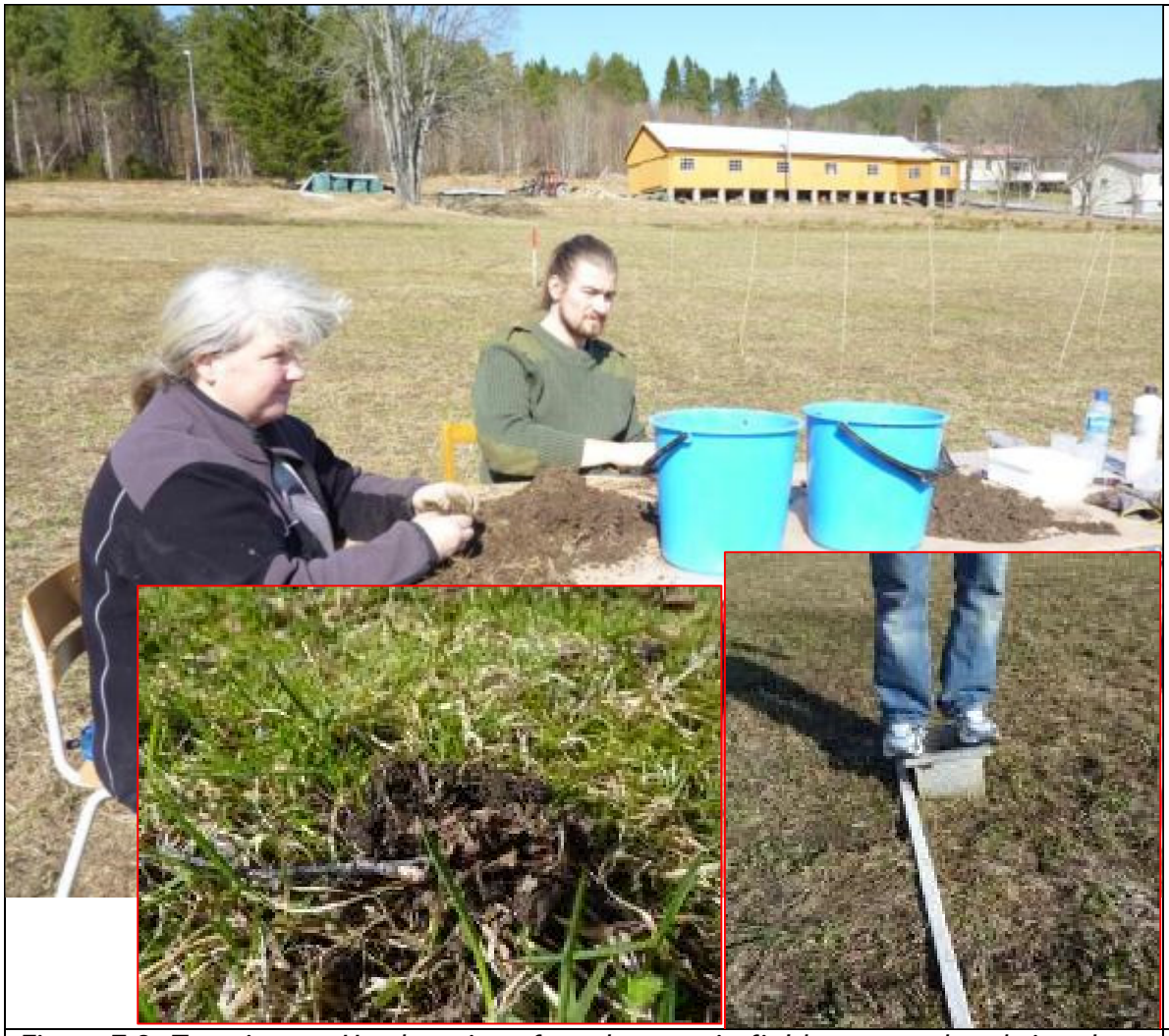

Figure 7.2. Top picture: Hand sorting of earthworms in field saves work to bring the soil back to the right plot again. April 13, 2011. Bottom left: Earthworm cast on the soil surface. Bottom right: A metal frame was used to excavate the soil cubes (20x20x20cm). Photos by Reidun Pommeresche.

Results

The soil water content at sampling varied between 15 and 30.9 %, with a mean value of 22.1 % (weight).

Altogether, 120 earthworms were found in the 24 cubes. Hence, the average earthworm density on the experimental field was 125 earthworms m^{-2}. Similarly to the result for 2010, the earthworm density was higher in the grass system (133 earthworms m^{-2}) than in the arable system (117). The differences between the two systems were lower than found in the autumn of 2010.

The mean biomass was somewhat higher in the arable system (mean 63.5 g m^{-2}) than in the grass system (mean 42.1 g m^{-2}). For these samples, no significant relationship was found between biomass and SOM content in any of the systems. A possible explanation for the higher biomass in the arable system is that the large species *Octolacion cyaneum* was more frequently found in the arable system.

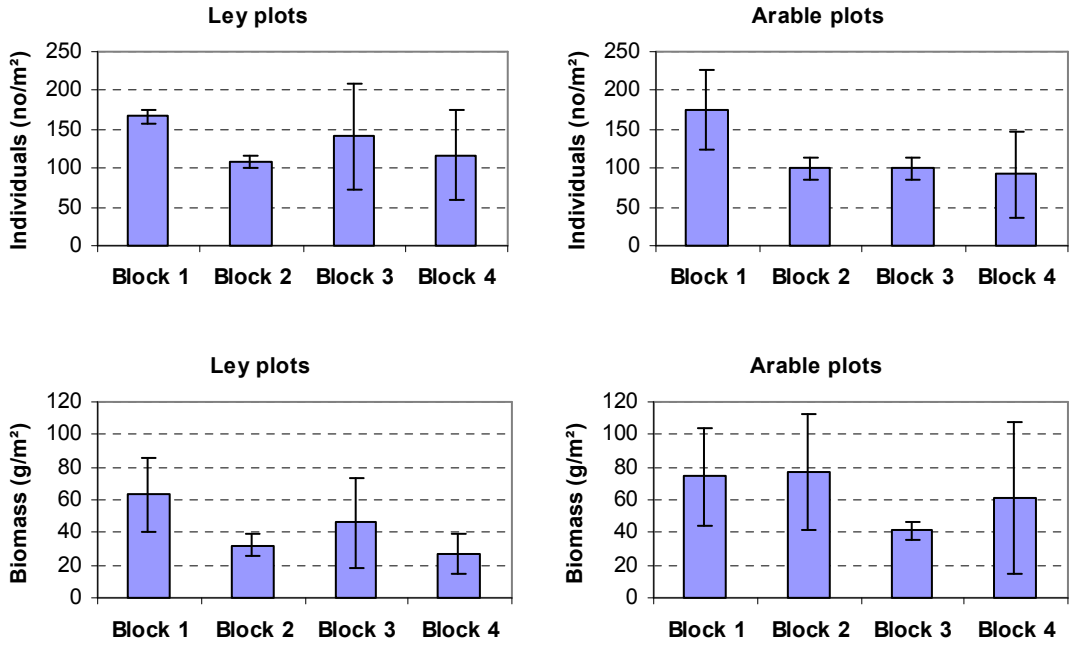

Figure 7.3. Number of individuals and biomass of earthworms in grass (ley) and arable plots of the SoilEffects field experiment on April 13, 2011, distributed across blocks (1-4) in each system. The standard error of means are shown as bars, n=3.

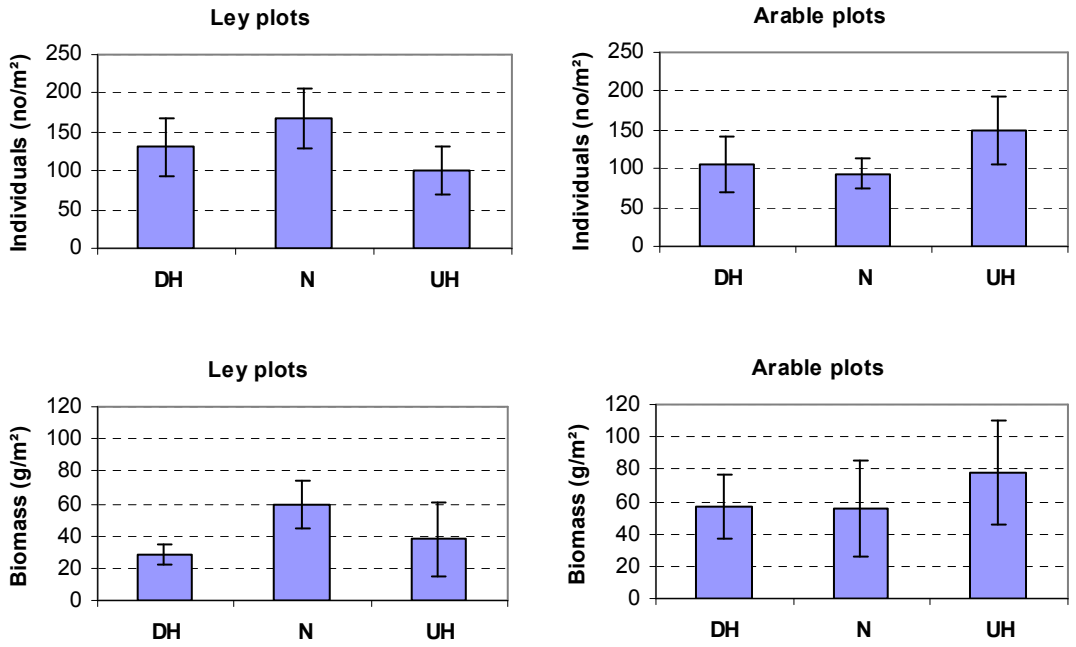

Figure 7.4. Number of individuals and biomass of earthworms in grass (ley) and arable plots of the SoilEffects field experiment on April 13, 2011, distributed across treatments. The standard error of means are shown as bars, n= 4.

Figure 7.5. Field worm, also called grey worm (Aporrectodea caliginosa, upper left) is the most common species in the experimental field. Dew worm (Lumbricus terrestris, upper right), is also present. The pictured worm was about 3 cm long, and newly hatched from the lemonshaped cocoon seen above it. Blue-grey worm (Octolacion cyaneum, lower right) is present in the arable system part of the experiment. This worm is bluish-grey with a characteristic yellow/white tip of the tail. Photos by Reidun Pommeresche.

80% of the individuals were juvenile, and most of them were identified to species. Altogether, five species were found (Fig. 7.6, 7.7). As expected, *Aporrectodea caliginosa* (Fig. 7.5) and *Lumbricus terrestis* dominated with 61 and 21 % of the individuals. Two further species, also common in agricultural soil, were *A. rosea* with 7.5 % of the individuals, and *L. rubellus* with 1.7 %. An interesting result was a significant number of *Octolasion cyaneum* with 9.2 % of the individuals. *O. cyaneum* is usually found on wet locations in limnic soils (pH 3.5 to 8.2), wet sands, under stones in water, in moss, but occurs also in gardens, pastures, arable land, woodland and caves (Sims and Gerard 1999). This species was found in all blocks of the arable system, and in block 4 of the grass system. This species has been recorded at Landvik (Bakken et al. 2006), in Tromsø (Haraldsen and Engelstad, 1994), but formerly not in Møre og Romsdal. In Tingvoll farm, *O. cyaneum* seems to be a quite common species, and has been observed in the demonstration garden on Tingvoll farm, and in a private garden 300 m east of the experimental field (Sissel Hansen) and in the garden of the kindergarten at Meisingset (Reidun Pommeresche).

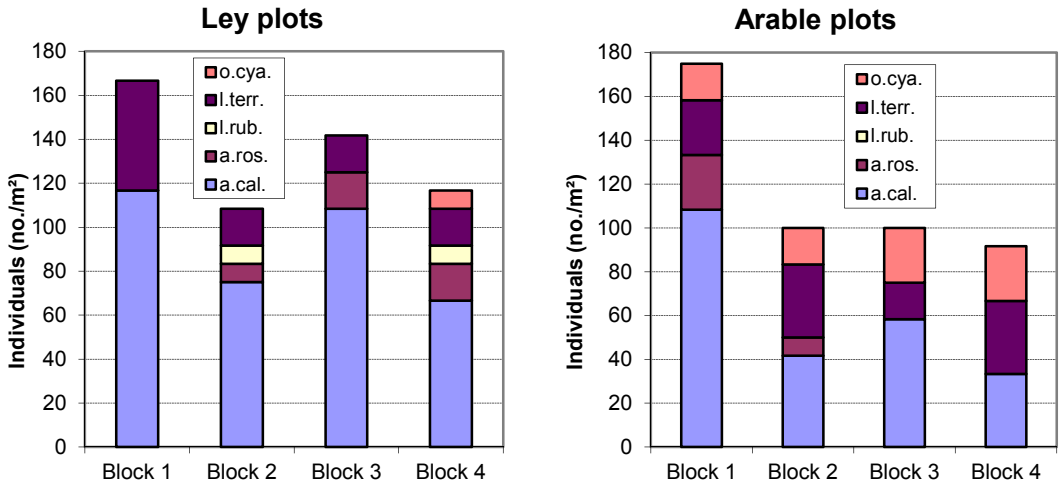

Figure 7.6. Earthworm species identified during the start characterization of the SoilEffects field experiment. Juveniles and adults are included. a.cal= Aporrectodea caliginosa, a.ros = A. rosea, l.rub= Lumbricus rubellus, l.terr= L. terrestris, o.cya= Octolasion cyaneum.

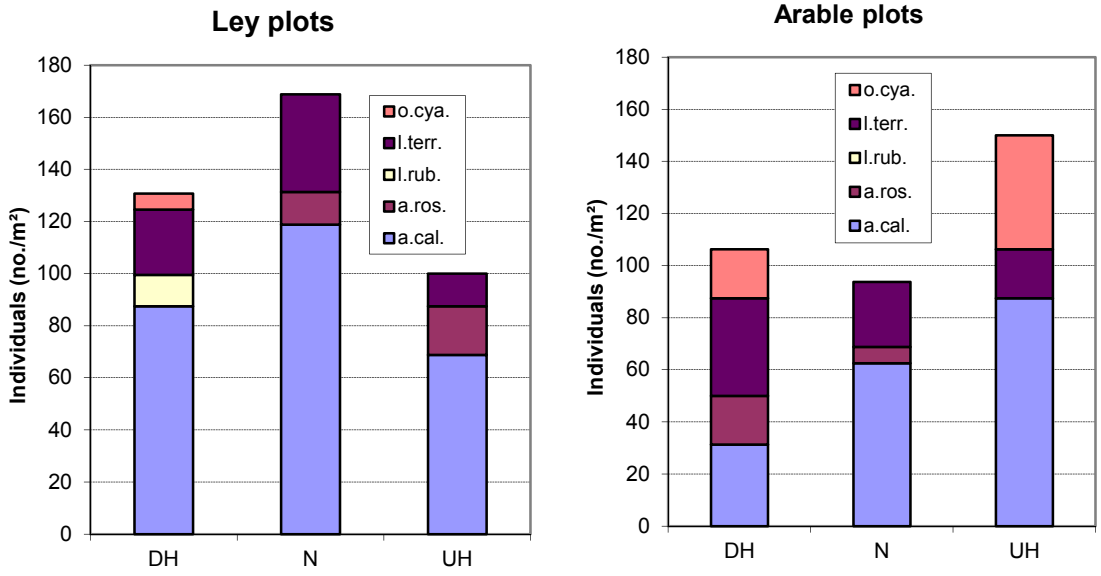

Figure 7.7. Distribution of earthworm species from the start characterization of the SoilEffects field experiment, from plots where high level of digested slurry (DH), high level of undigested slurry (UH) or no manure (N) will be applied (n=4). Juveniles and adults are included. For abbreviations of species, see Fig 7.6.

7.3 Start characterization: Collembolans

Collembolans (springtails) are a group of small animals (1-3 mm) living in vegetation, litter and in soil cavities to a depth of about 15 cm. They are important decomposers of dead plant material, and they feed on fungi, algae and microorganisms in soil and on organic debris. Their grazing and decomposing activity contribute significantly to the nutrient circulation in the soil. The name springtail refers to a furca on their back, usually folded in under the body, but released in need of a rapid escape. In one jump, collembolans can jump more than 50 times their own body length.

In Norway 334 species are known; in the world more than 6000. Collembolans are divided into two main groups; *Entomobryomorpha* consisting of species with a clearly elongated segmented body, and *Symphyleona* consisting of species with a more or less globular body with fused segments, reminding of small "rabbits". Whereas species living in the vegetation and in the upper soil litter are often pigmented, have long antennas, large furca and visible eyes, soil living species are smaller (< 0.5 mm), and have shorter extremities and lack eyes or have small eyespots. They are often white or grey in color. The general distribution of species in Norway has been extensively studied by Arne Fjellberg (Fjellberg 1998; Fjellberg 2007). Detailed studies of this group of animals have not been carried out in arable soil in Norway, and the knowledge about collembolans in arable soil is generally quite restricted.

Methods

Collembolans were sampled from the same 12 grass system plots that were used for earthworm studies; the control treatment (N) and treatments with high amounts of digested (DH) or undigested (UH) slurry. The sampling occurred on April 28 2011, before manure application (on May 4). The aim of this start characterization was to achieve an overview of the collembolan fauna on the experimental site.

Figure 7.8. Sampling and extraction of collembolans. From left: Soil and sward is sampled by means of a metal cylinder that is tapped into the ground and excavated. The sample is carefully placed, upside down, on a nylon net which allows collembolans to pass through, and placed under a lamp which forces the animals to redraw through the soil and further through the net. Animals are caught via a funnel in a bottle below the lamp. The bottle contains 80 % ethanol to conserve the animals.

We used steel cylinders (pF-rings , Ø = 5.8 cm, h = 3.8 cm) to take out the soil and sward samples. From each experimental plot, one cylinder was taken (Fig. 7.8). The cylinder was placed on the right side of the small pits (20 x 20 x 20 cm) where the soil cubes for earthworm studies had been excavated, with a distance of 5 cm between the pit and the left side of the cylinder. The weather conditions on the sampling date were satisfactory,

with sunny weather and a temperature of 8°C. The cylinders were gently tapped with a rubber hammer into the soil. Immediately after, a plastic lid was placed on top of the cylinder to prevent the animals from escaping. The soil in front of the cylinder was cut with a knife to be able to put a flat brick layer spade under it, and excavate the cylinder with soil and sward. Excess soil and roots were cut off to make the soil sample fit inside the cylinder. Thereafter a plastic lid was put over the bottom of the sample.

The soil fauna was set to extraction within one hour after sampling. A variant of Berlese funnels method vas used (Klingen et al. 2008), where desiccation of the soil under a light bulb (40 W) forces the fauna to move away and become trapped. The samples were placed with the sward down on a fine meshed nylon net (mesh size 0.8 mm x 0.8 mm), which was placed on a metal sieve sized 3 mm x 3 mm that was attached to a funnel (Fig. 7.8). The net prevented the soil to fall into the bottle below. The funnel size was in the top Ø =25 cm and in the bottom Ø = 2.6 cm. Funnels were placed in a rack of metal rings; Ø = 23 cm, h= 11.5 cm. The tip of each funnel was inserted through a hole in the lid of a plastic cup containing 80 % ethanol which had to be refilled due to evaporation. The extraction period was one week (7 days) with continuous light.

A micro-sieve was made to sieve out the collembolans from the alcohol solution. A piece of nylon net with mesh size 45 micrometer was clued between to small plastic tubes . The collembolans were stored in 80 % alcohol in small glasses with plastic lids. All sampled material was boiled for 10-20 seconds to dissolve their external wax layer, to get a better preservation. The collembolans were identified by observations in both binocular and microscope. For most species, it was required to make them transparent by putting a droplet of a lactic acid - glycerol mixture (3/1) over the animal on a microscope slide and heat it carefully until transparency was achieved. Then a cover glass was gently placed on the top.

For most individuals, it was possible to identify them to species, using the keys of Arne Fjellberg (Fjellberg 1998; Fjellberg 2007). Some individuals were only identified to genus. For 8 individuals classified as *Isotomurus italicus*, the identification was somewhat uncertain and hence marked by "?" (Fig. 7.9). Only one individual could not be classified at all, and is listed as "indet" (indetermined).

The soil in the cylinders was weighed before and after the soil fauna extraction. Thereafter, the soil samples were dried at 105 ºC until constant weight was achieved, and the original water content was calculated.

The sample volume of each cylinder was 100.348 cm^3, or about 0.1 dm^3. The volume of a 1 m^2 soil layer with 3.8 cm depth is 38 000 cm^2. Hence, the numbers of collembolans per m^2 may be calculated as the number in the cylinder times (38 000/100) = 380.To test if larger samples could have extracted more animals, we excavated four larger samples, two from each side of the grass system plots. We used plastic cylinders Ø = 10 cm, h = 6.5 cm with a sampling height of 5 cm, soil volume sampled = 392.5 cm^3. Such large samples did not fit well to the available funnel system. The soil did not dry out, and the surface molded. Some collembolans were extracted, but not as many as would have been expected from the size of the sample as compared to the 0.1 dm^3 cylinder samples.

Results

The average water content in the soil at the sampling date varied from 33.1 to 48.8 %, with a mean value of 39.8 % (weight %). After extraction of the collembolans, the soil was further dried at 105 ºC until constant weight was achieved. It then varied from 1.9 to 3.9 %, with a mean value of 2.3 %. This means that the extraction in the funnel system almost

dried the soil completely, and indicates that the sample size was well adapted to the design of the funnel system.

In total, 251 individuals of collembolans were found and identified, in cooperation with Arne Fjellberg. Altogether, 17 species were identified (Fig. 7.9). The material comprised a large variation in colors and body forms.

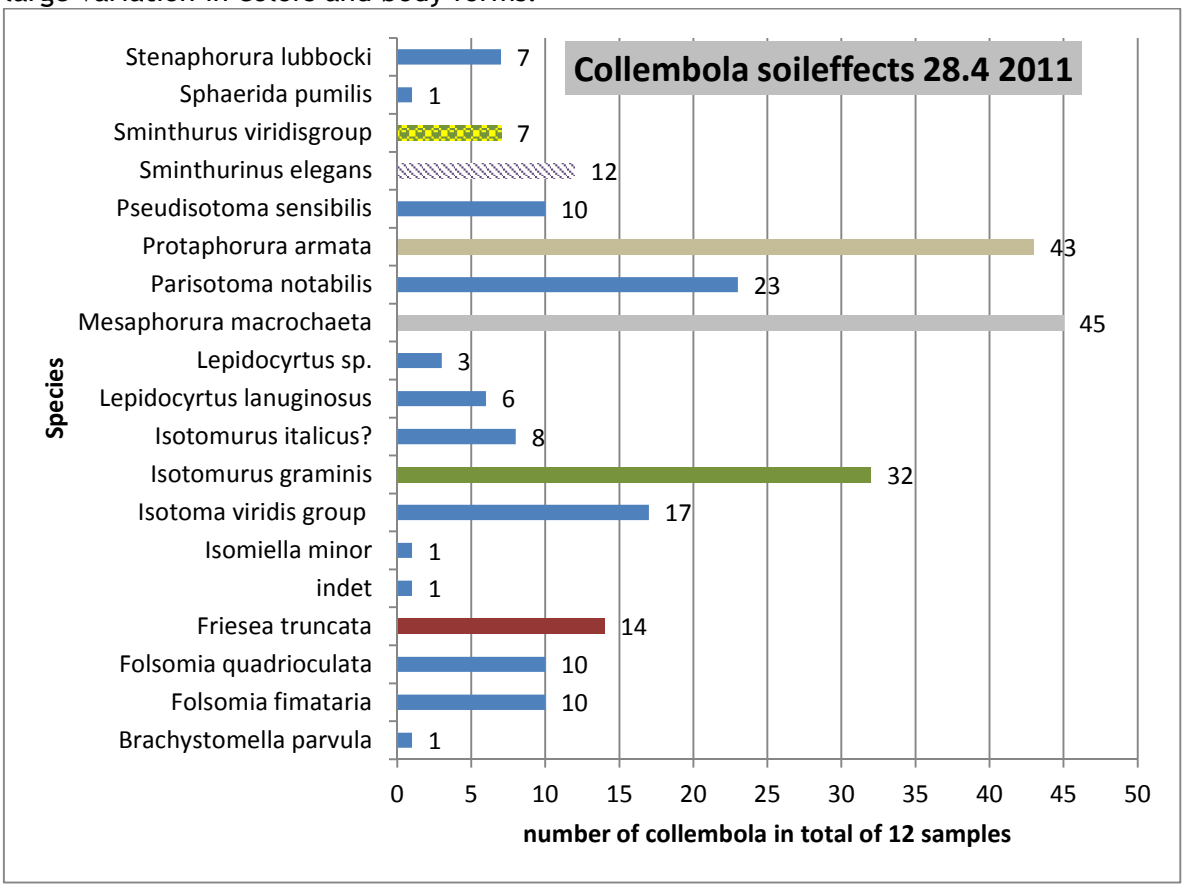

Figure 7.9. Total catch of collembolans from the grass system of the SoilEffects field experiment, distributed among 17 identified species. Sampling on April 28 2011, before manure application.

On average, 21 individuals were found in each cylinder sample, corresponding to 7980 individuals m^{-2}. The density varied between the blocks and between the placement of the planned treatments (Fig. 7.10).

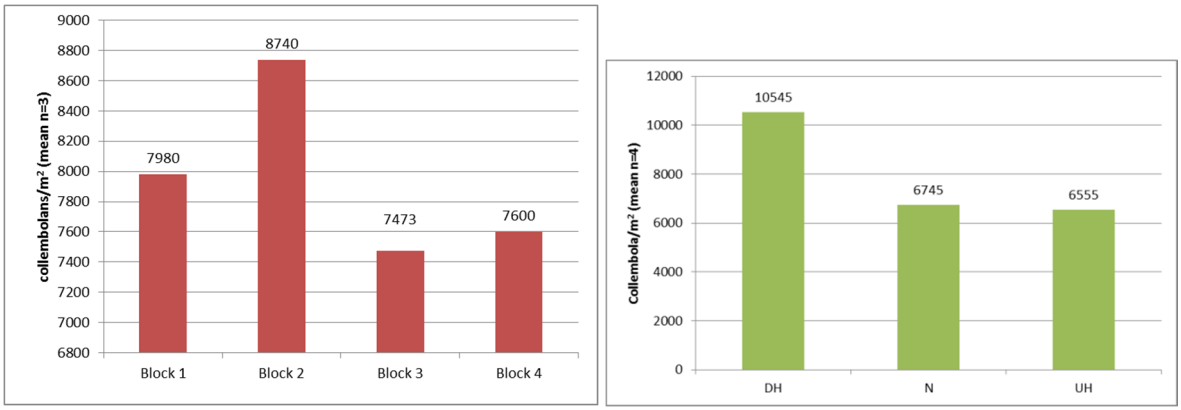

Figure 7.10. Frequency of collembolans per block (n=3) and treatment (N, UH, DH, n=4).

We found species belonging to both groups, *Entomobryomorpha* (elongated shape) and *Symphyleona* (rounded shape). Identification was easiest for the species with most evident

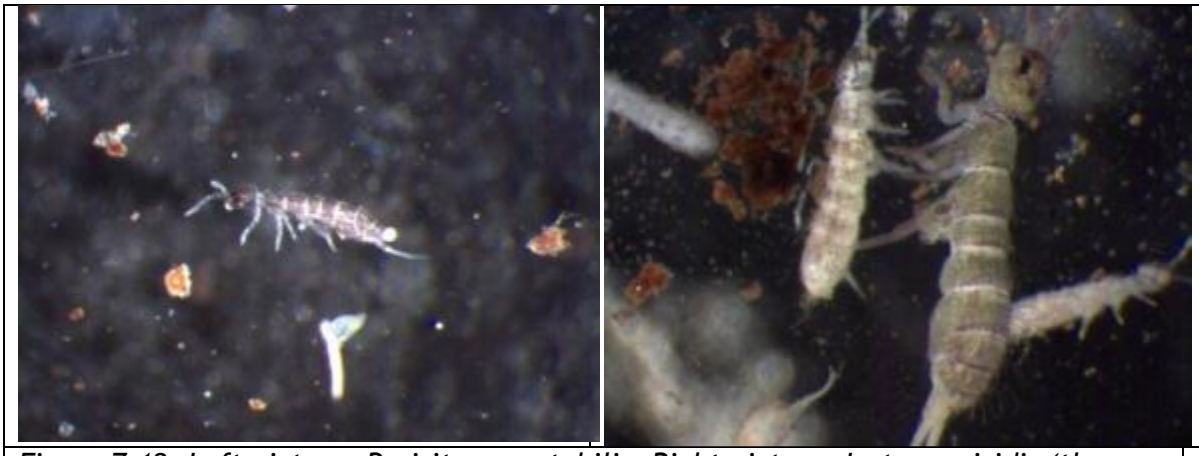

Figure 7.12. Left picture: Parisitoma notabilis. Right picture: Isotoma viridis (the large one). Photo: Reidun Pommeresche.

characteristics, and the further presentation follows the "road of learning" of collembolan taxonomy. Within the elongated group, a numerous species was *Isotomurus graminis*, with a greenish color, medium long antenna and a solid furca (Fig. 7.11). *Parisotoma notabilis* was also quite common (Figs. 7.11, 7.12). This species is small (1 mm) with a characteristic dot as eyespot, black/grey pigmentation and a curly furca. *Isotoma viridis* (Figs. 7.11, 7.12) is also greenish, but with purple edges of each segment. In this species, the body hairs are better preserved in alcohol than mostly found for *I. graminis*. *I. caerulea* is almost identical to *I. viridis*, but most likely these species were *I. viridis* since this species is common in this part of Norway.

Figure 7.11.Left picture: Isotomurus graminis (green and large), Isotoma viridis (bright purple, upper left), Pseudisotoma sensibilis (dark purple, upper right), Parisotoma notabilis (small, just below the I. graminis). To the right, an unidentified mite. Right picture: Several individuals of I. graminis made transparent by lactic acid-glyserol mixture. Photos: Reidun Pommeresche.

Among the smaller, white soil living species in the elongated group, *Protaphorura armata* and *Mesaphorura macrochaeta* (Fig. 7.13) were the most numerous species. A few *Stenaphorura lubbocki* were found. «Aphorura» means «without tail». All these species have reduced furca and thorns on the back.

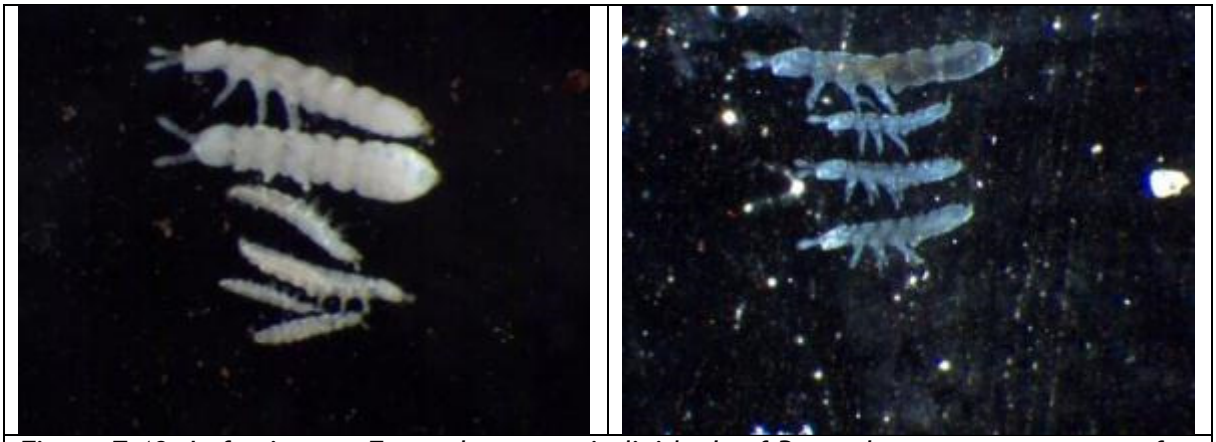

Figure 7.13. Left picture: From above two individuals of Protaphorura armata, two of Stenaphorura lubbocki and two of Mesaphorura macrochaeta. Right picture: Protaphorura armata made transparent in lactic acid-glycerol mixture. Pictures by Reidun Pommeresche.

In the rounded shape group, many species are found in the vegetation and the upper parts of the soil. We found some individuals of *Sminthurus viridis* (Fig. 7.14). This species is large and green when adult. *Disurtoma minuta* is a chacteristic species with yellow colour, black dot and a short 4th segment of the antenna (Fig. 7.14). This species was not found in the grass plots, but in the field border vegetation, in June 2011.

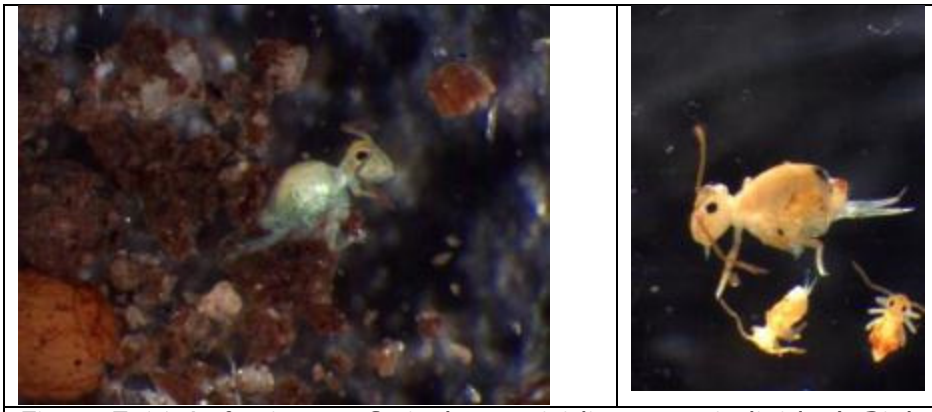

Figure 7.14. Left picture: Sminthurus viridis, young individual. Right picture: Disurtoma minuta. Pictures by Reidun Pommeresche.

The distribution of species varied between blocks, with different species dominating in different blocks (Fig. 7.15, Fig. 7.16). *Mesaphorura macrochaeta* dominated in block 4, and *Protophorura armata* in block 1. The distribution of species between treatments was more even, and 11 out of 17 species were represented in all treatments.

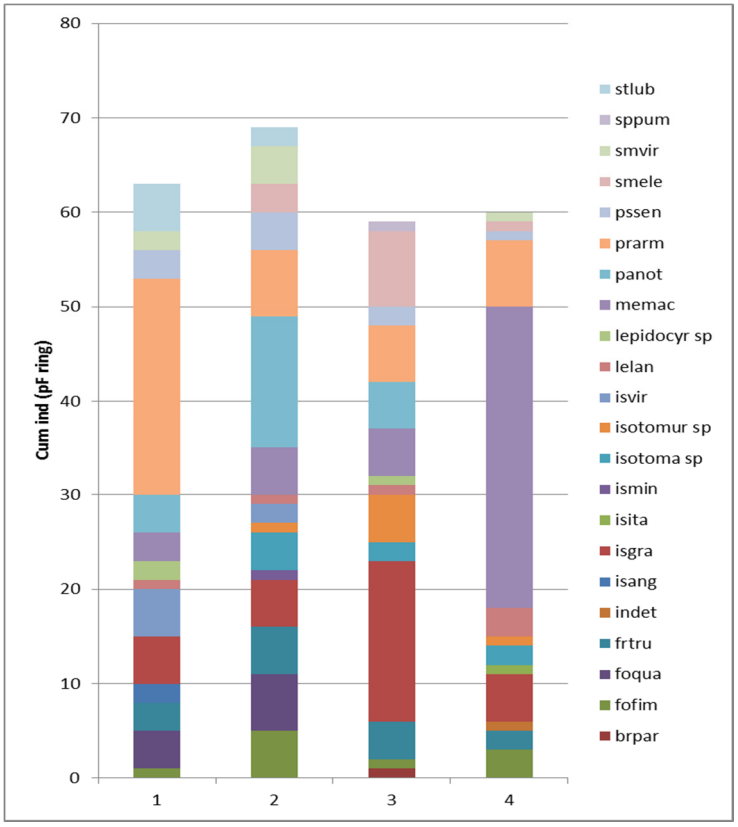

Figure 7.15 Distribution of collembolan species across blocks (1-4). Species names are abbreviated as the two first letters in the genus and the three first letters in the species name. Full names are shown in Figure 7.9.

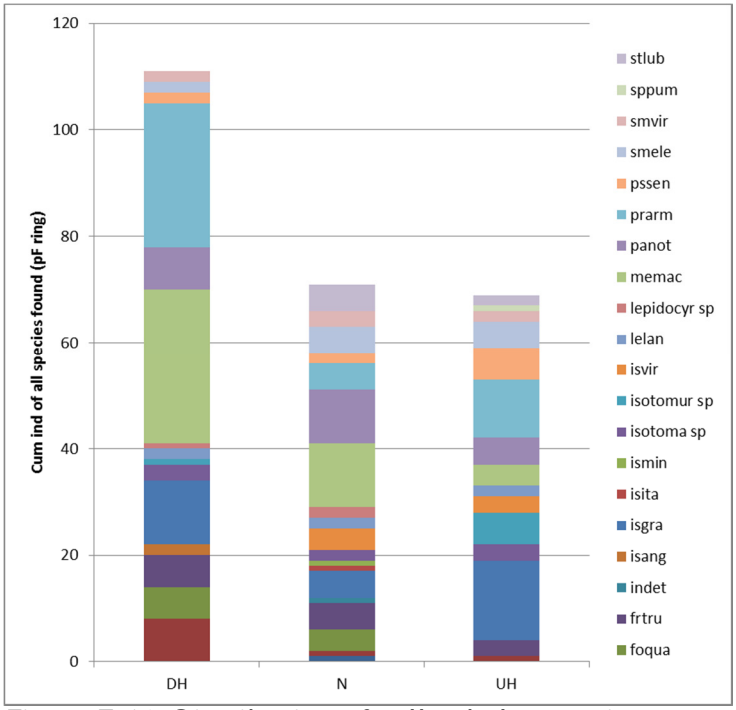

Figure 7.16. Distribution of collembolan species across treatments (N, DH, UH). Species names are abbreviated as the two first letters in the genus and the three first letters in the species name. Full names are shown in Figure 7.9.

7.4 Soil fauna, summary and conclusions

Five earthworm species have been identified in the field experiment. *Apporrectodea caliginosa* was the most common, but also *Lumbricus terrestris* was abundant. *Octolasion cyaneum* was found mainly in the arable system. The average density was 133 earthworms m^{-2} in the grass system and 117 in the arable system. The average biomass was somewhat higher in the arable system (63.5 g m^{-2}) than in the grass system (42.1 g m^{-2}).

17 species of collembolans were found in the grass system, with an average density of and 7950 individuals m^{-2}. The variation in species composition and density was high, and larger between treatments than between blocks. 11 species were found in all treatments (N, DH,UH).

The most numerous collembolan species were the soil dwelling, white *Mesaphorura macrochaeta* and *Protaphorura armata*, and the litter dwelling greenish *Isotomurus graminis*.

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