Nitrogen dynamics in an organic green manure - cereal rotation and mineralization of clover leaves at low temperature

Nitrogendynamikk ved bruk av grøngjødsel i økologisk korndyrking og mineralisering av kløverblad ved låg temperatur

Philosophiae Doctor (PhD) Thesis

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Preface

The work presented in this thesis focuses on the nitrogen dynamics within an organic green manure - cereal rotation and mineralization of clover leaves at low temperature. The work was a part of the project “Improving barley yields in organic stockless farming systems through innovations in green manure management – BYGGRO”, funded by The Research Council of Norway (Project No.184970).

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Tingvoll 2015

Randi Berland Frøseth

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List of papers

Paper I:

Frøseth R B, Bakken A K, Bleken M A, Riley H, Pommeresche R, Thorup-Kristensen K, Hansen S. 2014. Effects of green manure herbage management and its digestate from biogas production on barley yield, N recovery, soil structure and earthworm populations. European Journal of Agronomy. 56: 90–102

Paper II:

Frøseth R B, Bleken M A. 2015. Effect of low temperature and soil type on the decomposition rate of soil organic carbon and clover leaves, and related priming effect. Soil Biology and Biochemistry. 80: 156–166

Paper III:

Frøseth R B, Thorup-Kristensen K, Hansen S, Bleken M A. Mineralization of clover leaves at low temperatures in contrasting soil types. Manuscript.

Summary

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In northern temperate regions, grass-clover green manure leys are commonly used as a break crop in stockless organic cereal rotations in order to provide soil fertility and reduce the pressure of weeds, pests and diseases, thereby enhancing the yield of subsequent crops. The green manure leys are mown repeatedly and the herbage is left as mulch. The mulched herbage contains a substantial amount of nitrogen (N), which is at risk of being lost when the herbage decomposes in the field. Such losses reduce the fertilizer value of the mulch and have negative environmental impacts.

The overall aim of this work was to increase our knowledge about the possibilities to improve the N recovery from green manure leys on contrasting soil types under cold climate conditions. A field experiment was run at four different locations in the eastern and central parts of Norway, in order to evaluate the effect of various green manure herbage strategies on the yield and N recovery of a subsequent spring barley crop, and their short-term effects on soil structure and earthworm populations. The strategies involved different options for on-site herbage management and the application of anaerobically digested green manure herbage. Two of the soils, a silty clay loam and a sandy loam, were also used in an incubation experiment in order to study the effects of low temperature (0–15 °C) and soil type on carbon (C) and N mineralization from soil organic matter and clover leaves. The data from this experiment were further used to investigate whether it was possible to improve the ability of a soil-crop model to simulate the N mineralization of clover leaves at low temperatures.

The C mineralization rates of clover leaves and soil organic matter were found to be higher in the sandy soil than in the clay soil, but the sensitivity of the rates to temperature was not affected by soil type. The mineralization rate of clover was somewhat less temperature sensitive than that of soil organic matter. A positive priming effect occurred after incorporation of clover leaves, even at 0 °C. The priming effect correlated with clover leaf decomposition. For modelling purposes, parameterized equations are presented for the mineralization rates of soil organic matter and clover leaves as function of soil temperature.

Relatively little of the total plant N was mineralized either in the field or in the laboratory experiment, yet conspicuous net N mineralization was observed below 5 °C in both investigations. In the incubation experiment, rapid and substantial net N mineralization from the clover leaves took place over the first few days, and this was unaffected by temperature. This rapid mineralization was followed by a phase of slow net N mineralization in the sandy loam and net N immobilization in the silty clay. Immobilization was greater at higher than at lower temperatures, and also the ratio of inorganic N to mineralized C was higher at low temperatures than at high temperatures during the first weeks of decomposition. This shows that N mineralization is not simply a function of C mineralization. The model was unable to mimic the N dynamics in the soil during the six weeks after clover incorporation, although model improvements were attempted by raising the decomposition rate of the fast decomposing litter pool at low temperature. More knowledge of how low temperature affects soil biology, and especially microbial growth, is needed for improving prediction of the net N mineralization of newly incorporated N-rich plant material.

The field experiment showed that removal of the herbage reduced the barley grain yield. Removal of the herbage without the use of any fertilizer application to the subsequent crop is therefore not recommended, unless the soil is very fertile. Compared with mulching and removal of the herbage, the digestate strategy turned out to be the most promising option with regard to reduced risk of N losses and improved N recovery by a subsequent spring barley crop. Digestate also improved the soil aggregate stability. The earthworm population decreased after herbage removal, but the digestate application had no effect. At the farm scale, by applying only part of the N in the removed herbage to the area from which it was removed, excess digestate remains available for use as fertilizer to crops on other fields. However, cost-efficient and practical solutions are needed for running small herbage-based biogas plants under cold climate conditions.

Samandrag

Frøseth, R B. 2016. Nitrogendynamikk ved bruk av grøngjødsel i økologisk korndyrking og mineralisering av kløverblad ved låg temperatur. Norges miljø- og biovitenskapelige universitet, Philosophiae Doctor (PhD) avhandling 2016:25, ISSN: 1894-6402, ISBN: 978-82-575-1335-1.

Grøngjødsel vert brukt i vekstskiftet i husdyrlause økologiske dyrkingssystem for å betre jordfruktbarheita og førebyggje problem med ugras, skadedyr og sjukdomar, og vil såleis bidra til større avling i etterfølgjande vekstar. Heilårs grøngjødsel under nordiske klimatilhøve er ofte ei eittårig eng samansett av kløver og gras. Enga vert slått fleire gongar og grønmassen vert liggjande på stubben for å rotne. Grønmassen inneheld ei stor mengd nitrogen som kan tapast til luft og vatn når plantematerialet vert brote ned ute på marka. Tapa representerer reduksjon i gjødselverdien av grønmassen og har negative konsekvensar for miljøet.

Hovudmålet med dette arbeidet var å auke kunnskapen om moglegheiter for å betre nitrogenutnyttinga frå grøngjødseleng i ulike jordtypar under kalde klimatilhøve. For å evaluere effektar av ulik grøngjødselhandtering vart det gjennomført eit feltforsøk på to stadar i Trøndelag og på to stadar på Austlandet. Etter kvar slått vart grønmassen liggjande, fjerna eller fermentert i biogassreaktor og delvis tilbakeført som gjødsel til bygg neste vår. Effektar av ulik grøngjødselhandtering på nitrogenutnytting og kornavling i bygg, samt effektar på jordstruktur og meitemark vart registrert.

Jord frå to av felta, siltig mellomleire og siltig sand, vart også nytta i eit laboratorieforsøk for å studere effekten av låg temperatur (0-15 °C) og jordtype på karbon- og nitrogenmineralisering frå jordas organiske materiale og frå kløverblad. Data frå dette forsøket vart vidare brukt for å undersøkje om det var mogleg å betre modellsimuleringa av nitrogenmineralisering frå kløverblad i jord ved låge temperaturar.

Karbonmineraliseringa frå kløverblada og det organiske materialet i jorda var raskare i sandjorda enn i leirjorda, men temperaturresponsen var lik for dei to jordtypane. Mineraliseringa frå kløverblada var litt mindre hemma av låge temperaturar enn mineraliseringa frå det organiske materialet i jorda. Som eit resultat, til bruk i modellar, er det presentert parametriserte likningar for mineralisering av organisk materiale i jord og kløverblad som funksjon av jordtemperatur. Ei ekstra frigjering av karbon, som ikkje kom

frå plantemateriale, vart funne etter innblanding av kløverblada. Denne såkalla primingeffekten vart funne sjølv ved 0 °C, og viste samanheng med nedbrytinga av kløverblada.

Ei relativt lita mengd av det totale nitrogeninnhaldet i plantematerialet vart mineralisert i forsøka, men netto mineralisering av nitrogen vart likevel funne ved temperaturar under 5 °C. Laboratorieforsøket viste rask, temperaturuavhengig og betydeleg netto nitrogenmineralisering frå kløver dei første dagane. Deretter kom ein fase med lite netto mineralisering i sandjorda og netto immobilisering i leirjorda. Immobiliseringa var større ved høge enn ved låge temperaturar, og høvet mellom mineralisert nitrogen og karbon var større ved låge enn ved høge temperaturar. Dette viser at nitrogenmineralisering ikkje er berre ein funksjon av karbonmineralisering. Den valde vekstskiftemodellen kunne ikkje simulere nitrogendynamikken som fann stad i løpet av dei seks første vekene etter innblanding av plantematerialet i jorda. Dette til trass for det vart prøvd å betre simuleringa ved å auke nedbrytningsfarten ved låg temperatur til den raskt nedbrytbare delen av det tilførte plantematerialet. Meir kunnskap om korleis låg temperatur påverkar jordbiologien, og spesielt mikrobiell vekst, trengst for å betre modellering av netto nitrogenmineralisering frå nyleg innblanda nitrogenrikt plantemateriale.

Feltforsøket viste at fjerning av grønmassen reduserte byggavlinga. Fjerning av grønmassen utan å gjødsle til neste års vekst vert derfor ikkje tilrådd, med mindre jorda er svært fruktbar. Samanlikna med å la grønmassen liggje eller å fjerne han, er strategien med biorest det mest lovande alternativet med omsyn til redusert risiko for nitrogentap og betra nitrogenutnyttig av ein etterfølgjande byggkultur. Biorest betra også aggregatstabiliteten i jorda. Meitemarkpopulasjonen vart redusert då den liggjande grønmassen vart fjerna, men ingen effekt vart funne av biorest. Dersom ein vel strategien med fjerne grønmassen for biogassproduksjon og tilbakeføre bioresten som gjødsel, vil ein ha nok biorest til å kunne gjødsle andre areal på garden også. For å kunne setje dette ut i praksis på den einskilde gard trengs det kostnadseffektive og praktiske løysingar for plantebaserte biogassanlegg under kalde klimatilhøve.

Introduction

Background

Organic farming in Norway

Agricultural policy in Norway over the last sixty years has resulted in regions dominated by either arable or livestock production. The main regions for cereal production are in eastern and central Norway, covering in total 30 % of the 1 million hectare agricultural area in the country (SSB 2012).

Organic farming in Norway amounts at present to ca 5 % of the total agricultural area. The aim of Government is to achieve 15 % organic production and consumption by 2020 (Meld. St. 9 2011). Up to now, the main conversion to organic farming has been in livestock production, especially dairy and cattle farming (SSB 2012). At the same time, organic dairy milk production has been intensified by increased use of concentrates (TINE Rådgivning 2014). Since there is no mandatory requirement to be self-sufficient with all feed on the organic farms, farmers give priority to roughage production and purchase the concentrates. Despite the evident demand for organic cereals, the domestic organic cereal production, which in 2014 was about 6600 hectares or 14 % of the organically farmed land, far from meets this demand (Debio 2015). Cereals for direct human consumption amount to only 8 % of the total organic cereal production (Landbruksdirektoratet 2015).

Organic cereal production

Most of the organic cereal production in Norway takes place on stockless farms after several decades of conventional cereal monocropping. Although the climate, machinery park, and infrastructure in these areas favours cereal cropping, there are some agronomic constraints when converting to organic cropping. During the years of arable cropping, the soil structure quality and the amount of soil organic matter have declined (Riley and Bakkegard 2006; Riley et al. 2008). Aggregate stability, which is an indicator of soil structure, is mediated by soil organic carbon (C), soil biota, ionic bridging, clay and carbonates (Bronick and Lal 2005). Decline in soil structure has impact at both local and global scales, as it reduces water infiltration, soil cation exchange capacity and CO_2 sequestration inof the soil, and thereby enhances the risk of soil erosion, pollution of water and the CO_2 emission from cultivated soil. Good soil structure and high organic matter provides good rooting conditions and habitat for soil organisms, which are the drivers of nutrient turnover. This is even more essential in organic farming, since there is no possibility to compensate with inorganic fertilizers. According to the rules for organic farming, the use of conventional animal manure is allowed (with restrictions on amount and type), but this is not an option when the access to animal manure in restricted. There are some organic fertilizer products in trade, but the arable organic cropping systems rely mainly on biological nitrogen (N) fixing plants. Due to the high temperature requirements (for growth and ripening) of legume cash crops like beans and peas, their cropping is climatically restricted to the southern part of the cereal cropping region, and the N-fixing crops in use are non-cash crops such as clover. Therefore, the aim in practice is to produce as much cereals in the crop rotation as possible without reducing the stability of the crop rotation; i.e. to provide enough N, secure good soil structure and avoid problems with weeds, plant diseases and pest infestation. Sub-optimal nutrient supply is the main constraint for increasing the organic cereal production without access to animal manure.

Spring barley

Spring barley is by far the most common cereal crop in Norway, and is mainly used in the concentrate feed industry. The cultivars in use are well adapted to the climate, with low temperature requirement and short growth cycle. However, under nutrient limited conditions, which occur in organic arable farming, barley performs less well than the other cereals, and farmers often prefer to grow oats rather than barley in stockless farming systems (Abrahamsen 2006). Consequently, the demand for organic barley is higher than the domestic supply, and a relatively larger proportion has to be imported for the organic concentrates than for conventional concentrates (Adler and Løes 2014).

The amount and timing of N supply to the cereal crop strongly affects the grain yield. The development of leaf area for dry matter production is related to N uptake, and the sink capacity in grain depends upon good nutrient supply during the initiation of the florets (Hay and Walker 1989). The period for the development of leaf area in spring barley is shorter than that in the other cereals. This is especially challenging when relying on organic nutrient sources (Hauggaard-Nielsen et al. 1998). In addition, barley is very sensitive to poor soil structure (Martino and Shaykewich 1994; Arvidsson 1999).

Green manure

Green manure crops are grown primarily for improving soil fertility and thereby provide a nutrient source for the subsequent crops in organic arable farming systems. There are several additional benefits of green manure as a break crop in an arable crop rotation, e.g.

improved soil structure, enhanced soil organic matter content, more earthworms, suppression of weeds, pests and diseases (Cherr et al. 2006; Riley et al. 2008; Thomsen 2011). Green manure crops with deep roots may be grown specifically to loosen the soil or to bring nutrient resources up from deeper soil layers, to make them available to shallower-rooted crops (Thorup-Kristensen 2006). The choice of species and management depends on the purpose, climatic conditions and cost.

Legumes are commonly grown as green manure crops, either singly or in mixture with other species, due to their ability to supply the farming system with N through biological N fixation. Mixtures have a yield advantage over pure stands because of mutual grass-legume interactions which stimulate N acquisition of the legumes and the grasses, as well as of the efficiency of transforming acquired N into biomass (Nyfeler et al. 2011). In organic cereal production in Norway, green manures are commonly grass-clover leys. The leys are usually established in spring as an undercrop in cereals. This ensures good establishment, and the ley can act as a cover crop between the growing seasons. The following season, the ley is often repeatedly mown, chopped and mulched on the stubble, and ploughed under in late autumn or the next spring, before a new cereal crop is sown. The green manure may also be sown in spring in the green manuring year, which allows use of annual species and varieties. Studies have shown that when a mulched grass-clover ley is the only N source, more than 25 % of crop rotation has, under Norwegian conditions, to be leguminous green manure in order to balance N input and N off-take at harvest (Løes et al. 2011; Korsaeth 2012).

Herbage management

Mowing is done to control weeds and to keep the crop in a vegetative state and thus sustain high N_2 fixing activity in the legumes (Dahlin and Stenberg 2010). The mulching is intended to be a nutrient source for the ley regrowth, enhancing microbial activity in the soil and thereby contributing to the following crop`s nutrient supply. However, when the green manure herbage was removed, Hatch et al. (2007) found increased N fixation. On the other hand, Dahlin and Stenberg (2010) found no such effect on N fixation. Neither one of these studies included the effect of mulch removal on subsequent crops. Several Scandinavian field trials have shown that on-site mulching contributes only slightly to the nutrient demand of the following crop (Solberg 1995; Engström et al. 2007; Frøseth et al. 2008). This suggests that N from herbage is either lost from the cropping system or incorporated in a more recalcitrant form in soil organic matter, and removal of the herbage may therefore have little effect on the subsequent crop.

The chopped plant material can also be moved to another field and used as surface mulch, in for example vegetable production. Under Scandinavian conditions, Riley et al. (2003) found that the apparent N recovery from such material in the vegetables was only 13%, while Båth et al. (2006) found 25–28% N recovery. This method is, however, only suitable in crops that have a long period of nutrient uptake, such as brassicae. For cereals, it is too late to delay mulching until the first cut of the green manure ley.

Alternatively to the common practice, the nutrients in the herbage can be redistributed later by removing the herbage and treating it in a way that conserves them, e.g. by ensiling, drying, composting or anaerobic digestion in a biogas plant, followed by incorporation into the soil before the next crop. The time span for the release of plant-available N is affected by the conservation method (Carter et al. 2014). Of the various methods, only the anaerobic digestion in a biogas plant increases the amount of plant available N. In biogas plants, the easily degradable organic matter is digested and methane for heating or fuel is released together with residues (digestate) that can be used as fertilizer. This strategy seems therefore to be the most promising option for improving cereal yields and N recovery (Möller and Müller 2012; Stinner et al. 2008), and also from an energy self-reliance perspective (Halberg et al. 2008). This strategy has not previously been compared with different green manure herbage treatments under Nordic conditions. However, compared to mulching and the other conservation strategies, the higher concentration of ammonium in digestate may be toxic to earthworms (Curry 1976) and a lower content of easily degradable C may affect the soil biota negatively due to substrate shortage.

Potential nutrient losses from green manure

Agriculture relies on biological processes that may also have negative environmental impacts at both the local and the global scale. The atmospheric concentration of the greenhouse gas CO_2 is increasing. The soil may function as a sink or a source for atmospheric CO_2, depending upon management. Use of legumes in the cropping systems contributes to soil C sequestration (Jensen et al. 2012). However, a grass-clover green manure ley may increase N gaseous emissions, surface runoff and leaching of nitrate, soluble organic N or other nutrients, such losses reduce its fertilizer value and have negative impacts on the environment. It is of particular interest to improve the N recovery from green manure so that the amount of plant available N and the timing of the delivery of N from plant residues conforms with the demand of the subsequent crop. The progress and the level of the N losses are strongly influenced by the weather conditions, but also by the soil and crop management. Removing the herbage from the field and conserving it through the winter reduces the amount of N that can be potentially lost from the field, but N loss may occur during storage and application (Möller and Stinner 2009; Möller 2015).

Nitrate-N originating from the breakdown and further mineralization of green manure may be lost from the cropping system if it is leached beyond the root zone. The risk of leaching is especially high outside the growing season. The nitrate ions, are not adsorbed by the negatively charged colloids. If not taken up by the roots, nitrate moves downward with the drainage water to the ground-water or waterways where it may contaminate drinking water or cause eutrophication. The leaching loss depends mainly on the amount of precipitation and the amount of nitrate in the soil, but it is modified by type of crop, duration of crop cover, soil type, soil structure and drainage conditions. In general, sandy soils are more prone to leaching than clay soils. Use of catch crops are an example of measures that reduces N leaching (i.e. Askegaard and Eriksen 2008). A green manure ley may act as a catch crop, but Askegaard et al. (2005) recorded particularly high nitrate leaching losses from organic rotations with green manure on coarse sandy soil. For reducing the risk of N leaching from green manure, spring or late autumn incorporation on heavy clay soils is preferable (Känkänen 1998).

Heavy rainfall and pulses of meltwater in winter and spring may also cause nutrient loss by surface runoff and erosion. In the northern temperate climate, Sturite et al. (2007) found substantial losses of N (6–68 %) and phosphorus (11–60 %) from above-ground perennial crops during winter. The losses varied greatly from year to year.

N may also be lost to the atmosphere as ammonia gas produced in the soil-plant system. Typical sources are fertilizers, animal manures, digestate, decomposing plant material and living plants. The volatilization of ammonia from the foliage of growing plants is generally less than 2 kg ha^{-1} and year, whereas emissions from decomposing mulched plant material can be substantial. Whitehead and Lockyer (1989) found that decomposing grass herbage placed on the stubble of a cut sward containing 3% N, lost 10% of its N as ammonia during 28 days with showery weather. Emission levels twice as high as this, or even up to 39%, have also been found from grass with lower N content (Larsson et al. 1998; Whitehead et al. 1988). The ammonia emission from decomposing plant material is affected by air temperature, N-concentration in the herbage and precipitation amount, with the latter being of most importance (Whitehead et al. 1988).

The main sources for soil born nitrous oxide (N_2O) emissions are nitrification of ammonium under aerobic conditions and denitrification under anaerobic conditions. N_2O is a potent greenhouse gas with a specific global warming potential about 300 times that of CO_2, and is also the dominant ozone-depleting substance (Ravishankara et al. 2009). The presence of organic N sources in soil under anaerobic conditions with high temperature favours denitrification (Paul and Clark 1989). The pH influences whether the output of denitrification is N_2 (higher pH) or N_2O (lower pH). This is because low pH inhibits the enzyme nitrous oxide reductase. Fertilization, mulching and decomposition of green manure ley are likely to cause emissions of N_2O. Larsson et al. (1998) found the N_2O emissions during three months from N-rich herbage mulch on bare soil to be 1 % of the applied N. Further, it is common to estimate that 1 % of the N input and the ammonia volatilization turn to N_2O (IPCC 2006).

Decomposition and mineralization of plant material and soil organic matter

The decomposition of organic matter in soil takes place through leaching of soluble materials, fragmentation by soil animals and chemical alteration by microorganisms. The factors controlling decomposition in soil are the physical environment (temperature, moisture and soil properties), the substrate (quality and quantity) and the microbial community composition. Mineralization of organic matter to inorganic components (e.g. CO_2 and NH_4) and transformation of organic matter into recalcitrant organic compounds are products of decomposition. When organic matter decomposes, the most labile compounds decompose before the more recalcitrant compounds, thus the decay rate declines over time. The C/N ratio is found to be the most important factor determining the mineralization from fresh plant material (Thorup-Kristensen 1994; Marstorp and Kirchmann 1991). The decomposition is mainly a consequence of the energy and N requirements of the decomposer organisms, fungi, bacteria and soil micro fauna. The hyphal network of the fungi enable them to grow into substrates, whereas bacteria depend more on transport processes in the soil by for example water movement. Bacteria produce exo-enzymes that must reach the substrate, and the product of decomposition must diffuse ‘back’ to the bacteria. Thus, moisture content influences the decomposition, and indirectly also by influencing the oxygen content. Further, decomposition occurs more rapidly in neutral soils than acid soils, which are more dominated by fungi. Different soil types provide contrasting environments for decomposition of organic matter. Mineral particles and soil aggregates act as chemical and physical protection of organic substrates and microorganisms, and clay soils show slower decomposition rates of both soil organic matter and added readily decomposable substrate than do sandy or silty soils (Van Veen et al. 1985; Saggar et al. 1996). There is little knowledge about the effect of soil type on the N dynamics of decomposing N-rich plant residue, but it might be expected that N mineralization, similar to C mineralization, is affected by soil type. However, Müller (1988) found that soil type varying from sandy loam to clay only slightly affected the release of clover N from mesh bags during Finnish winter conditions.

According to kinetic theory, the decomposition of substrate with high molecular complexity, i.e. soil organic matter, is more sensitive to temperature than is substrate with low molecular complexity, i.e. fresh plant material (Davidson and Janssens 2006). In general, decomposition is found to be more affected by temperature in the lower temperature range (<10 ºC) than at higher temperatures (Kirschbaum 1995; Kätterer et al. 1998). The adsorption of soil organic C to mineral particles is expected to increase the effect of temperature on the decay rate, since higher activation energy is needed to break the bonds between the organic matter and the mineral particles (Conant et al. 2011).

N mineralization is closely linked to C mineralization, but at low temperature, the ratio of these processes has been found to be altered; C mineralization is depressed more than net N mineralization (Kirschbaum 1995; Magid et al. 2001; Schütt et al. 2014). The suggested reason is that gross microbial growth (immobilization) is restricted more by low temperature than is gross N mineralization (Andersen and Jensen 2001; Magid et al. 2001).

The decomposition of soil organic matter can be influenced by the addition of readily decomposable plant residues and root exudates (e.g. Löhnis (1926) referred by Kuzyakov et al. 2000; De Graaff et al. 2010; Lukas et al. 2013). Kuzyakov et al. (2000) has defined the priming effect as short term increased or decreased turnover of soil organic matter caused by any soil treatment. An increased C mineralization may originate from an increased turnover of the soil organic matter (a real priming effect) or an increased turnover of the soil microbial biomass (an apparent priming effect) (Blagodatskaya and Kuzyakow 2008; Kuzyakow et al. 2010).

Predicting N dynamics in cropping systems

Models are developed as tools to predict effect of different agronomic measures in the complex agroecosystem. Models can be useful means of providing a better understanding of the N dynamics and of supporting decision-making at tactical and strategic levels. Field-scale soil-crop models for predicting N dynamics are composed of different modules, of which organic matter decomposition and mineralization, crop growth and water are the basic modules. Soil properties and weather data are input information, in addition to soil characteristics and crop management.

In general, models assume that the soil organic matter can be divided into a number of more and less decomposable pools and that their decomposition is proportional to the amount of substrate, i.e. that it follows first order kinetics (Manzoni and Proproato 2009). Thus the amount C of an initial pool C_0 remaining after time t can be expressed as

$$C = C_0 e^{-kt}$$

where k is the decay rate constant. Arrhenius equation is one of the most commonly used functions for describing the dependency of decomposition on temperature (Davidson and Janssens 2006):

$$k = A e^{-Ea/(RT)}$$

where A is the pre-exponential factor, E_a is the required activation energy, R is the universal gas constant and T is the temperature in Kelvin. For the purpose of modelling, the temperature sensitivity can be expressed as a temperature function $f(T)$ that modifies a decay rate k_{ref} at a reference temperature (Kutsch et al. 2009; Moyano et al. 2009; Davidson et al. 2012):

$$k = k_{ref}\ f(T)$$

Thus, the sensitivity to temperature can be seen as the change in decay rate relative to the decay rate at another temperature (Reichstein and Janssens 2009). The same modifying temperature function is often applied to all substrates in a soil (Hansen et al. 2002; Coleman and Jenkinson 1999; Jansson and Karlberg 2004). The most commonly used temperature factor is the Q_{10}, which expresses the relative change in k when temperature increases by 10 °C (Kirschbaum 2006):

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)}$$

Where k_2 and k_1 are respiration rates observed at temperatures T_2 and T_1. Kätterer et al. (1998) found Q_{10} of 2 to be adequate for organic matter decomposition in the temperature range 5–35°C. However, Q_{10} decreases with increasing temperature, and for the lower temperature range (<10 ºC) Q_{10} is found to be greater and almost 8 at 0 ºC (Kirschbaum 1995; Leifeld et al. 2005; Farrar et al. 2012). This is also predicted by the Arrhenius equation (e.g. Davidson and Janssens 2006), and complicates the use of Q_{10} as factor for modelling the decomposition of organic matter (Reichstein and Janssens 2009).

Objectives

The overall aim of the work was to increase the knowledge on the possibilities to improve the N recovery from green manure leys on contrasting soil types under cold climate conditions. Specific objectives within this scope were to:

i. evaluate the effects of various strategies for green manure management, including herbage-based digestate as fertilizer, on the yield and N recovery of a subsequent spring barley crop, and its short-term effects on soil structure and earthworm populations.
ii. estimate the effects that low temperature and soil type have on the decomposition of soil organic C and newly incorporated clover leaves, and thereby improve the temperature response function used in models of the C mineralization of such substrates.
iii. acquire empirical data to improve model simulation of N mineralization of N-rich plant residue at low temperatures in soil types representative for Norwegian cereal growing.

An original objective was also to track the N leaching from the different green manure measures by using the EU-Rotate_N simulation model. It turned out that the chosen model could not be adapted to reproduce the observed N mineralization without fundamental changes in the model, beyond the scope of this thesis. The plans were therefore modified to concentrate on studying the C mineralization because of its near relationship with N mineralization.

Materials and methods

A field experiment was run for three years at four different locations with contrasting soil types, to evaluate the effect of selected alternative green manure herbage strategies on the yield and N recovery of subsequent spring barley crops, and their short-term effects on soil structure and earthworm populations (Paper I). Two of these soils, a silty clay loam and a sandy loam, were used in an incubation experiment to study the effect of low temperature (0 to 15 °C) and soil type on C and N mineralization from soil organic matter and added plant material (Paper II and III). The data from the incubation experiment were further used to investigate whether it was possible to improve the ability of a soil-crop model to simulate the N mineralization of added plant material at low temperatures (Paper III). Finally, a simulation of the field experiment was attempted, in order to evaluate the effect of the green manure measures on N-leaching.

Field experiment

Study site characteristics and experimental design

The green manure herbage strategies were studied in a field experiment from spring 2008 to spring 2011 at four different sites in the two main cereal cropping regions in Norway; the eastern and the central part, from 59°39’N to 63°29’N (Paper I). The sites were Ås, Apelsvoll, and the neighbouring sites Kvithamar and Værnes (Fig. 1). The normal values (1961–1990) for annual precipitation at Kvithamar/Værnes, Apelsvoll and Ås are 896, 600 and 785mm, respectively; of which half occurs during the growing season (May–September). The normal values for air temperature in the growing season are 12–13°C.

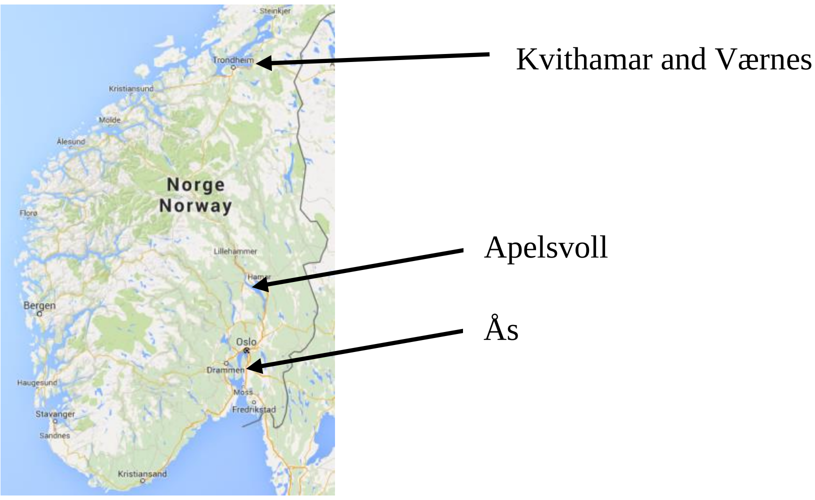

Fig. 1. The four locations of the field experiment.

The soil at Kvithamar is a silty clay loam (27% clay and 3% sand) classified as Mollic Gleysol, whilst the soil at Værnes is a sandy loam (6% clay and 51% sand) classified as Arenic Fluvisol (IUSS Working Group WRB 2006). At Apelsvoll the soil is a sandy loam (14% clay and 55% sand) classified as Endostagnic Cambisol and at Ås the soil is a clay loam (35% and 22% sand) classified as Typic Endoaqualf.

The previous crop rotations at the sites Kvithamar, Apelsvoll and Ås were mainly organically managed arable crops (mainly cereals) with undersown clover or green manure ley breaks. At Værnes, the crop roatation was dominated by conventionally managed grass.

The green manure ley was established in 2008 as an undersown crop in unfertilized spring barley. The sown green manure was a mixture of 20% red clover, 10% timothy, 35% meadow fescue and 35% perennial ryegrass (on a seed weight basis). In 2009, the ley was cut three times. The herbage was treated in three different ways: chopped and left on the stubble (mulched, G-3M), removed twice and mulched after the last cut (G-1M), or removed after each cut (G-0M, Table 1). In addition, a further treatment included removal of the herbage and application of herbage-based digestate as fertilizer in 2010 (G-0M-D). A control treatment with repeated cereal cropping was also included; unfertilized spring barley in 2008, unfertilized spring oats in 2009 and spring barley fertilized with either digestate (C-D) or inorganic fertilizer (C-I) in 2010. The unfertilized cereal treatments were chosen to estimate the above-ground N uptake from soil without any green manure in the rotation. The inorganic fertilizer contained 8 g total N per m^2 and the digestate contained 11 g total N per m^2 and 6 g ammonia N per m^2. The amount of total N in digestate applied was originally planned to be 8 kg, but it turned out to be higher. The amount of N in the applied digestate represented about 45 % of the N in the harvested herbage. Due to the lack of small biogas plants at the sites, the digestate used at all four sites came from the same batch of a grass-clover ley based digestate produced at a biogas plant in Sweden.

Table 1 Overview of treatments in the crop rotation. 2008 was a preparatory year to establish green manure (G) undersown in spring barley. In 2009 the green manure was removed or mulched (M), with unfertilized oats (C) as a control treatment. In the following spring, green manure was ploughed under and barley was sown, either unfertilized or fertilized with digestate (D) at 11 g N m^{-2} or inorganic fertilizer (I) at 8 g N m^{-2}.

Term	2008	2009	2010
G-3M	Barley with G undersown	G 3 cuts mulched	Barley
G-1M	Barley with G undersown	G 2 cuts removed,3rd mulched	Barley
G-0M	Barley with G undersown	G 3 cuts removed	Barley
G-0M-D	Barley with G undersown	G 3 cuts removed	Barley + digestate
C-D	Barley	Oats	Barley + digestate
C-I	Barley	Oats	Barley + fertilizer

The experimental layout was a randomized block design with four replicates, but for practical reasons the two control treatments were always placed beside each other. Depending on the site, the gross size of individual plots varied from 48 to 60 m^2.

Sampling and analysis

Plants were sampled to determine total biomass and N content at each grain harvest (2008, 2009 and 2010), at all ley cuts and late ley regrowth in the autumn (2009) and twice early in the growing season of 2010. In addition, species composition was determined manually by sorting the harvested green manure herbage at each ley cut.

The soil was sampled to determine inorganic N, total N and C. The samples used to determine inorganic N content were taken on 12 dates selected for their likelihood of tracking mineral N fluxes caused by the different treatments and showing the plant availability of N. The sampling depth was normally either 30 or 20 cm (Paper I), but late every autumn and in spring after the green manure ley, soil was also sampled to 80 cm (not included in the papers).

The samples were extracted with 1M KCl, and the supernatant was analysed by spectrophotometry on a FIAstar™ 5000 Autoanalyser (Foss Tecator AB, Höganäs, Sweden, Application Notes 5232 and 5226 (2001) for NO_3-N and NH_4-N, respectively). The total C and N in soil and plant material were analysed using the Dumas combustion method (Bremmer and Mulvaney 1982) on a Leco CHN 1000 analyzer (LECO Corp., St. Joseph, MI, USA).

N balance[1]

An N surface balance of the three-year crop rotation was calculated as input of N by biological N fixation (BNF), fertilizers or digestate and atmospheric deposition minus the N removed in harvested green manure and cereals. The BNF was estimated by the model for grass-clover mixtures presented by Høgh-Jensen et al. (2004):

$$\text{BNF} = DM_{\text{legume}} \text{ x } N\% \text{ x } P_{\text{fix}} \text{ x } (1 + P_{\text{root+stubble}} + P_{\text{transsoil}} + P_{\text{immobile}})$$

where

$\text{DM}_{\text{legume}}$ = amount of legume shoot dry matter above defoliation height

N% = concentration of N in the dry matter of the legume

P_{fix} = fixed N_2 as proportion of total N in the shoot dry matter of the legume

$\text{P}_{\text{root+stubble}}$ = fixed N in the root and stubble as proportion of totally fixed shoot N at the end of the growing period

$\text{P}_{\text{transsoil}}$ = below-ground transfer of fixed legume N located in the grass in mixtures as the proportion of total fixed shoot N at the end of the growing period

$\text{P}_{\text{immobile}}$ = fixed N immobilized in an organic soil pool at the end of the growing period as the proportion of fixed shoot N at the end of the growing period

The proportion values used (P) for mown 1–2 year old grass red clover ley were those given in table 1 of Høgh-Jensen et al. (2004) was used:

$$\text{BNF} = DM_{\text{legume}} \text{ x } 0.030 \text{ x } 0.95 \text{ x } (1 + 0.25 + 0.05 + 0.50(\text{clay}) \text{ or } 0.25(\text{sand}))$$

Soil structure

In order to assess possible effects on soil structure of two years with green manure crops versus continuous cereal growing, soil sampling was performed after tillage and digestate application but before sowing of the barley in spring 2010 (Paper I). Samples of 5–6 litres of soil from the seedbed down to 5–6 cm depth were taken on each plot with treatments G-3M, G-0M-D and C-D. The samples were air dried before analysis of aggregate size distribution

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[1] Not included in Paper I

and aggregate stability. The aggregate size distribution was found by dry sieving for two minutes on a reciprocating shaker containing sieves with mesh openings of 2, 6, 10 and 20 mm. Aggregate size groups were calculated on a weight basis and the mean weight diameter was expressed using the formula of Van Bavel (1949), assuming a maximum clod size of 35 mm. The stability of the aggregates to simulated rainfall was measured for aggregate sizes of 2–6 and 6–10 mm, using similar apparatus as that described by Njøs (1967). Two subsamples (40 g) of each size group were placed within a radius of 0.15 m and subjected to simulated rain for 2 minutes (pressure 1 bar, Hardi 4110–20 nozzles, nozzle height 0.35 m and ca. 70 passes). Aggregate stability was given as the weight percentage of aggregates remaining on the sieve.

Earthworms

In order to assess possible effects on earthworms of the green manure treatments and the digestate application, earthworms were sampled in September 2009 and 2010 at Kvithamar and Værnes, in all replicates of the four treatments G-3M, G-0M, G-0M-D and C-D (Paper I). Two soil cubes (0.2 m x 0.2 m x 0.2 m) per plot were removed and the earthworms were sorted by hand. Juvenile and adult worms were counted and species were identified. Their total biomass was recorded as g m^{-2}.

Incubation experiment

The C and N mineralization from N-rich plant material in contrasting soil types under low temperature were studied in soil samples from Kvithamar (silty clay loam) and Værnes (sandy loam), with and without added ^{13}C labelled clover leaves, incubated at 0, 4, 8.5 or 15 °C.

The bulk samples of the topsoil were sieved while moist through a 2 mm mesh and pre-incubated under aerobic and moist conditions at about 15°C for 4 1/2 months. Prior to incubation, the soil was further moistened to 75% of pore volume at field bulk densities. For three days prior to incubation, the soil was kept at the final incubation temperature.

The moist soil samples were next gently mixed with dried red clover leaves (*Trifolium pratense* L.), corresponding to 4 g dry matter kg^{-1} dry soil, and recompacted to the field bulk densities. The clover leaves contained 46.3% C and 4.8% N. The plant material was cut into small pieces and sieved through a 2 mm mesh. The clover leaves were enriched with ^{13}C, in order to estimate the priming effect when the clover was added to the soil.

C mineralization

The decomposition of SOC and of newly incorporated clover leaves was studied for 142 days in gas tight 1 l chambers with soil equivalent to 400 g dry soil per chamber, and with or without the clover leaves (Paper II and III). It was three replicates within each temperature. In addition, two chambers without soil, one at 0 °C and one at 15 °C, were used as blanks. A CO_2 trap, which consisted of 10 ml 2 M NaOH, was placed in a plastic tube in the middle of the chamber. The sampling and replacement of the trap was done through a pipe in the cork on days 0, 3, 8, 15, 30, 52, 80, 134 and 142. The lye was collected in pre-evacuated glass vials, and 1 ml of it was transferred to a 10 ml glass vial prefilled with 1.5 ml 2M H_2SO_4 for releasing the CO_2. This was used for determination of the CO_2 amount on a gas chromatograph (GC) (Model 7890A, Agilent, Santa Clara, CA, US), and the fractional abundances of ^{13}C on a gas chromatograph isotope ratio mass spectrometer (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany). Another pipe in the cork was used for sampling the atmosphere of the chamber and amending the oxygen level to around 20% when needed. The oxygen content was also measured on the GC. The amounts of total C and N in the soil and clover leaves were analysed using the Dumas combustion method mentioned earlier.

The mineralized C was calculated on the basis of the measured fractional abundances of ^{13}C in the soils, the ^{13}C-labelled clover leaves and in the CO_2 evolved in the chambers. Since the plant material was artificially labelled, the equation for mass-balance was used to estimate the plant-derived and the soil-derived CO_2 (Hayes 2004). The decomposition rate for soil organic matter and clover leaves was calculated for time intervals in which the slope of the decay curve was close to a straight line, i.e. when first order decay could be assumed (Paper II). Estimation of the relative response of the mineralization rate to temperature was attempted by using Arrhenius function and a linear function of temperature (Paper II).

N mineralization

Contemporarily with the chambers used for recording CO_2 mineralization, smaller jars were prepared in order to measure net N mineralization, and incubated in the same cabinets for 80 days (Paper III). Moist soil equivalent to 50 g dry soil was placed in 200 ml plastic jars with the lids left loose, to allow some aeration. Clover leaves were mixed into the soil in half of the jars, in the same proportion as that used in the CO_2-chambers. There were four replicates for each combination of soil, temperature and sampling occasion. The jars were sampled destructively on days 0, 3, 8, 15, 30, 52 and 80, when 130 ml 2M KCl as added to each jar. After shaking, the extract were analysed by spectrophotometry on a FIAstar^{TM} 5000 Autoanalyser (Foss Tecator AB, Höganäs, Sweden, Application Notes 5232 and 5226 (2001) for NO_3-N and NH_4-N, respectively). The net mineralized N from the clover leaves was calculated by the difference method; subtracting inorganic N in soil without clover leaves from inorganic N in soil with added clover leaves.

Simulations with the EU-Rotate_N model

The field-scale soil-crop model EU-Rotate_N (Rahn et al. 2010) was used in two ways, firstly to improve the model prediction of N mineralization of N-rich plant material at low temperatures by simulating the incubation experiment, and secondly to evaluate the effect of the different green manure measures on N leaching by simulating the field experiment.

The EU-Rotate_N model was developed as a tool for assessing the effects of different fertilizers and crop rotation practices on N losses to the environment, across Europe (Rahn et al. 2010). The mineralization module is based on routines used in the Daisy model (Hansen 2002). The soil organic matter is divided into three main pools; dead native soil organic matter, microbial biomass and added organic matter. Each pool is further divided into two sub pools: one with slow turnover and one with faster turnover. The decay rate for each pool at the actual situation is calculated by multiplying a reference decay rate by factors for abiotic conditions, including soil temperature, soil water content and clay content (the latter only for the microbial pool). The same temperature function is applied to all organic matter pools in the model.

N mineralization of clover leaves at low temperatures

The EU-Rotate_N model was adapted to allow variable temperature functions for the different organic matter pools, and improvements were attempted by raising the decomposition rate of the fast decomposing litter pool at low temperature (Paper III).

The model was run for each of the sites from which the soil for the incubation experiment was taken, Kvithamar and Værnes. The soil properties, a crop sequence resembling the actual field history and weather conditions were used as input data. Two years preceding the experiment were simulated first to initiate organic pools in the soil. Thereafter, the incubation conditions were imitated with constant temperature (0, 4, 8.5 or 15 °C) and clover leaves added in the amount and quality as used in the incubation study.

The model was first run with its original temperature function, and then compared with simulations in which the temperature function for the fast decomposing pool of the added organic matter (clover leaves) was adjusted to higher mineralization rates at low temperatures. The N mineralization performance of the different adjustments was evaluated by using the temperature response pattern relative to 15 °C.

An attempt to track N leaching in the field experiment

The EU-Rotate_N model was run for each experimental site with its actual soil properties, crop management and weather conditions as input data. A crop sequence for the five years preceding the experiment was simulated to initiate organic pools in the soil using default model parameters (Hansen 2002; Bruun et al. 2003). A soil mineral N update was run for late autumn 2008 to adjust the simulated level of inorganic N to the measured level. The model over-simulated the levels of mineral N in soil at all four sites and both the simulated crop dry matter production and the N uptake were too high. Improvements were attempted by adjusting some of the default parameters:

- Decomposition rate of soil organic matter: Based on measured inorganic N content in soil of the control treatment and the decay rates found in the soils at Kvithamar and Værnes presented in Paper II, the decomposition coefficient for the fast SOM pool were reduced from 0.000140 per day to 0.0000516 at Kvithamar and Ås, to 0.0000983 at Værnes and to 0.0000805 per day at Apelsvoll. The adjustment for Apelsvoll and Ås was based on the clay content in the soils and the relationship between clay content and its influence on decomposition as presented in Hansen (2002).

- Crop growth parameters: Simulations of the grass-clover ley with the default crop growth parameters showed too little dry matter production early in the growing season, and too much in autumn, compared to what had been measured. Improvements were attempted by reducing the base temperature from 8 to 4 °C, in order to simulate the growth start earlier in spring. Until the second cut, this gave comparable yields to what were measured. Then the ley was simulated mixed into the soil and a new green manure ley was sown. For this crop, the parameters for the daily growth rate were adjusted to fit the measured yields in the fields. The mulch was simulated as an organic fertilizer input after each cut. The input was parameterized to match the observed yield and C/N content of the herbage. For cereals, the crop-specific coefficients for determining the minimum N content required in plants for maximum growth (Eq. 5 and Table 1 in Rahn et al. 2010) were adjusted towards what the N status was in the young barley plants (Fig. 1, Paper I). The measured N uptake and dry matter yields in unfertilized oats were used as a guideline for the adjustments.

The model was run with its original temperature response function and with the adjusted temperature response for the added organic matter pool (Paper III).

Main results

Effect of herbage management on a subsequent barley crop

The green manure herbage strategies tested affected both the subsequent barley yield and the apparent N recovery. Early in growing season, N deficiency was observed in barley plants after herbage removal, particularly on the two clay soils. At all four sites, there were a consistent trend for grain dry matter and N yields in the order: inorganic fertilizer without green manure ≥ herbage removal and digestate application ≥ herbage mulched ≥ herbage digestate without green manure ≥ herbage removal. Depending on the site, removal of green manure herbage reduced the barley grain yield by 0% to 33%, compared to leaving it on-site. The two sites with sandy loam were most negatively affected by herbage removal. The earthworm density and biomass at both measured sites were also lower when the herbage was removed instead of mulched (Paper I).

Applying digestate, containing 45% of the N in harvested herbage, as fertilizer for barley gave the same yields as those when all herbage had been mulched the preceding season. The application of digestate increased the proportion of herbage N recovered in the barley crop. Overall, the apparent N recovery was enhanced from 7% when all herbage was mulched, to 16% when it was returned as digestate. The application of digestate contributed to higher soil aggregate stability, but had no significant overall effect on the earthworm population (Paper I).

Effect of herbage management on inorganic N in deeper soil layers[2]

The measurements of inorganic N in soil did not show any high levels to have been caused by mulching, either in the topsoil (Paper I, Fig. 2) or in deeper layers (30–80 cm) on any of the sampling dates (Fig. 2). The maximum level of NO_3–N in the soil layer 30–80 cm ranged from 1.3 g m^{-2} at Kvithamar to 2.4 g m^{-2} at Apelsvoll (including data not shown for 60–80 cm for G–3M at Apelsvoll).).

In the first year (2008), the green manure ley showed a catch crop effect in late autumn, as the levels of NO_3–N in the whole soil profile (0–80 cm) were at all sites except Kvithamar lower on plots with green manure than on the control plots with barley stubble only (Fig. 2). Deeper soil layers were not measured in the following spring, but the levels of NO_3–N at 0–20 cm were still highest on the plots without green manure (Paper I).).

In late autumn 2009, after the different green manure herbage treatments, mulching raised the levels of NO_3–N in soils (0–80 cm) compared to removing the herbage, but the levels were below 2 g m^{-2} (Fig. 2). There was no difference between the amount of inorganic N in soil with green manure herbage removed and the control treatment with oats, except at Apelsvoll, where it was almost twice as high in the oat treatment. From late autumn 2009 to spring 2010, the levels of inorganic N at 0–80 cm increased in all treatments by 1.5–2.7 g N m^{-2}. An exception was the oat treatment at Apelsvoll, where the inorganic N content decreased. The temperature at 10 cm depth after soil sampling in autumn was on average 2°C for 1.5–2 months, thereafter it fell to below freezing for 3.5 months and finally increased to 2–4°C for 10–20 days before sampling in spring.).

In spring 2010, before the green manure was ploughed down, there was at all sites a higher level of inorganic N in soil with mulched green manure, compared to the other treatments. This difference was maintained at autumn sampling and again in the spring of 2011, though in most comparisons it was not statistically significant. The effect was also seen in the deeper parts of the soil profile, where all green manure treatments showed higher inorganic N content than the control treatments, except at Kvithamar.

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[2] Not included in the papers.

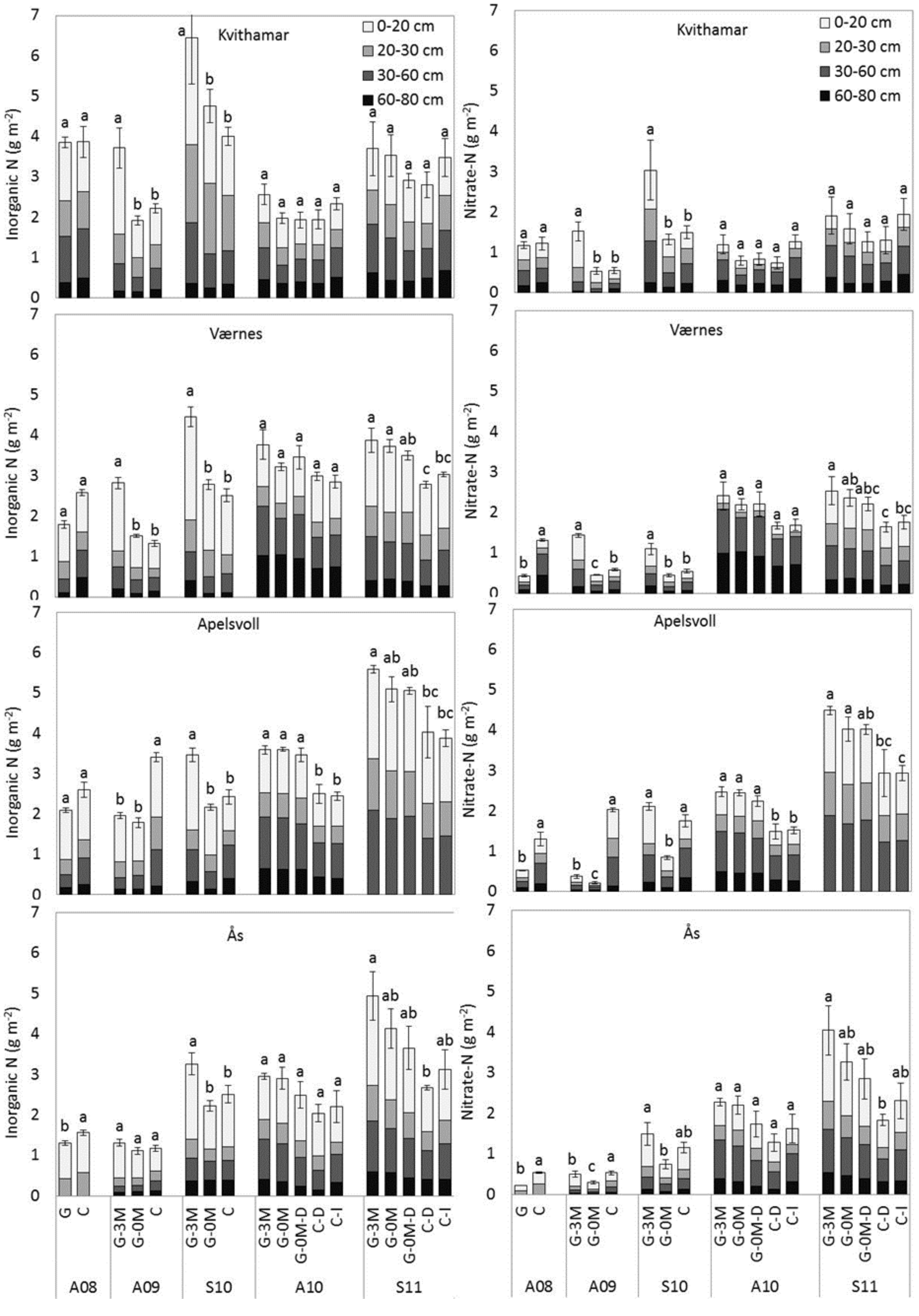

Fig. 2 Inorganic N (± SE) in soil (0–0.8 m) in late autumn 2008 (A08), after green manure treatments or oats in late autumn 2009 (A09), in the subsequent spring before ploughing (S10), in late autumn 2010 after barley (A10) and in May 2011 (S11). Abbreviations for the treatments are explained in Table 1. Bars (± S.E) within each sampling which do not have any letter in common are significantly different (P < 0.05) according to the Tukey HSD method.

Table 6 N surface balance for the 3-year crop rotation. 2008: Establishing of green manure (G) in spring barley or spring barley monoculture (C). 2009: Green manure was removed (0M) or mulched one (1M) or three times (3M). Unfertilized oats (C) was control. 2010: Spring barley fertilized with biogas residue (D), inorganic fertilizer (I) or unfertilized. Biological N fixation (BNF) in the green manure ley was estimated by the model by Høgh-Jensen et al. (2004). Both grain and straw were removed from the field.

	Kvithamar						Værnes						Apelsvoll						Ås					
	G-3M	G-1M	G-0M	G-0M-D	C-D	C-I	G-3M	G-1M	G-0M	G-0M-D	C-D	C-I	G-3M	G-1M	G-0M	G-0M-D	C-D	C-I	G-3M	G-1M	G-0M	G-0M-D	C-D	C-I
Atm. dep year^{-1}	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2008:																								
BNF	8.3	8.3	8.3	8.3			5.8	5.8	5.8	5.8			1.8	1.8	1.8	1.8			3.1	3.1	3.1	3.1		
Removed*	3.1	3.1	3.1	3.1	4.3	4.3	3.9	3.9	3.9	3.9	5.0	5.0	5.0	5.0	5.0	5.0	6.0	6.0	5.9	5.9	5.9	5.9	6.3	6.3
G**	8.9	8.9	8.9	8.9			6.7	6.7	6.7	6.7			1.7	1.7	1.7	1.7			3.0	3.0	3.0	3.0		
Uptake in aerial plant parts	12.3	12.3	12.3	12.3	4.6	4.6	10.8	10.8	10.8	10.8	5.2	5.2	7.0	7.0	7.0	7.0	6.3	6.3	10.6	10.6	10.6	10.6	8.0	8.0
Input-removed	**5.6**	**5.6**	**5.6**	**5.6**	**-3.9**	**-3.9**	**2.3**	**2.3**	**2.3**	**2.3**	**-4.6**	**-4.6**	**-2.7**	**-2.7**	**-2.7**	**-2.7**	**-5.5**	**-5.5**	**-2.3**	**-2.3**	**-2.3**	**-2.3**	**-5.8**	**-5.8**
2009:																								
BNF	27.4	32.7	32.7	32.7			31.6	30.8	30.8	30.8			26.6	32.2	32.2	32.2			25.6	27.6	27.6	27.6		
Removed*		17.4	25.1	25.1	4.8	4.8		16.9	22.5	22.5	3.9	3.9		17.4	20.5	20.5	2.5	2.5		11.5	19.5	19.5	3.8	3.8
G mulched	26.3	7.8					22.9	5.6					19.0	3.1					19.7	7.9				
Uptake in aerial plant parts	26.3	25.1	25.1	25.1	5.7	5.7	22.9	22.5	22.5	22.5	4.5	4.5	19.0	20.5	20.5	20.5	2.9	2.9	19.7	19.5	19.5	19.5	5.5	5.5
Input-removed	**27.8**	**15.8**	**8.0**	**8.0**	**-4.4**	**-4.4**	**32.0**	**14.3**	**8.8**	**8.8**	**-3.5**	**-3.5**	**27.1**	**15.2**	**12.2**	**12.2**	**-2.0**	**-2.0**	**26.1**	**16.5**	**8.6**	**8.6**	**-3.3**	**-3.3**
2010:																								
Fertilizer***				10.7	10.7	8				10.7	10.7	8				10.7	10.7	8				10.7	10.7	8
Removed*	3.7	3.1	2.3	3.3	3.3	5.7	7.6	5.6	5.5	7.2	6.0	8.4	6.6	5.2	4.7	7.3	5.3	7.3	6.5	6.6	6.2	7.8	7.7	9.7
Uptake in aerial plant parts	4.2	3.6	2.7	3.7	3.8	6.1	8.3	6.3	6.3	7.9	6.7	9.1	6.8	5.5	5.0	7.5	5.5	7.5	6.8	6.9	6.5	8.1	7.9	9.9
Input-removed	**-2.9**	**-2.4**	**-1.9**	**7.8**	**7.8**	**2.7**	**-7.2**	**-5.2**	**-5.1**	**3.9**	**5.1**	**0.0**	**-6.1**	**-4.7**	**-4.2**	**3.9**	**5.9**	**1.2**	**-6.0**	**-6.1**	**-5.7**	**3.4**	**3.5**	**-1.2**
2008-2010:																								
Input	36.9	42.2	42.2	52.9	11.9	9.2	38.6	37.8	37.8	48.5	11.9	9.2	29.9	35.5	35.5	46.2	12.2	9.5	30.2	32.2	32.2	42.9	12.2	9.5
Uptake in aerial plant parts	42.8	41.0	40.1	41.1	14.0	16.4	42.0	39.6	39.5	41.2	16.4	18.9	32.9	32.5	35.0	33.0	15.2	16.7	37.1	36.9	36.5	38.2	21.4	23.4
Removed*	6.8	23.6	30.5	31.5	12.4	14.7	11.5	26.4	31.9	33.6	14.9	17.3	11.6	27.6	30.2	32.8	13.8	15.8	12.4	24.0	31.5	33.2	17.8	19.8
Input-removed	**30.1**	**18.6**	**11.7**	**21.4**	**-0.5**	**-5.5**	**27.1**	**11.4**	**5.9**	**14.9**	**-3.0**	**-8.1**	**18.4**	**7.9**	**5.3**	**13.4**	**-1.6**	**-6.3**	**17.8**	**8.2**	**0.7**	**9.7**	**-5.6**	**-10.3**

*Grain and straw, except only grain at Apelsvoll in 2009, **In above-ground biomass before frost, ***Digestate/inorganic fertilizer

Effect of herbage management on crop rotation N balance[3]

The estimated total BNF for the green manure ley ranged from 28 to 41 g N m^{-2} (Table 2). The surface N balance for the three years of crop rotation showed N surpluses for all green manure treatments, with an especially high N surplus for mulched green manure (G–3M, range 18–30 g N m^{-2}). Removing all herbage reduced the N surplus to 1–12 g N m^{-2} depending on site (Table 2).

Simulating the field experiment with the EU-Rotate_N model

The adjustment of the crop growth parameters, soil organic matter decomposition rates and the temperature response of the fast decomposing pool of added organic matter did slightly improve the simulations, but not sufficiently to be used for estimating the N losses in the field experiment.

The measured soil inorganic N and N uptake in cereals in the control treatment (C-I) was used as an important reference for the simulations of the green manure ley, since there were two years with cereal crops relaying on only mineralized N from the soil. The correlation between the simulated and measured crop N uptake was relatively good, except from the mulched ley treatment, which was over-simulated (Fig. 3). In general, there were no good fit for the measured and the simulated soil inorganic N content in this treatment (Fig. 4) or in any of the treatments (Fig. 5). Soil inorganic N contents below 2.5 g m^{-2} were over-estimated, while higher contents were under-estimated. Best fit was found for Værnes, and poorest was found for Apelsvoll.

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[3] Not included in Paper I

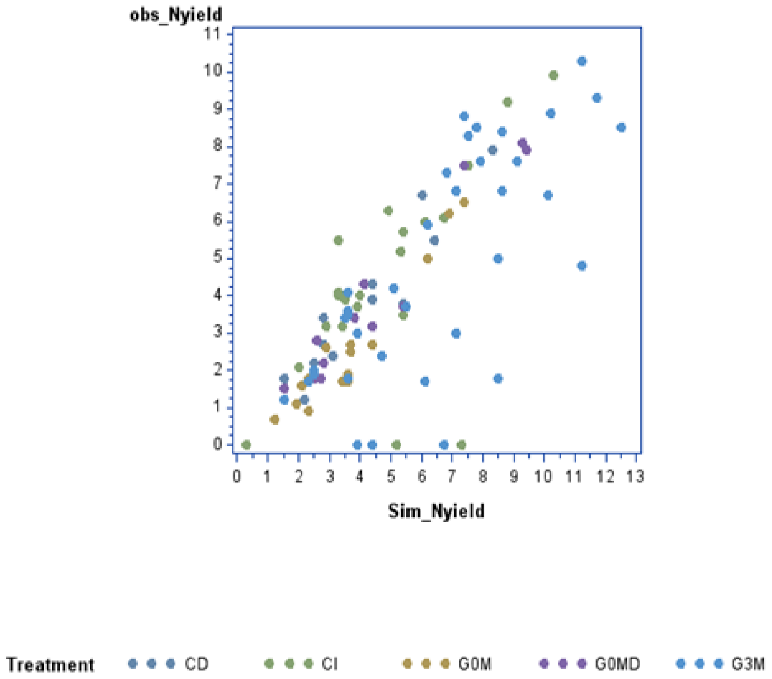

Fig. 3 Observed crop N yield vs. simulated at all sites. Abbreviations for the treatments are explained in Table 1.

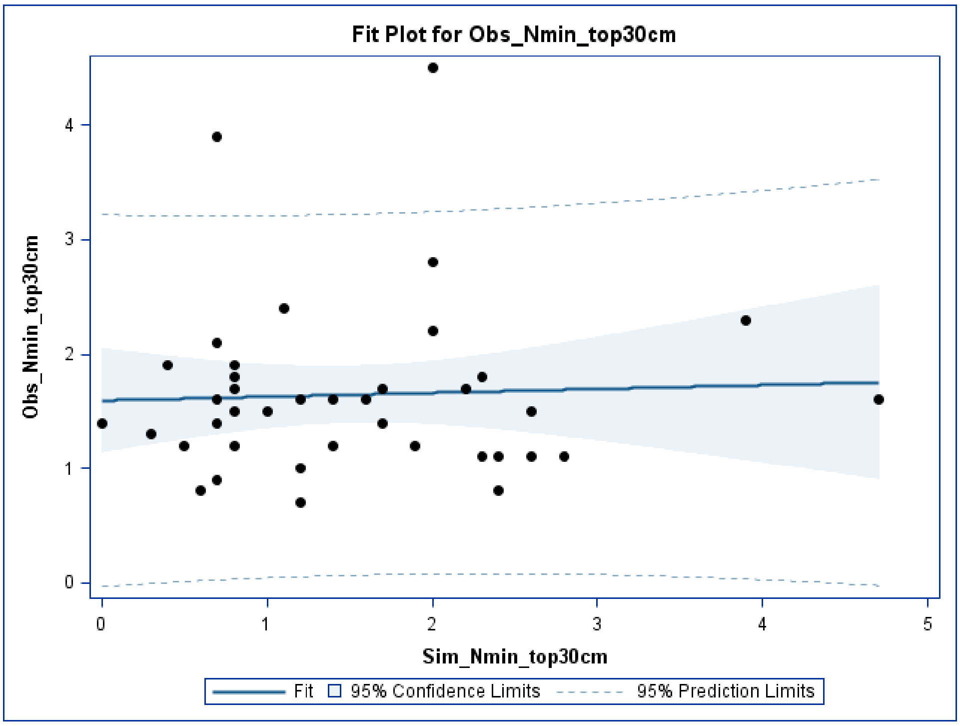

Fig. 4 Observed soil inorganic N content in 0-30 cm depth vs. simulated for the control treatment with cereals (C-I).

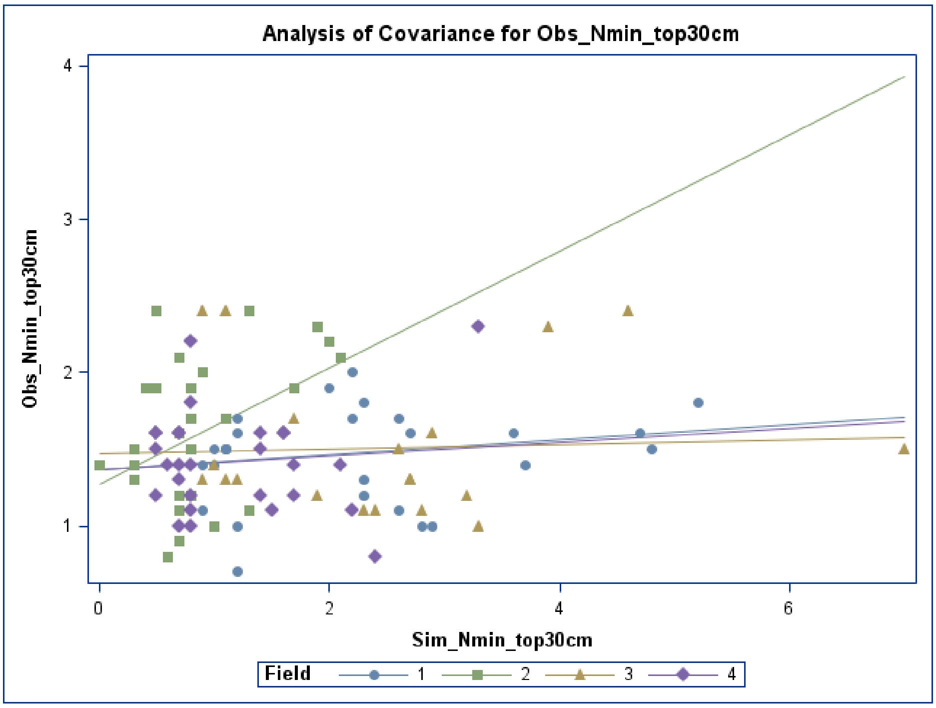

Fig. 5 Observed vs. simulated soil inorganic N content in 0-30 cm depth for observations < 2.5 g m^{-2} for all treatments and fields (1: Kvithamar, 2: Værnes, 3: Apelsvoll and 4: Ås).

Effect of low temperature and soil type on soil organic C and clover decomposition rates

Incubation of clover leaves for 142 days in the temperature range 0–15 °C in a silty clay loam and a sandy loam, showed that soil type affected the decay rates of both soil organic C and clover, but the sensitivity to temperature of the decay rates was not affected by soil type (Paper II). The temperature sensitivity of the decomposition was in the order: priming < clover leaves < organic C in sandy loam = organic C in silty clay loam.

The decomposition rate of soil organic C, in the absence of added clover, followed a first order reaction that was twice as fast in the sandy soil as in the clay soil. The relative response of soil organic C mineralization rate to temperature was the same in both soils. It was well described by an Arrhenius function and could also be approximated as a linear function of temperature. For the purpose of modelling, we present parameterised equations for the mineralization rates of soil organic C and clover leaves as functions of the soil temperature range 0–15 °C (Table 3, Paper II).

The C mineralization of clover leaves was affected by soil type, and was slower in the silty clay loam than in the sandy loam. Also the initial temperature sensitivity of the clover decomposition (up to 18% decomposition) could be approximated by a linear function that was similar for the two soil types.

Soil organic C mineralization was enhanced (thus showing a priming effect) by the presence of clover. The relative increase was most conspicuous at 0 °C and decreased with increasing temperature. At the start of the incubation and up to 52 days of incubation, the priming effect was correlated with clover leaf decomposition.

Effect of low temperature and soil type on N mineralization of N-rich plant residues

Incubation of clover leaves for 80 days in the temperature range 0–15 °C in a silty clay loam and a sandy loam showed substantial net N mineralization during the first three days, irrespective of temperature and soil type. This was, for about 4 weeks, followed by slow mineralization in the sandy loam and net N immobilization in the silty clay loam, which was more severe at the higher than at the lower temperatures. During this phase, the ratio of net mineralized N to C was higher at lower than at higher temperatures and higher in the sandy loam than in the silty clay loam. Thereafter, mineralization increased, positively related to temperature, and the N/C ratio became constant. At the end of the incubation, only 13–22% of the N added with clover had been net mineralized, and about half of this was mineralized already by the 3rd day.

It was not possible, using the EU-Rotate_N model, to simulate the N dynamics that took place in the early phase of decomposition of plant residue simply by raising the decomposition rate at low temperature of the fast decomposing added organic matter pool. The simulation of the temperature responses improved at the end of the incubation, i.e. more N was mineralized from incorporated plant residue at low temperature than when using the original temperature response. However, the simulated N level was still too high, compared to the measured values.

General discussion

The main objective of this thesis was to increase our knowledge about possible ways to improve the recovery of N from green manure leys grown on contrasting soil types under cold climate conditions, within the frame of cereal production in a stockless organic cropping system. The study focus was on the effect of the common mulching practice vs. removal of the green manure herbage and the use of herbage based digestate as fertilizer, with respect to the N recovery in a subsequent barley crop. Furthermore, the study examines how C and N mineralization of clover leaves respond to low temperature in contrasting soil types. The hypotheses behind the specific objectives are discussed in detail in Papers I-III. The results are discussed further within the framework of the overall aim.

Herbage N recovery and barley yield

The green manure herbage in the field experiment contained a substantial amounts of N (19-26 g total N m^{-2}), in line with that expected from three cuts of organic grass-clover leys in Norway (Steinshamn et al. 2015). However, the apparent N recovery from the mulched herbage in the above-ground biomass of the subsequent barley crop was low (7 %). Under comparable field conditions, Müller and Sundman (1988) found that a subsequent spring barley crop (including roots) took up around 20 % of the N from red clover and timothy buried in mesh bags in late autumn. Otherwise, there are few comparable studies on N recovery in cereals from herbage mulched and decomposed on ley stubble. Removal of the herbage and use of half of the N it contained in digestate as fertilizer, improved the apparent N recovery to 30 %, when calculated in relation to the amount of NH_4-N applied, rather than total N applied. This is similar to levels found when using animal slurry in an organic arable farming experiment (Olesen et al. 2007), and 4–12 % lower than when using mineral N fertilizer for barley and oats in Norway (Riley et al. 2012). The improvement of apparent N recovery by using digestate instead of mulch was mainly due to the fact that less N was applied, rather than increased yield, since the barley yield was not significantly affected. We did not expect that removal of the herbage would affect the yield of the subsequent barley crop, because we expected that the main contribution from the ley came from roots and stubble, and we expected higher clover proportion and increased N_2 fixation of the ley in absence of mulch manure. Contrary to what we expected, the results showed an overall positive effect of mulching on the subsequent barley yield, compared to removal of the green manure herbage. This was found despite there being no increase in dry matter yield, N yield or clover fraction in the mulched green manure ley. In fact, rather the opposite was found in some cases at Apelsvoll and Kvithamar. The effect of the mulched herbage was also observed as increases in the number and biomass of earthworms, already shortly after the last cut of the green manure ley. This indicates that at least part of the N in herbage was organically bound in wormcasts and earthworm biomass for a shorter or longer period. Furthermore, the amount of inorganic N in the soil in late autumn was almost twice as high when the herbage was mulched as when it was removed (Fig. 2). Before ploughing in the subsequent spring, higher soil contents of inorganic N were found after mulching compared to removal of the herbage, and a net increase in inorganic N had taken place during the winter.

C and N mineralization as affected by low temperature and soil type

Relatively low amounts of the N in the incorporated green manure ley seemed to mineralize in early growing season, which indicate a temporary immobilization, in line with what Baggs et al. (2000) found when incorporated green manure and cover crops in a field experiment in Scotland. In contrast, rapid N mineralization have been found after spring incorporation of green manure and catch crops under Danish conditions (Willumsen and Thorup-Kristensen 2001; Thorup-Kristensen and Dresbøll 2010).

Also low amounts of the total clover N were mineralized during the incubation experiment, compared to other incubation studies (Van Schöll et al. 1997; Cookson et al. 2002). The pretreatment of the plant material may influence decomposition, as found by Sørensen et al. (1996) who tested the effect of ground and unground plant materials. However, Cookson et al. (2002) found higher total net N mineralization from dried clover material that consisted of larger pieces and had slightly higher C/N ratio than the clover leaves used in our experiment. However, the mineralization pattern with an initial rapid net mineralization followed by a short period of net immobilization and then a net mineralization was similar to the pattern found when Jensen et al. (2005) a group of plant material with an average C/N ratio of 10. The incubated soils were the same as on two neighbouring sites in the field experiment, and the relatively low amount of clover N mineralized in the incubation experiment support that there was small effect of the incorporated green manure ley on N uptake in young barley plants.

The green manure ley is expected to have positive effects on crop yield beyond the first year. The contents of inorganic N in late spring 2011 support that there were still mineralization of the ley, but no effect was seen by the different herbage measures. The levels of inorganic N in 0–80 cm depth in late spring one year after incorporation of the ley showed that in general the level was higher when green manure had been present compared to cereals only, but the difference was only significant if the green manure had been mulched. However, carry-over effects in 2011 were not tested by crop N uptake this growing season. Løes et al. (2011) found 28% yield increase in wheat the second year after a mulched green manure ley, but the effect of the mulch itself was not tested in this experiment.

Temperature

The C decomposition of soil organic matter and the initial decomposition of the clover leaves (to 18 % decomposed) followed first order kinetics, which indicated that a relatively homogenous pool decomposed. It was therefore possible to make estimates for the modifying temperature function for the two soil types and the initial phase of decomposition of the clover leaves. The presented equations can be used for modelling purposes in the tested temperature range.

In line with several other studies, the incubation experiment showed that the Q_{10} values was considerable higher in the temperature range 0–4 °C than for the higher temperatures, and that the use of Q_{10} as temperature factor for modelling is not useful in the low temperature range.

We found substantial C and N net mineralization at temperatures below 5 °C in both experiments, especially in the early phase of mineralization. In line with several other studies, we observed a shift between net mineralization and net immobilization that took place between 3–5 °C and 8.5–11 °C (Andersen and Jensen 2001; Magid et al. 2004; He et al. 2014). Temperature affects the microbial community and the soil biology in general. Schimel et al. (2007) support our findings by explaining that a metabolic shift in microorganisms occur above freezing point, and that this shift is large enough to change the overall pattern of ecosystem C and N cycling from net N immobilization to net mineralization. As an adaptation to low temperature, the decomposition may also be more dominated by fungi than bacteria (Pietkäinen et al. 2005).

Soil type

The sandy loam soils responded more to the different green manure treatments than the clay loam soils. This indicate a slower net mineralization of the plant material in the clay soils compared to the sandy soils, and this was confirmed by the incubation experiment, where the decomposition rates were found to be twice as high in the sandy loam than in the silty clay loam. Slower decomposition in the clay soil may also be the reason for higher density of the soil-eating earthworm *A. caliginosa* at Kvithamar one year after mulching, compared to at Værnes. Net immobilization was found to take place in the clay soil, even after incorporation of more N-rich plant material (leaves) than root and stubble of the green manure ley consisted of.

A comparison between the clay loam and the sandy loam in the two experiments was attempted by comparing the net mineralized N during incubation (Fig. 1, Paper III) with the net increase in soil inorganic N and N uptake in barley in early growing season 2010 (Fig. 2; Fig. 2 and Table 6 Paper I). According to the measured soil temperature in 10 cm depth in this period, the incubation data for 8.5 °C was most comparable.

Table 3 Net increase in soil inorganic N from ploughing to one week after barley germination and N uptake in barley plants at tillering and booting in 2010 at the sites Værnes, Kvithamar and Ås for the treatments G-3M, G-0M and C-I (explanation in Table 1). The net N mineralization during incubation (Inc) of soil with clover leaves are included for a comparable time span.

	At first leaves developing				At tillering				At booting			
	Inc	G-3M	G-0M	C-I	Inc	G-3M	G-0M	C-I	Inc	G-3M	G-0M	C-I
Værnes	6.0	1.2	1.4	9.1	10.0	1.8	0.9	3.2	11.0	3.7	1.7	5.2
Kvithamar	1.0	0.7	0.8	8.8	5.0	1.2	0.7	2.1	7.5	1.7	1.1	4.0
Ås	3.0	1.8	1.9	7.5	7.0	2.1	1.8	3.9	8.5	3.0	2.5	5.7

In the field, the amount of inorganic N in sandy loam (Værnes) was about 1.5 (1-2.2) times higher than in the silty clay loam (Kvithamar) (Table 3). This was comparable to the ratio found after 40 days of incubation (1.5–2.0), but not earlier because of the net immobilization in the clay loam. Assuming that the clay loam at Ås would have the similar mineralization pattern as at Kvithamar, only half of the inorganic N amount was found at Kvithamar compared to Ås, ranging from 0.3 to 0.9 in the incubation experiment and 0.4 to 0.7 in the field experiment. The difference between the two sites was likely due to lower soil temperature and more precipitation at Kvithamar compared to Ås in spring 2010.

We studied the decomposition rates at temperatures down to 0 °C, but when the temperature decreases below 0 °C, Clark et al. (2009) found that clay soil had higher mineralization rates than loamy soil because more unfrozen water remain in the clay soil.

Priming effect

In presence of plant material, we found a positive C priming effect that correlated with decomposition of plant litter, but responded to temperature less than litter decomposition did. In the lower temperature treatments (0 and 4 °C), the priming effect more than doubled the total mineralization of soil organic C. Thus, the effect of plant litter on soil priming should be considered in C and N mineralization models as it can have an important effect on the amount and on the C/N stoichiometry of the decomposition products. In lack of a labelled N pool, we could not quantify priming effect on N mineralization from soil organic matter. C and N turnover are closely linked to each other, and priming of soil organic N are likely to have happened when adding organic substrates (Kuzyakov et al. 2000).

Priming effects are located to microbial hotspots, and when plant roots are present, the rhizosphere hotspot is great importance for priming (Kuzyakov et al. 2010). According to Gärdenäs et al. (2011), the influence of rhizosphere priming may be large enough to influence C balances at both ecosystem and global scale.

Modelling N mineralization

Crop rotation models must be used with care when approaching the limits of the environment they are developed for. The EU-Rotate_N model was developed to optimize N use in horticultural crop rotations across Europe. The model was chosen because it was adapted to Norwegian cropping conditions by validation of its snow and frost module and validation of N leaching and N requirement of vegetable crops in three crop rotations in contrasting soil types in southern Norway (Rahn et al. 2010). The crop rotations were conventional farming systems with use of mineral N fertilizers, but earlier Nendel and Riley (2005) concluded that one standard parameter set was sufficient for simulation of background N mineralization in vegetable cropping systems all over Europe. The exception was sandy soils, where the N mineralization was overestimated. The model was also successfully tested and evaluated in Britain for organic vegetables with ley as pre-crop (Rayns et al. 2006). In general, the measured inorganic N level in this experiment was comparable to the levels found in our field experiment. However, N dynamics in organic green manure – cereal rotations on contrasting soil types under Norwegian climate conditions was not simulated earlier. The EU-Rotate_N model gave poor simulation of the measured low level of inorganic N and the N dynamics in the soil in the field and the incubation experiment. It was especially difficult to simulate the growth pattern of the green manure ley, the mulching and the decomposition of the green manure ley. Challenges with simulating the residual effects of ley were also found when using the whole farm model FASSET, but in that case the model under-simulated the inorganic N content on coarse sand (Berntsen et al. 2006). If the models fail to simulate the short-term dynamics of N mineralization from easily decomposable plant material under low temperatures, this can lead to erroneous estimates of N leaching and early N supply to the succeeding crop. Improvement of this simulation should be attempted, not only for the management of organic farming systems, but also for better fertilization management and N use efficiency in conventional farming systems.

The results show that basic model changes are needed for simulating effects of low temperature on N mineralization from fresh plant material. We suggest to alter the ratio between net N and C mineralization by including a temperature response function for microbial growth, as the latter is more inhibited by low temperature than is the decomposition of plant material. This is in line with Henriksen and Breland (1999) who improved a simulation model by reducing the microbial growth efficiency at low temperatures.

N losses under contrasting herbage managements

Measurements of N leaching and gaseous emissions were not performed in the field experiment. For this reason, we planned to estimate N losses by model simulations. However, as it turned out that the chosen model could not be adapted to simulate the observed N mineralization, it could not be used for estimating the N losses either. Possible N losses are therefore discussed on the basis of the measured soil inorganic N contents and the crop N uptakes, in relation to weather conditions and soil properties.

Ammonia emissions

Ammonia emissions from decomposing mulched herbage can be substantial, especially from N-rich plant material after precipitation at high temperatures, whilst they are negligible under dry conditions (Whitehead et al. 1988; Whitehead and Lockyer 1989; Larsson et al. 1998). Depending on field location in our trials, the average air temperature was 13–17 °C from the first cut until 30 days after the second cut. The precipitation during the four weeks after the first cut ranged between 40–85 mm, with occasional dry conditions in between, whilst it was twice as high over the four weeks after the second cut. In general, higher ammonia emissions may therefore have taken place after the second cut than the first. During the four weeks after the third cut, precipitation was 250 mm at the northern sites and

40 mm at the southern sites, but the temperature was low (2.5–5.3 °C) at all sites. Assuming the same amount of ammonia emission for three mulching occasions in our field experiment as that found by Whitehead and Lockyer (1989), i.e. 10% of herbage N, the estimated N loss as ammonia from the herbage was around 20 kg per hectare in the year it was mulched. Based on ten years of field experiment at Apelsvoll, Korsaeth (2012) estimated the mean annual N loss as ammonia from mulched herbage to be 6.7 kg N per hectare from an organic arable cropping system with 25 % of the area used as green manure. This ammonia N loss amounted to 18 % of the total N loss. Korsaeth (2012) set the ammonia emission from the first cut to 39 % of the herbage N, in accordance with Larsson et al. (1998), and to 20, 10 and 5 % for later cuts. Larsson et al. (1998) measured ammonia emission from mulch on bare soil, while Whitehead and Lockyer (1989) placed the mulch on the stubble of a ley, similar to what we did in our experiment. This suggests that their figure is more relevant in our case. Nevertheless, mulching practice under weather conditions with normally 300–450 mm precipitation during the growing season, as in our case, does involve a high risk of N losses as ammonia. By removing the herbage for biogas production, the ammonia emissions and other N losses can be better controlled by management, since N losses then mainly occur during storage and at application. Möller (2015) state that, to get the full potential advances from implementation of biogas plants in terms of improvement of nutrient use efficiency and reduction of greenhouse gas emissions, gas-tight closure of the digestate stores and direct soil incorporation of the field-applied digestate should be adopted. At the Apelsvoll site, the digestate was applied using the Direct Ground Injection technique. Contrary to the other sites, there was no positive effect of digestate on N status in young plants at Apelsvoll, although the final grain yield was improved. The relatively large row-spacing of the application equipment may have hampered the early N uptake from digestate by young barley plants with small roots.

N leaching and runoff

The dates for soil sampling to 80 cm depth were selected on the basis of their suitability for tracing N leaching; i.e. late autumn and early spring, before ploughing. In general, no high levels of nitrate were found on these sampling dates in either topsoil or deeper soil layers. The green manure ley acted as a catch crop for nitrate N in the year of establishment (Fig. 2). This was also the case in 2009, when the herbage was removed, but not when it was mulched at the two northern sites. At the sandy loam at Værnes, a small decrease in nitrate during the winter (less than 3.5 kg N per hectare at 0–80 depth) indicated a leaching N loss, probably due to 40 mm precipitation that occurred over a short period in the early spring. A net N loss was also observed in the control treatment with oats in the sandy loam at Apelsvoll, probably due to rainy weather in late autumn. Apart from these two cases, at soil temperatures below 5 °C from late autumn 2009 until spring 2010, the inorganic N content increased, which indicates that net mineralization had occurred. This interpretation is supported by the net mineralization and the delayed nitrification rate that were found at low temperatures in the incubation experiment.

Sandy soils are more prone to leaching than clay soils. In the field experiment, indications of leaching losses were found in trials on sandy loam, and the catch crop effect of the green manure ley was also more pronounced on these soils than on the clay soils. Askegaard et al. (2005) demonstrated that the nitrate leaching loss from an arable crop rotation with grass-clover green manure, was three times higher on coarse sand (with 5 % clay) than on sandy loam (with 16 % clay). With respect to grassland, Korsaeth et al. (2003) found that soil type had a greater effect on N leaching than the source of N input (inorganic fertilizer or manure). N leaching losses were in the range of 5–23% of the N input in their experiment. Svoboda et al. (2013) found that the N leaching potential of digestate was similar to that of animal slurry. In our field experiment, 64 tonnes of digestate containing 4.3 % dry matter were applied per hectare, which is twice to three times as much as the amounts of animal slurry that are commonly applied on organic livestock farms. This relatively large amount of liquid applied at one time is equivalent to a rainfall event of >60 mm, and could thus cause leaching losses. To reduce the risk of leaching losses, the digestate could have been distributed over larger areas.

The incorporation time of green manure and catch crops influences the nutrient supply to the succeeding crop, and should be related to soil and climate conditions at the individual farm (Thorup-Kristensen and Dresbøll 2010). Field studies have shown that rapid N mineralization from N-rich plant material occurs even at low temperatures (Breland 1994; Thorup-Kristensen). Spring incorporation has therefore been recommended to increase the N recovery by subsequent crops. In our field trial, spring ploughing was performed, but the mineralization of the green manure ley seemed to be too low to meet the N demand of the young barley plants. For this reason autumn ploughing of catch crops has also been suggested as an alternative under northern climatic conditions (Torstensson and Aronsson 2000; Korsaeth et al. 2002). Autumn ploughing starts the decomposition and mineralization of the ley. If done in late autumn, at low temperatures, the nitrification may be inhibited, resulting in a higher ammonium/nitrate ratio, as found in the incubation experiment. This reduces the risk of N leaching. However, autumn ploughed soils are more susceptible to erosion and runoff than soils ploughed in spring.

Loss of N due to surface runoff from poorly overwintering plant material may influence the effect of incorporation time (Korsaeth 2002). Mulched plant material is susceptible to such losses, especially in autumn. Surface N runoff from a cereal cropping system on loam soil with mulched green manure ley was found to be only 3 % of the N leaching losses to drainage, which were 31 kg N per hectare and year (Korsaeth 2008). An alternative, not tested in our field experiment, could be to omit the last cut and let the plants prepare for the winter earlier. However, the N loss from leaves of perennial crops may also be substantial. Sturite et al. (2007) found that the N loss from above-ground biomass of white clover and grass varied greatly between years, within the range 6–68 %.

Nitrous oxide emissions

Nadeem et al. (2012) measured the N_2O emissions in the field experiment at the Ås site during the green manure year (2009) and the following year. They found high N_2O emission levels irrespective of treatments. Use of green manure ley increased the N_2O emissions compared to the cereal reference (80 kg fertilizer N per hectare), by 20% when the herbage was removed and by 35% when the herbage was mulched. The N_2O emissions from the green manure ley declined by 11 %, when the herbage was removed rather than mulched. No residual effect of herbage management in N_2O emissions was found the following year. The N_2O emissions caused by mulching were estimated to 0.37 kg N_2O-N per hectare. This corresponds to 0.2 % of the total herbage N, which is lower than the found by Larsson et al. (1998) or commonly estimated of N input by IPCC (2006). Digestate application did not increase the N_2O emissions, but emission data shortly after digestate application were lacking. Möller and Stinner (2009) found that digestate application of grass-clover herbage boosted the N_2O emissions shortly after incorporation. However, as an overall effect of the cropping system, Möller and Stinner (2009) found 38% decrease in N_2O emissions when crop residues and green manure herbage were harvested and digested, instead of being mulched and incorporated.

Development of organic farming systems

Development of organic farming systems must be sought within the principles of organic agriculture (IFOAM 2005). The principles point out that organic agriculture should be based on ecological processes, biodiversity and recycling, and managed in a precautionary and responsible manner that sustains and enhances the health of soils, plants, animals, humans and the whole environment. However, new management strategies will not be implemented by farmers unless they are practical and economic feasible.

Anaerobic digestion of herbage

In line with the review of Möller (2015), the digestate strategy turned out to be the most promising option as regards enhancing N recovery and reducing the risk of N losses. However, running farm scale biogas plants based solely on green manure herbage is challenging in cold climate regions, with regards to getting the process running at a high enough temperature, and thereby attaining a sufficient energy balance (Briseid and Bergersen 2012; Fjørtoft et al. 2014). When studying the suitability of anaerobic digesters on organic farms in Britain, Clemets (2013) concluded that there is a need for new systems to be assessed, especially on small farms. Simple high-solid reactor systems may be an option when biogas production is based upon crop residues, as suggested by Svensson et al. (2005) under Swedish conditions. Another possibility may be to cooperate with biogas plants fed continuously throughout the year with, for example, household waste or animal manure.

Digestates contain more mineral N and less C than for example fresh plant residues or non-digested animal manures. Consequently, digestate reduces soil microbial activity compared to non-digested plant residue or animal manure (Johansen et al. 2013; Thomsen et al. 2013). In our field trial, the earthworm populations (both in number and biomass) were decreased by removing the herbage in autumn 2009, whilst no further effect on earthworm population was found upon application of the digestate. The main effects of digestate on soil biota seem to be short-term, but there is little information about long-term effects (Möller 2015).

Green manure

The green manure ley performs multiple services, and this that a broad approach should be used when evaluating its usefulness (Cherr et al. 2006). Green manure ley in a cereal rotation represents a break crop that may prevent problems with pests, diseases and weeds.

As an example, green manure ley has been found to reduce the growth of the perennial weed *Cirsium arvence* more than mechanical operations (Thomsen et al. 2011).

In a short-term land use perspective, mulched green manure ley is an expensive practice, because it gives no direct income, only the expectancy of higher income from future crops grown on the field. An alternative to using one growing season for a green manure crop, is to repeatedly use clover sub-cropping in cereals. Løes et al. (2011) studied the grain yields and N supply after a mulched green manure ley versus repeated use of sub-crops. They concluded that in order to obtain acceptable yields under Norwegian conditions, the crop rotation should include both green manure ley and clover or clover-grass sub-crop. When not using green manure catch crops, more than 25 % of rotation should be used for green manure ley. Kayser et al. (2010) concluded that the positive effect of a one-year grass-clover ley on cereals yield was rather short-lived compared to that of a three-year ley. This is in line with Johnston et al. (1994) who found a significant increase in wheat yields with increasing ley length up to three years. They suggest that neither the length of the ley nor that of the arable period should exceed three years.

The feed quality of the green manure leys at the four sites implied that the herbage could also have been used as roughage for ruminants (Steinshamn et al. 2015). In areas with a high proportion of livestock farms, it may therefore be an option to sell the grass-clover herbage to such farmers. Cooperation with neighbouring livestock farmers may also be possible, by combining the total area of the farms and including cereals on the livestock farm as well as ley on the arable farm. This may improve the crop rotations at both farms.

Introduction of green manure leys for biogas production may also be an option for conventional farmers. Tidåker et al. (2014) used the life cycle assessment (LCA) methodology to evaluate the environmental impact of including a two-year grass-clover ley in a conventional five-year cereal crop rotation. The herbage of the ley was used for biogas production and it was calculated that the digestate could replace 57 % of the mineral fertilizer N requirement. The indirect land use caused by displaced cereal crops showed a substantial impact on the LCA analysis. However, C sequestration and the pre-crop yield effect of the ley could to some extent mitigate greenhouse gas emissions of the indirect land use.

The farming system

Organic cereal production in Norway is mainly an indirect land use of the organic livestock production. The sustainability of these two farming systems should therefore be evaluated together. However, investigations are usually performed within only one of the farming systems. Borgen et al. (2012) applied an ecosystem model to evaluate N dynamics in an organic green manure – cereal rotation in Norway. They found only modest potential for improvement of the system by management changes in ploughing time and crop rotation. Use of anaerobically digested green manure herbage as fertilizer or reintroduction of livestock on the arable farms were suggested as ways to substantially improve the organic cereal production system. Our study supports the suggested improvement by the use of herbage based digestate as fertilizer. Recycling of nutrients is an important aim in organic agriculture. There is also a large potential for the utilization of various organic wastes from society as nutrient sources, e.g. anaerobic digestion of household waste (Haraldsen et al. 2011).

Effects of climate change must also be taken into consideration in the evaluation of the alternative herbage management strategies. The annual mean temperature and precipitation are increasing in Northern Europe, and also the frequency of intense rainfall events (Olesen et al. 2011; Hov et al. 2013). Consequently, there will probably be an increase in the number of occasions on which N losses by leaching, runoff and gaseous emissions are likely to occur. This underlines the importance of removing the herbage, conserving its nutrients and returning them as fertilizer in the subsequent the growing season.

Conclusions and final remarks

This thesis analysed the N and C mineralization of soil organic matter and clover leaves in contrasting soil types under cold climate conditions, and how selected green manure herbage management alternatives influenced the N dynamics and the subsequent spring barley yield. In relation to the specific objectives, it can be concluded:

i) Of the various green manure herbage measures tested, the digestate strategy turned out to be the most promising option with regard to reduced risk of N losses and improved N recovery by a subsequent spring barley crop. In addition, the digestate improved the soil aggregate stability. At the farm scale, by applying only half of the N in the removed herbage, additional fertilizer as digestate remains available for application to crops on other fields as well. However, farmers are unlikely to adopt the digestate strategy unless cost-efficient and practical solutions become available for running herbage-based biogas plants under cold climate conditions.

Removal of the herbage reduces the risk of N losses form the crop rotation, since such losses are highly dependent upon the N input and weather conditions, particularly the amount and distribution of precipitation. The low apparent N recovery we found after mulching the green manure herbage suggests that the herbage should be removed and utilized. However, removal of the herbage reduced the barley grain yield, especially on the sandy loam soils. Removal of the herbage without any fertilizer application to the subsequent crop is therefore not recommended, unless the soil is very fertile.

ii) Soil type affected the decay rates of soil organic C and of clover leaves, but the sensitivity to temperature of the decay rates was not affected by soil type. The decay rate of clover was somewhat less temperature-sensitive than was that of soil organic C. The decomposition rates were higher in the sandy soil than in the clay soil, and this was also reflected in the field experiment. For modelling purposes, parameterized equations were presented for mineralization rates of soil organic C and clover leaves as functions of soil temperature in the range 0–15 °C. A positive priming effect occurred after incorporation of clover leaves, even at 0 °C. Regarding the modelling of such priming, there is further scope for relating it to litter decomposition.

iii) Relatively little amount of plant N mineralized in either the field or the laboratory experiment. However, substantial net N mineralization were found at temperatures less than 5 °C. The incubation experiment showed an initial rapid and substantial net N mineralization from the clover leaves, which was not reduced by low temperature. This was followed by slow net N mineralization in the sandy loam or net N immobilization for about four weeks in in the silty clay loam. The ratio of inorganic N to mineralized C was higher at low temperatures than at high temperatures during the first weeks of decomposition, showing that N mineralization is not simply a function of C mineralization.

The EU-Rotate_N model gave very poor simulation of the measured low level of inorganic N and the N dynamics in the soil during the six weeks after clover incorporation, although improvements were attempted by raising the decomposition rate of the fast decomposing litter pool at low temperature. A suggested approach for further model improvements is to alter the ratio between net N and C mineralization, by including a temperature response to microbial growth that would make the latter more inhibited by low temperature than is the decomposition of plant material.

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Paper I

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Effects of green manure herbage management and its digestate from biogas production on barley yield, N recovery, soil structure and earthworm populations

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ABSTRACT

In repeatedly mown and mulched green manure leys, the mulched herbage contains substantial amounts of nitrogen (N), which may only slightly contribute to the following crops' nutrient demand. The objective of the present work was to evaluate the effect of alternative strategies for green manure management on the yield and N recovery of a subsequent spring barley crop, and their short term effects on soil structure and earthworm populations. A field trial was run from 2008 to 2011 at four sites with contrasting soils under cold climate conditions. We compared several options for on-site herbage management and the application of anaerobically digested green manure herbage. Depending on the site, removal of green manure herbage reduced the barley grain yield by 0% to 33% compared to leaving it on-site. Applying digestate, containing 45% of the N in harvested herbage, as fertilizer for barley gave the same yields as when all herbage was mulched the preceding season. Overall, the apparent N recovery was enhanced from 7% when all herbage was mulched, to 16% when returned as digestate. A positive effect on earthworm density and biomass was seen after one season of retaining mulch material, rather than removing it. Digestate did not affect the earthworm population, but contributed to higher soil aggregate stability. In conclusion, for spring barley production after green manure ley, the digestate strategy increased N recovery and reduced the risk of N losses. The yield of the succeeding barley crop yield was reduced when N in herbage was not returned as mulch or digestate.

1. Introduction

Green manure leys are commonly used in organic cereal crop rotations to maintain soil fertility on stockless farms. Such full season grass-clover leys may increase yields through improved nitrogen (N) supply and through non-nutritional benefits such as improved soil structure, suppression of diseases and weeds, more earthworms and increased mycorrhizal activity (Cherr et al., 2006; Janzen and Schaalje, 1992; Riley et al., 2008). The green manure leys are generally grown as set-aside; managed by leaving the chopped herbage as mulch after frequent mowing during the growing season (Cormack et al., 2003; Stopes et al., 1996). The mowing is done as a means to control weeds and to keep the clover in a vegetative state and thus sustain high N_2 fixating activity and low C/N ratio (Dahlin and Stenberg, 2010).

Due to the accumulation of easily degradable N in green manure crops, current practice with repeatedly mowing and mulching means that substantial amounts of N in the herbage are at risk of being lost from the cropping system, both as gaseous emissions (NH_3, N_2O, NO and N_2) and through surface runoff or leaching of nitrate (NO_3^-) and soluble organic N (Askegaard et al., 2005; Korsaeth, 2012; Larsson et al., 1998; Möller and Stinner, 2009). Further, it is an expensive practice, using the land, establishing and managing the green manure for a whole season with no direct income, only the expectance of higher income from future crops on the field.

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That on-site mulched herbage contributes only slightly to the fulfilment of the following crops' nutrient demand has been demonstrated in several Scandinavian field trials (Engström et al., 2007; Frøseth et al., 2008; Solberg, 1995). As a consequence of herbage removal, one might expect decreased soil inorganic N availability for the green manure crop, which could enhance clover and N_2 fixation and thereby compensate for the lack of mulching in N pre crop effect. Hatch et al. (2007) found that removing cuttings from a grass-clover ley increased fixation, compared with mulching, but Dahlin and Stenberg (2010) found no differences. Neither of these studies included the N effect of these strategies on subsequent crops.

In spring barley, availability of inorganic N at the early tillering stage is a key factor for N uptake and dry matter (DM) yield (Hauggaard-Nielsen et al., 1998). Growing spring barley, based on the nutrients from a preceding green manure crop and without any additional nutrient input, is challenging under the cold Nordic climatic conditions with a short growing season. Borgen et al. (2012) concluded that there is a limited potential for improving N-use efficiency by management changes, in for example the time of ploughing and/or crop rotation, in stockless organic cereal production systems in Norway. For more substantial improvements, alternative strategies appear to be necessary. Application of digestate from green manure foliage digested anaerobically in a biogas plant may be a promising option for improving yields and N recovery instead of mulching (Möller and Müller, 2012; Stinner et al., 2008). In biogas plants, the easily degradable organic matter is digested, releasing methane for heating or fuel and residues (digestate). The latter contain plant available nutrients that may be applied as fertilizer in the subsequent season. To our knowledge, this strategy has not been compared previously with other strategies for green manure management under Nordic conditions.

Soil structure is important for the development of the barley crop (Arvidsson, 1999), both to create good conditions for root growth and for the turnover of soil organic matter (Breland and Hansen, 1996). The processes and mechanisms involved in soil aggregation is complex and can be affected through management practices (Bronick and Lal, 2005). Earthworm activity influences and normally improves soil structure and aggregate stability (Bronick and Lal, 2005; Edwards and Lofty, 1977; Marinissen, 1994). Although earthworm species have different feeding strategies, their excrements (casts) contain more plant available nutrients than does bulk soil (e.g. Buck et al., 1999; Haynes et al., 2003; Pommeresche and Løes, 2009). This finding supports the idea that one intensive year of "feeding" the soil with mulch material may improve soil structure and soil nutrient status.

The effects on earthworms when green manure herbage is removed and subsequently returned as digestate, instead of being mulched, have been little studied. Because the easiest available carbohydrates are converted to methane and removed, less energy and organic carbon (C) will be available for earthworms and other soil fauna. Ammonium and sulphide, which are toxic to earthworms (Curry, 1976) are formed by anaerobic digestion. Thus mulched green manure herbage may be more favourable to earthworms than anaerobically digested herbage.

The objective of the present work was to evaluate the effect of various strategies for green manure management on the yield and N recovery of a subsequent spring barley crop, and its short term effects on soil structure and earthworm populations in contrasting soils under cold climate conditions. The strategies involved different options for on-site herbage management and the application of anaerobically digested green manure herbage. The following hypotheses were tested:

- Removal of herbage, compared with mulching, will not affect the yield of a subsequent spring barley crop.
- Digestate applied as fertilizer for spring barley, compared with mulching the preceding season, will increase the crop yield and the proportion of N input by the green manure herbage that is recovered.
- Compared to herbage removal, mulching will not increase the amount of soil N available for a subsequent spring barley crop. On the contrary, digestate application will increase plant available N.
- Soil structure and earthworm populations will be negatively affected by removing the green manure herbage or by one application of digestate.

2. Materials and methods

2.1. Experimental sites, soil and weather conditions

Four field trials were established in 2008 at sites differing in soil characteristics and climatic conditions.

2.1.1. Weather and climate

The two neighbouring sites Kvithamar (63°29′ N, 10°52′ E) and Værnes (63°27′ N, 10°57′ E) share the same humid coastal climate in central Norway. Apelsvoll (60°42′ N, 10°51′ E) is situated inland, in eastern Norway with a drier climate and lower winter temperature. Ås (59°39′ N, 10°46′ E), in southeast Norway, represents an intermediate climate with respect to precipitation and winter temperature, but has the highest summer temperature of the sites. The normal values (1961–1990) for annual precipitation at Kvithamar/Værnes, Apelsvoll and Ås are 896, 600 and 785 mm, respectively, of which respectively 465, 319 and 382 mm occur during the growing season (May–September). The amounts of rainfall during the growing seasons of 2008/2009/2010 were 351/624/401 at Kvithamar/Værnes, 376/404/421 at Apelsvoll and 463/433/489 mm at Ås. The mean corresponding growing season temperatures in 2008/2009/2010 were 12.5/12.8/11.7, 12.8/13.1/12.4 and 13.4/13.8/13.1 °C, which are close to or above the normal values. During the winter prior to the barley crop (October 2009 – April 2010), the mean temperatures were −1.4, −3.2 and −1.7 at Kvithamar/Værnes, Apelsvoll and Ås. The corresponding amounts of precipitation were 534, 461 and 324 mm.

2.1.2. Soil properties

The soil at the sites is classified as a Mollic Gleysol, Arenic Fluvisol, Endostagnic Cambisol and Typic endoaqualf (IUSS Working Group WRB, 2006) for Kvithamar, Værnes, Apelsvoll and Ås. The soils at Ås and Kvithamar are derived from marine clay with relatively high silt contents, whilst that at Værnes overlies a coarse freshwater alluvium and that at Apelsvoll is developed from glacial till. The silty clay loam topsoil at Kvithamar is highly drought-resistant, but it overlies a very compact plough pan layer and compact subsoil with gley spots, both of which have low air and available water capacities. The clay loam topsoil at Ås is relatively drought-resistant and has a moderate air capacity, whereas the deeper soil layers are more compact, with low air and available moisture-holding capacities. At Værnes the soil is sandy loam and reasonably drought-resistant and well-aerated down to 0.5 m, but deeper layers have very low water-holding capacity and support little root growth. The soil at Apelsvoll is well-aerated sandy loam and relatively drought-resistant at all depths, and has few physical limitations to plant growth. The deeper subsoil (>0.6 m) is very compact. Information on the basic physical properties within soil profiles at the trial sites was obtained from previous studies performed at or close to the present locations (Table 1).

The topsoil at Kvithamar has a high C content, whereas the content is moderate at Apelsvoll and Ås and low at Værnes (Table 2). The C content in deeper horizons is very low, especially at Værnes and Ås. The level of total N is considerably higher at Kvithamar than

Table 1
Soil particle size distribution, bulk density, air capacity and moisture retention capacity.

Depth (m)	Sand	Silt	Clay	Gravel in whole sample	Bulk density	Air capacity[a]	Available water[b]	Wilting point[c]
	% of fine earth			%	g cm^{-3}	vol%		
Kvithamar[d]								
0–0.21	3	70	27	3	1.13	6	36	15
0.21–0.36	2	64	34	2	1.81	3	13	17
0.36–0.65	0	63	37	2	1.71	5	10	24
Værnes[e]								
0–0.30	51	43	6	0	1.53	8	27	6
0.30–0.50	73	25	2	0	1.62	9	26	3
0.50–0.62	92	7	1	0	1.54	33	4	1
Apelsvoll[f]								
0–0.25	55	31	14	11	1.47	12	21	10
0.25–0.50	55	32	13	12	1.52	14	18	9
0.50–0.60	51	32	17	11	1.5	16	16	10
Ås[g]								
0–0.30	22	43	35	8	1.36	9	20	19
0.30–0.39	14	46	40	4	1.62	5	9	27
0.39–0.70	15	46	39	4	1.58	5	10	26

[a] At 10 kPa.
[b] 10–1500 kPa.
[c] >1500 kPa.
[d] From profile no. 6 in Sveistrup et al. (1994).
[e] From profile 20177 in Solbakken (1987).
[f] Six profiles from Riley (unpublished).
[g] Østre Voll in Sveistrup et al. (1994).

at the other sites. In general the total N level followed that of C, with C/N ratios mostly in the ranges of 11–15 at Kvithamar, Værnes and Apelsvoll and 8–11 at Ås. The C and N was analysed using the Dumas combustion method (Bremmer and Mulvaney, 1982) on a Leco CHN 1000 analyzer (LECO Corp., St. Joseph, MI, USA).

The soil reaction in the topsoil, measured in water, was slightly above pH 6 at all sites. The topsoil contents of plant-available phosphorus and potassium were measured in 2008 by the ammonium acetate lactate method (AL, 0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75, ratio of soil weight to solution volume of 1:20, Égner et al. (1960)) as practice in Norway. The phosphorus level (P-AL) was very high at Værnes (155 mg kg^{-1}), medium at Kvithamar and Apelsvoll (66 and 75 mg kg^{-1}) and low at Ås (40 mg kg^{-1}). The potassium level (K-AL) was low at Værnes (36 mg kg^{-1}) and medium at the other sites (75 mg kg^{-1} at Ås, 89 mg kg^{-1} at Apelsvoll and 98 mg kg^{-1} at Kvithamar).

At Kvithamar, the soil had been farmed organically and according to a rotation dominated by grass-clover leys fertilized with animal manure from 1993 until 2003. From 2003 and until the start of the present experiment, the site had been cropped with cereals with low rates of fertilization and with breaks of green manure leys. At Værnes the soil had not been farmed organically. Here the crop rotations were dominated by annual crops until 2000 and by grass from 2001 to 2006. In 2007 the crop was cereals. The previous rotation at Apelsvoll was dominated by organically managed arable crops. In 2007 the field was fallowed with repeated harrowing. At Ås the soil had been managed organically since 1993 with ley-arable crop rotation until about 2000, then mainly with spring cereals and undersown clover every year until 2008. Chemical weed control with glyphosate was performed before ploughing in spring 2008.

2.2. Crop management and experimental treatments

The green manure was a grass-clover ley established in spring as an undercrop in cereals. The year after, the ley was cut several times and the herbage was chopped and left on the stubble (mulched), which is according to the most common practice for management of green manure leys in Norway. In addition to mulching, the treatments included removal of the herbage and application of digestate in the following year (Table 3). A control treatment with repeated cereal cropping was also included. An overview of crops and management is given in Table 4.

In spring 2008 the green manure plots were sown with 2 g m^{-2} seed mixture consisting of 20% red clover (*Trifolium pratense* L. cv. Nordi), 10% timothy (*Phleum pratense* L. cv. Grindstad), 35% meadow fescue (*Festuca pratensis* L. cv. Fure) and 35% perennial ryegrass (*Lolium perenne* L. cv. Napoleon), as an undercrop in unfertilized spring barley (*Hordeum vulgare* L. cv. Sunnita at 16 g m^{-2}). On the control plots, unfertilized spring barley was sown in pure stand. The barley straw was removed after grain harvest.

In 2009 the green manure leys (G) were cut three times. The chopped herbage was either mulched after all cuts (mulched three times = G-3M), or removed twice and mulched after the last cut (mulched one time = G-1M), or removed after all three cuts (mulched zero times = G-0M). The first cut was carried out when

Table 2
Means (±SD) of total C and N (% of fine earth) at various sampling depths (cm) at the four sites. Samples taken from all treatments were pooled blockwise before analysis ($n = 4$).

Depth	Total-C				Total-N			
	Kvithamar	Værnes	Apelsvoll	Ås	Kvithamar	Værnes	Apelsvoll	Ås
0–20	4.90 (1.57)	1.39 (0.07)	2.14 (0.17)	2.08 (0.08)	0.40 (0.11)	0.11(0.01)	0.21(0.03)	0.20 (0.01)
20–30	4.40 (1.37)	1.26 (0.05)	1.76 (0.25)	1.61 (0.26)	0.36 (0.10)	0.09 (0.01)	0.17 (0.03)	0.15 (0.02)
30–60	1.08 (0.47)	0.38 (0.04)	0.79 (0.14)	0.46 (0.08)	0.09 (0.04)	0.01[a]	0.07 (0.02)	0.05 (0.01)

[a] $n = 1$.

Table 3
Overview of treatments in the 3-year crop rotation. 2008 was a preparatory year with establishing of green manure (G) in spring barley. In 2009 the green manure herbage was removed (0 M) or mulched (1 M or 3 M). Unfertilized oats (C) was control. In the following spring, green manure was ploughed under and barley was sown and fertilized with digestate (D) at 11 g N m^{-1}, inorganic fertilizer (I) at 8 g N m^{-1} fertilization or unfertilized.

Term	2008	2009	2010
G-3M	Barley with G undersown	G 3 cuts mulched	Barley
G-1M	Barley with G undersown	G 2 cuts removed, 3rd mulched	Barley
G-0M	Barley with G undersown	G 3 cuts removed	Barley
G-0M-D	Barley with G undersown	G 3 cuts removed	Barley + digestate
C-D	Barley	Oats	Barley + digestate
C-I	Barley	Oats	Barley + fertilizer

timothy reached late stem elongation, i.e. just before inflorescences were visible on 10% of the shoots, next after 600–650 day degrees (base temperature 0 °C) from the first cut and the third in mid-September. The stubble height of the green manure was 5–8 cm.The control plots (C) were sown with unfertilized oats (*Avena sativa* L. cv. Gere, Table 3). The oat straw was removed at all sites except Apelsvoll. After grain harvest in plots without undercrop in 2008 and 2009, some native white clover (*Trifolium repens* L.), couch grass (*Elymus repens* L.) and dicotyledonous weeds emerged. These were removed by hand weeding or frequent mowing using a small lawn mower with a rotor working at the soil surface.

In spring 2010 the leys were ploughed under, and spring barley (*Hordeum vulgare* L. cv. Tiril at 20 g m^{-2}) was sown (Table 4). Prior to sowing, herbage-based digestate (D) was applied to half of the plots where green manure herbage had been removed (G-0M-D), and to half of the plots with preceding oats (C-D, Table 3). The digestate contained 11 g total N and 6 g ammonia N m^{-2}, which corresponded to about 45% of the total N harvested as herbage, and in addition 1.6 g P m^{-2} and 7.7 g K m^{-2}. It was applied on the soil surface and harrowed down on the same day, or by the Direct Ground Injection (DGI) technique to 6–8 cm depth in 0.3 m rows (Apelsvoll). On control plots (C-I), 8 g N, 1.5 g P and 9 g K m^{-2} were applied as inorganic fertilizers. The proportion of NO_3-N of the total fertilizer N, varied from 20% (Apelsvoll) to 90% (Ås).

After harvesting of barley grain and straw, the fields were harrowed to control perennial weeds, except at Kvithamar where such

Table 4
Overview of crops and operations at the four sites during the experiment.

	Treatment dates			
	Kvithamar	Værnes	Apelsvoll	Ås
Preparatory year 2008				
Ploughing	28 October 07	15 April	30 October 07	19 May
Soil sampling	28 April	29 April	21 May	20 May
Barley sowing	28 April	29 April	21 May	20 May
Green manure sowing	29 April	29 April	21 May	22 May
Barley harvest	12 August	16 August	18 September	1 September
Green manure sampling	22 September	23 September	6 October	7 October
Soil sampling	22 October	23 October	23 October	4 November
Green manure 2009				
Soil sampling	28 April	29 April	6 May	4 May
Oats sowing	29 April	29 April	6 May	5 May
Green manure first cut	1 June	5 June	17 June	3 June
Soil sampling	1 June	5 June	17 June	8 June
Soil sampling	11 June	15 June	26 June	15 June
Soil sampling	20 June	25 June	7 July	24 June
Irrigation[a]	–	–	2 June	26 June, 2 July
Green manure second cut	17 July	22 July	6 August	17 July
Oats harvesting	27 August	25 July	21 August	23 August
Green manure third cut	15 September	15 September	24 September	15 September
Green manure sampling	21 October	21 October	19 October	24 October
Soil sampling	21 October	21 October	13 October	30 October
Barley cropping 2010				
Soil sampling	23 April	22 April	5 May	22 April
Ploughing	7 May	26 April	6 May	23 April
Digestate application	11 May	6 May	24 May	12 May
Fertilizer application	14 May	8 May	20 May	12 May
Harrowing	14 May	7 May	–	12 May
Barley sowing	14 May	12 May	24 May	14 May
Weed harrowing	21 May	19 May	31 May	–
Soil sampling	31 May	27 May	7 June	31 May
Barley sampling	20 June	15 June	26 June	11 June
Barley sampling	5 July	1 July	5 July	28 June
Irrigation[b]	–	–	–	29 June, 9 July
Barley harvesting	26 August	19 August	2 September	31 August
Harrowing	–	10 September	6 September	7 September
Soil sampling	27 August	20 August	3 September	1 September
Soil sampling	29 October	2 November	15 October	26 October
Carry over effect 2011				
Soil sampling	16 May	18 May	19 May	20 May

[a] Apelsvoll: 30–35 mm; Ås: 25 mm each time.
[b] Ås: 20–25 mm and 25–30 mm.

a treatment was considered to increase the growth of weeds and native clover (Table 4). No further management was carried out until field trials were terminated at the end of May 2011.

The experimental lay out was a randomized block design with 4 replicates, but the plots with the two control treatments were always placed beside each other for practical reasons. The gross size of individual plots depended on the implements available at each site, and varied from 48 to 60 m^2.

2.3. Harvest, plant and soil sampling

2.3.1. Plant sampling

Yields of barley (grain and straw separately) and of the three leys cuts were harvested with experimental plot harvesters on 1.5 m × 4–5 m subplots. From the harvested grass-clover herbage (and barley straw), representative subsamples were sorted manually and later dried for determination of species composition. N and moisture content in the straw and herbage were determined plotwise in other sub-samples dried at 60 °C. On G-3M and at the last cut of G-1M, the raw herbage was, after weighing, manually redistributed on the harvested area and finely chopped with a stubble cutter.

Samples of the standing green manure biomass were taken as late as possible (late October) before frost in 2008 and 2009 (Table 4), by cutting plants at the soil surface on 0.25 m^2 within each plot. In 2010 above-ground biomass of barley was sampled twice early in the growing season (Table 4), by cutting two subplots of 0.25 m^2 at ground level on each plot, first at 250–330 day degrees from plant emergence (base temperature 0 °C), then at flag leaf sheath opening (growth stage 47 in the BBCH scale, Lancashire et al., 1991). The biomass of the stubble left after harvest was recorded similarly. It was only recorded on plots receiving digestate (G-0M-D and C-D), because stubble biomass is found to be relatively little affected by fertilizer treatments (Bleken, 1990).

Dried plant samples were finely milled (Cyclotec, mesh size 1 mm) before determination of total N using the Dumas method mentioned earlier.

2.3.2. Soil sampling

The content of inorganic N (NO_3-N and NH_4-N) was determined in soil samples collected to 20 cm depth on 12 sampling dates selected for their likelihood of showing differences in mineral N fluxes (dates are given in Table 4). In addition, late very autumn and in spring 2010 and 2011 soil was sampled in three more layers to 80 cm depth (20–30, 30–60 and 60–80). Composite sample of 6 soil cores were taken on each plot and stored frozen. Before all the differences between treatments were established, samples from equally treated plots within each block were pooled. Approximately 300–500 g of soil was coarsely ground without thawing and a subsample of 100 g was used for gravimetric determination of moisture content by drying at 105 °C. Another subsample of 40 g was extracted with 200 ml 1 M KCl, and the supernatant analysed by spectrophotometry on a FIAstar^{TM} 5000 Autoanalyser (Foss Tecator AB, Höganäs, Sweden, Application Notes 5232 and 5226 (2001) for NO_3-N and NH_4-N, respectively). Results were expressed on a dry weight basis and converted to area units using appropriate bulk density values (Table 1).

Total soil C and N were analysed in samples taken at the end of the trial, as described for inorganic N. Samples from treatments within the same block were pooled, ground in a mortar and analysed by the Dumas method mentioned earlier.

In order to assess possible effects on soil structure of two years with green manure crops versus continuous cereal growing, sampling was performed after ploughing and harrowing in spring 2010 soon after digestate application. Five subsamples were taken by spade, altogether 5–6 dm^3 of soil, from the seedbed down to 5–6 cm depth on each plot with treatments G-3M, G-0M-D and C-D. The samples were air dried in open containers at room temperature for several months before analysis.

Aggregate size distribution (5 groups: <2 mm, 2–6 mm, 6–10 mm, 10–20 mm, >20 mm) was found by dry sieving for 2 min on a reciprocating shaker containing sieves with mesh openings of 2, 6, 10 and 20 mm. Stones were removed. Aggregate size groups were calculated on a weight basis and the mean weight diameter was expressed using the formula of Van Bavel (1949), assuming a maximum clod size of 35 mm.

The stability of aggregates to simulated rainfall was measured for aggregate sizes of 2–6 and 6–10 mm, using similar apparatus as that described by Njøs (1967). These aggregate size fractions accounted for 50% of the total soil samples at Ås, 42% at Kvithamar, 37% at Apelsvoll and 24% at Værnes. Two subsamples (40 g) of each size group were placed within a radius of 0.15 m and subjected to simulated rain for 2 min (pressure 1 bar, Hardi 4110-20 nozzles, nozzle height 0.35 m and ca. 70 passes). Aggregate stability is given as the weight percentage of aggregates remaining on the sieve.

2.4. Apparent recovery of nitrogen

The apparent recovery of N in grain or above-ground biomass of barley from mulched green manure or digestate was assessed by subtracting total N yield in the treatment with no mulch left behind (N yield$_{G\text{-}0M}$) from the total N yield (N yield) in treatments receiving mulch (G-3M or G-1M) or digestate (G-0M-D), and expressed as a percentage of the amounts of N applied as mulch or digestate (N applied):

$$\text{Apparent N recovery}\,(\%) = 100 \times \frac{\text{N yield} - \text{N yield}_{G-0M}}{\text{N applied}} \tag{1}$$

2.5. Earthworm sampling and analysis

Earthworms were sampled at Kvithamar and Værnes after the last cut in 2009 and after grain harvest in 2010. Two soil cubes (0.2 m × 0.2 m × 0.2 m) were removed in all plots of the four treatments G-3M, G-0M, G-0M-D and C-D. The earthworms were sorted from the cubes by hand. Their total biomass was recorded as g m^{-2} after a short storage in 75% alcohol. The density, individuals m^{-2}, included both juvenile and adult worms. All earthworms were identified to species according to the identification key of Sims and Gerard (1999).

2.6. Statistical analysis

Analysis of variance (ANOVA) was performed using a general linear model (GLM) on soil inorganic N data, plant yields, N uptake, N concentrations and clover proportion. Analyses were performed for all sampling occasions for each site and in total using recordings for single plots as input data and block as random effect. For the barley yields in 2008, before the different treatments took place, we tested that the variance between plots was smaller than between blocks. For multiple comparisons tests, Tukey HSD procedure was used. The statistical software package R was used for these calculations (R Core Team, 2012).

For soil aggregate data ANOVA were performed, using a split-plot design with trial site as the main factor and green manure treatment as the split-plot factor (Minitab 15, Minitab Inc. State College, Pennsylvania, USA). For multiple comparisons the tests LSD procedure was used. For earthworm parameters, ANOVA was performed for each site and year separately, using the two samples in each plot as separate input data, and block as random effect. Biomass was analysed by a linear mixed model (MIXED), while the number of earthworms was analysed by the generalized linear

Table 5
Green manure 2009: Biomass (g DM m^{-2}), N content (g m^{-2}) and clover proportion in three consecutive cuts (±S.E), where the herbage was either mulched (G-3M) or removed (G-0M).

	1st cut	2nd cut		3rd cut	
	G-3M/G-0M	G-3M	G-0M	G-3M	G-0M
Kvithamar					
Biomass	296 (9)	461 (27)	495 (7)*	286 (13)	290 (8)
Nitrogen	7.1 (0.3)	10.3 (1.0)	10.8 (0.3)	8.4 (0.3)	7.8 (0.3)
Clover fraction	0.55 (0.10)	0.54 (0.10)	0.65 (0.05)	0.43 (0.04)	0.53 (0.04)
Værnes					
Biomass	321 (14)	439 (23)	429 (8)	173 (8)	178 (3)
Nitrogen	8.3 (0.5)	9.3 (0.5)	8.8 (0.6)	5.0 (0.1)	5.6 (0.3)
Clover fraction	0.71 (0.03)	0.84 (0.05)	0.83 (0.02)	0.69 (0.06)	0.64 (0.03)
Apelsvoll					
Biomass	355 (11)	316 (24)	383 (12)*	52 (6)	99 (4)*
Nitrogen	8.5 (0.5)	8.5 (0.6)	9.1 (0.5)	1.8 (0.3)	3.1 (0.3)*
Clover fraction	0.83 (0.03)	0.87 (0.02)	0.95 (0.01)*	0.63 (0.04)	0.70 (0.05)
Ås					
Biomass	366 (8)	174 (9)	182 (6)	326 (20)	327 (12)
Nitrogen	6.9 (0.3)	4.8 (0.3)	5.1 (0.3)	7.6 (0.6)	7.9 (0.1)
Clover fraction	0.34 (0.05)	0.67 (0.03)	0.74 (0.04)	0.79 (0.01)	0.85 (0.02)

* $P \leq 0.05$ for the test G-3M ≠ G-0M for each site × cut combination.

mixed model (GLIMMIX, with negative binomial distribution and *ln* as link function), both by SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA). For multiple comparisons tests, Tukey procedure was used.

In all tests, significance was assumed at *P*-levels < 0.05.

3. Results

3.1. Nitrogen and dry matter yields

3.1.1. Green manure and cereals, 2008–2009

In the establishment year, the green manure undercrop reduced grain yield compared to the pure stand, by 22% at Kvithamar, 15% at Apelsvoll and 7% at Værnes ($P = 0.058$), but no reduction was seen at Ås. At the four sites the mean grain DM yield of barley with undersown green manure ranged between 194 and 262 g m^{-2}. The standing herbage biomass and N content of the green manure in late autumn 2008 was markedly higher at the two northern sites than at the others, likely caused by earlier grain harvesting at the northern sites. The biomass was 297 and 240 versus 76 and 113 g DM m^{-2} at Kvithamar, Værnes, Apelsvoll and Ås, respectively. The corresponding N contents were 8.9 and 6.7 versus 1.7 and 3.0 g N m^{-2}.

All leys survived the winter well. In 2009, the average for the two treatments in accumulated herbage biomass for three cuts was 1071, 929, 808 and 873 g DM m^{-2} at Kvithamar, Værnes, Apelsvoll and Ås (Table 5). Herbage removal increased the DM yield of the second and third cuts at Apelsvoll and of the second cut at Kvithamar, but it did not affect the regrowth at Værnes and Ås. Similarly, herbage removal had either no effect on the total amount of N at the second and third cut, or slightly increased it in the case of Apelsvoll.

The estimated C/N ratio in the herbage ranged between 12 and 22, based on measured N (Table 5) and C contents in similar plant material analysed by Marstorp and Kirchmann (1991) and Thorup-Kristensen (1994). In general, the estimated lowest C/N ratio is for the herbage in the third cut.

Clover was already abundant at the first cut, and dominated over grasses at the second and third cuts (Table 5). Mulching significantly reduced the proportion of clover at Apelsvoll. Similar trends were seen at Kvithamar and Ås but not at Værnes. The clover biomass was significantly ($P < 0.05$) larger after herbage removal for Kvithamar, Apelsvoll and Ås averaged over the three sites (data not shown). The total N yield in the harvested herbage (G-0M) ranged between 19 and 26 g N m^{-2}, with the largest N yield at Kvithamar (Table 5). The standing biomass in late autumn 2009 contained between 2.4 and 4.7 g N m^{-2}. Again the highest biomass and N yield was observed at Kvithamar, and there was no effect of previous herbage management.

3.1.2. Barley, 2010

Early above-ground biomass and N content in the barley ranged the treatments approximately in the order C-I ≥ C-D ≥ G-0M-D ≥ G-3M > G-0M (Table 6). This indicates that green manure provided less readily available N than did digestate or the use of 8 g N m^{-2} fertilizer. Furthermore, removal of the herbage, rather than mulching, reduced the N supply to the young barley crop. Later, at the flag leaf stage, DM and N content still followed the same pattern. At Apelsvoll, however, digestate had a less positive effect on the young barley crop than at the other sites, with no differences between G-0M and G-0M-D. The biomass and especially the N uptake was higher on the sandy soil at Værnes than on the clay soil at Kvithamar, and the differences increased from the first to the second sampling date, in spite of the facts that the N yield of the green manure had been somewhat higher at Kvithamar (Table 5) and that the two sites were exposed to the same cold weather in early summer 2010. Early in the growing season, chlorosis typical of N deficiency was observed in the treatments where the herbage had been removed the previous year (G-0M and G-1M), particularly on the two clay soils (Kvithamar and Ås). It was most severe at Kvithamar, where the average soil temperature at 10 cm depth was 10.6 °C from plant emergence to 1st sampling, compared to 13.3 °C at Ås.

In order to explore the N state of the young plants we plotted them against published critical and minimum N dilution curves for winter and spring wheat (Justes et al., 1994; Ziadi et al., 2010). In all cases N concentration was far below the critical dilution curves, and very close or even below the minimum curves (Fig. 1), particularly in the case of plots where the green manure herbage had been removed (G-0M).

Barley grain yields in 2010 were close to the national average for conventional farming, about 300 g DM m^{-2}, except at Kvithamar, where the yield was only half of that. The barley crop there was particularly low on one of the blocks, apparently due to poor soil structure. At Ås, high precipitation after ripening delayed harvesting and reduced the recovered yield, especially on the most productive plots, where up to 90% lodging occurred and at least 10% of the ears remained on the ground after harvest. No correction was made for these losses.

Table 6
Biomass (g DM m^{-2}) and nitrogen (g m^{-2}) in barley plants in 2010 at 250–330 day degrees, DD, (with base temperature 0 °C) and at growth stage 47 (flag leaf sheath opened) according to the BBCH scale. Abbreviations for the treatments are explained in Table 3. Within a site, treatment means (±S.E) which do not share any letter in common are significantly different ($P < 0.05$) by Tukey HSD method. The highest value is shown as a.

	G-3M	G-0M	G-3M-D	C-D	C-I
	250–330 DD				
Kvithamar					
Biomass	43 (7) bc	31 (4) c	65 (7) ab	70 (7) a	58 (4) ab
Total N	1.17 (0.20) bc	0.72 (0.15) c	1.49 (0.23) abc	1.85 (0.22) ab	2.11 (0.14) a
Værnes					
Biomass	42 (9) bc	20 (1) c	79 (8) a	88 (10) a	70 (9) ab
Total N	1.80 (0.35) bc	0.92 (0.07) c	2.81 (0.38) ab	3.38 (0.49) a	3.18 (0.47) ab
Apelsvoll					
Biomass	60 (5) b	54 (6) b	53 (5) b	40 (8) b	110 (16) a
Total N	1.92 (0.13) ab	1.64 (0.14) bc	1.79 (0.15) bc	1.16 (0.20) bc	3.19 (0.54) a
Ås					
Biomass	70 (3) bc	64 (3) c	83 (3) bc	109 (2) ab	139 (2) a
Total N	2.05 (0.15) b	1.78 (0.15) b	2.16 (0.13) b	2.68 (0.02) b	3.88 (0.18) a
Growth stage 47					
Kvithamar					
Biomass	129 (22) bc	87 (7) c	159 (16) bc	186 (25) ab	245 (18) a
Total N	1.71 (0.29) b	1.11 (0.15) b	1.84 (0.23) b	2.20 (0.28) b	3.97 (0.32) a
Værnes					
Biomass	168 (10) b	62 (6) c	263 (12) a	270 (11) a	259 (20) a
Total N	3.68 (0.16) b	1.69 (0.14) c	4.29 (0.22) ab	4.28 (0.26) ab	5.20 (0.58) a
Apelsvoll					
Biomass	149 (13) b	125 (11) b	126 (9) b	118 (28) b	218 (16) a
Total N	3.45 (0.28) ab	2.59 (0.22) b	3.36 (0.20) ab	2.38 (0.36) b	4.02 (0.15) a
Ås					
Biomass	111 (15) bc	103 (26) c	136 (13) bc	177 (8) ab	226 (14) a
Total N	3.04 (0.45) ab	2.48 (0.31) b	3.20 (0.40) ab	3.89 (0.38) ab	5.66 (1.22) a

At all sites there was a consistent trend for grain DM and N yields in the order C-I ≥ G-0M-D ≥ G-3M ≥ C-D ≥ G-1M ≥ G-0M (Table 7). Relative to the early growth stage, the ranking of the G-3M and G-0M-D treatments had improved, but that of C-D was less good. N uptake after growth stage 47 up to maturity was lowest in treatment C-D and highest on treatment G-0M-D (1.8 and 4.6 g N m^{-2}, respectively, averaged over all sites). Furthermore, C-D had the lowest N harvest index, i.e. proportion of N in grain relative to the total N in the above-ground biomass (data not shown). The mulching of even a single cut (G-1M) improved grain DM and N yields, compared to G-0M, and mulching three times raised them evidently (Table 7). At site level, the latter was significant on the two sites with sandy loam (Værnes and Apelsvoll). The increased N yield in grain caused by previous green manure stubble and roots, as seen by comparing G-0M-D and C-D, ranged from 0.1 to 1.8 g N m^{-2} (Table 7).

The N concentration of the grain was higher after green manure than in C-I at the two northern sites (Kvithamar and Værnes), and a similar tendency was present at Ås (Table 7). This indicates that mineralization of green manure residues during late summer contributed positively to grain protein. At Kvithamar and Værnes, application of digestate on green manure (G-0M-D) diluted the grain N% to the same level as that of the C-I plots.

The biomass of the barley stubble, found in G-0M-D, and used for the calculations for the apparent N recovery, contained 0.46, 0.69, 0.26 and 0.28 g N m^{-2} for Kvithamar, Værnes, Apelsvoll and Ås.

3.2. *Apparent recovery of nitrogen*

The apparent N recovery from mulched green manure herbage or digestate in above-ground barley biomass or grain was low and in the order of G-0M-D > G-1M > G-3M (Table 8). The ranking reflects the level of N input to the systems, but also the lower N yield of G-1M relative to the other treatments.

3.3. *Inorganic nitrogen in soil*

No high levels of inorganic N caused by mulching were found in either the top-soil (Fig. 2) or in deeper soil layers (data not shown).

In spring 2009, one year after the green manure ley was established, the level of NO_3-N in the soil was lower in plots with green manure than in the control plots with barley stubble only, at all sites except Kvithamar (Fig. 2).

Ten and twenty days after the first cut, there were no significant differences in inorganic N in the soil layer 0–20 cm, whether or not the green manure herbage had been mulched.

At all sites, the NO_3-N or inorganic N in soil in late autumn 2009 was higher with G-3M than with G-0M, but both levels were below 1 g m^{-2}. From late autumn 2009 until spring 2010, the level of inorganic N at 0–20 cm increased in all treatments by 0.4–1.0 g N m^{-2}. The temperature at 10 cm depth after soil sampling in autumn was on average 2 °C for 1.5–2 months, then below freezing for 3.5 months and finally 2–4 °C for 10–20 days before sampling in spring.

In spring 2010, before the green manure was ploughed under, there was at all sites a higher level of inorganic N in soil with mulched green manure (G-3M), compared to the other treatments. However, 3–5 weeks after ploughing, two weeks after germination of the barley crop, there were no differences in the levels of inorganic-N in the top-soil between treatments G-3M and G-0M. Application of digestate (G-0M-D) tended ($P = 0.057$) to enhance the amount of inorganic N in the top-soil at Apelsvoll compared to mulching. No such differences at the other sites, and in general a lower soil inorganic N content, may be a consequence of higher precipitation from digestate application to soil sampling at these sites (45–78 mm) than at Apelsvoll (8 mm).

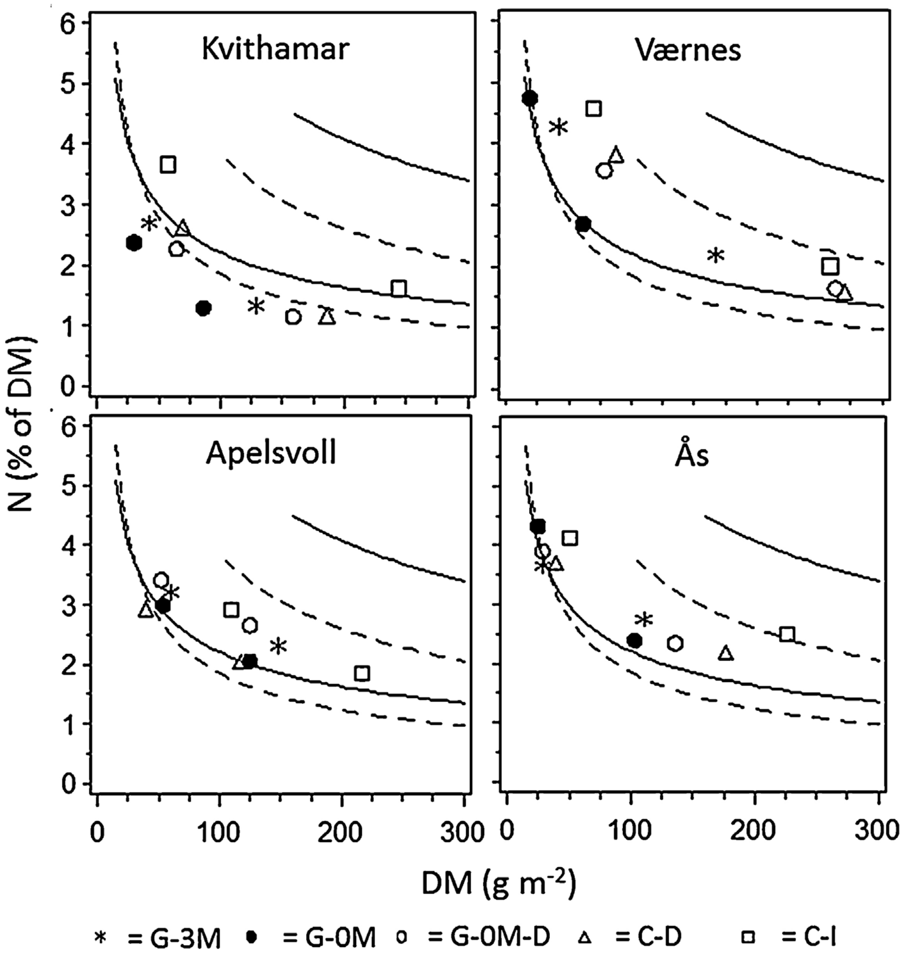

Fig. 1. N concentration (% of DM) versus standing biomass (g DM m^{-2}) at 250–330 day degrees and at growth stage 47. Weighted averages of fours replicates. Upper lines are the critical N dilution curve according to Justes et al. (1994) (continuous line) or Ziadi et al. (2010) (dashed line). Lower lines are the minimum N concentration curves by the same authors. Abbreviations for the treatments are explained in Table 3. The statistical differences between the treatments are given in Table 6.

Table 7
Barley grain and straw DM and N yield (g m^{-2}), and grain N concentration (%), at the different sites in 2010. Abbreviations for the treatments are explained in Table 3. Within a site, treatment means (±S.E) which do not share any letter in common are significantly different ($P < 0.05$) by Tukey HSD method. The highest value is shown as a.

		G-3M	G-1M	G-0M	G-0M-D	C-D	C-I
Kvithamar	Grain DM	140 (15) bc	122 (11) bc	89 (9) c	155 (20) b	156 (14) b	264 (8) a
	Grain N conc.	1.52 (0.05) a	1.50 (0.02) ab	1.50 (0.03) ab	1.29 (0.04) c	1.23 (0.04) c	1.35 (0.01) bc
	Grain N yield	2.1 (0.2) b	1.8 (0.1) b	1.3 (0.3) b	2.0 (0.2) b	1.9 (0.2) b	3.6 (0.1) a
	Grain + straw DM	360 (31) b	337 (26) b	276 (22) b	385 (32) b	386 (29) b	568 (11) a
	Grain + straw N yield	3.3 (0.1) b	2.8 (0.2) b	2.3 (0.1) b	3.3 (0.5) b	3.3 (0.3) b	5.7 (0.4) a
Værnes	Grain DM	327 (16) ab	260 (28) bc	217 (16) c	362 (11) a	310 (10) ab	392 (29) a
	Grain N conc.	1.83 (0.02) a	1.76 (0.03) a	1.84 (0.03) a	1.64 (0.02) b	1.48 (0.01) c	1.63 (0.03) b
	Grain N yield	6.0 (0.3) ab	4.6 (0.3) bc	4.0 (0.2) c	5.9 (0.4) ab	4.6 (0.1) bc	6.4 (0.5) a
	Grain + straw DM	613 (24) bc	481 (31) cd	474 (51) d	689 (22) ab	666 (20) b	803 (36) a
	Grain + straw N yield	7.6 (0.4) ab	5.6 (0.4) c	5.5 (0.6) c	7.2 (0.3) abc	6.0 (0.2) bc	8.4 (0.5) a
Apelsvoll	Grain DM	322 (11) ab	260 (15) bc	249 (10) c	347 (15) a	253 (21) bc	372 (14) a
	Grain N conc.	1.74 (0.05)	1.75 (0.13)	1.61 (0.02)	1.75 (0.02)	1.70 (0.01)	1.68 (0.14)
	Grain N yield	5.6 (0.2) a	4.5 (0.1) b	4.0 (0.2) b	6.1 (0.1) a	4.3 (0.4) b	6.2 (0.3) a
	Grain + straw DM	482 (22) b	376 (20) c	377 (22) c	523 (25) b	397 (27) c	573 (24) a
	Grain + straw N yield	6.6 (0.1) a	5.2 (0.1) b	4.7 (0.2) b	7.3 (0.3) a	5.3 (0.5) b	7.3 (0.3) a
Ås	Grain DM	254 (18) b	252 (25) b	241 (13) b	313 (24) ab	279 (12) ab	356 (18) a
	Grain N conc.	1.95 (0.05)	1.92 (0.07)	1.83 (0.05)	1.72 (0.05)	1.71 (0.04)	1.89 (0.08)
	Grain N yield	4.9 (0.4) ab	4.8 (0.3) b	4.4 (0.5) b	5.4 (0.4) ab	4.8 (0.3) b	6.7 (0.6) a
	Grain + straw DM	427 (38) d	424 (35) d	397 (18) d	499 (41) c	508 (24) b	637 (40) a
	Grain + straw N yield	6.5 (0.6) b	6.6 (0.4) b	6.2 (0.8) b	7.8 (1.2) ab	7.7 (1.0) ab	9.7 (0.7) a
All sites	Grain DM	260 (21) bc	223 (18) cd	199 (18) d	291 (23) ab	249 (17) bcd	346 (15) a
	Grain N yield	4.7 (0.4) bc	3.9 (0.3) cd	3.4 (0.3) d	4.8 (0.5) b	3.9 (0.3) cd	5.7 (0.4) a
	Grain + straw DM	402 (24) bc	336 (17) c	312 (21) d	453 (29) b	421 (25) bc	573 (21) a
	Grain + straw N yield	6.2 (0.4) b	5.3 (0.3) bc	4.7 (0.4) c	6.3 (0.6) b	5.6 (0.5) bc	7.8 (0.4) a

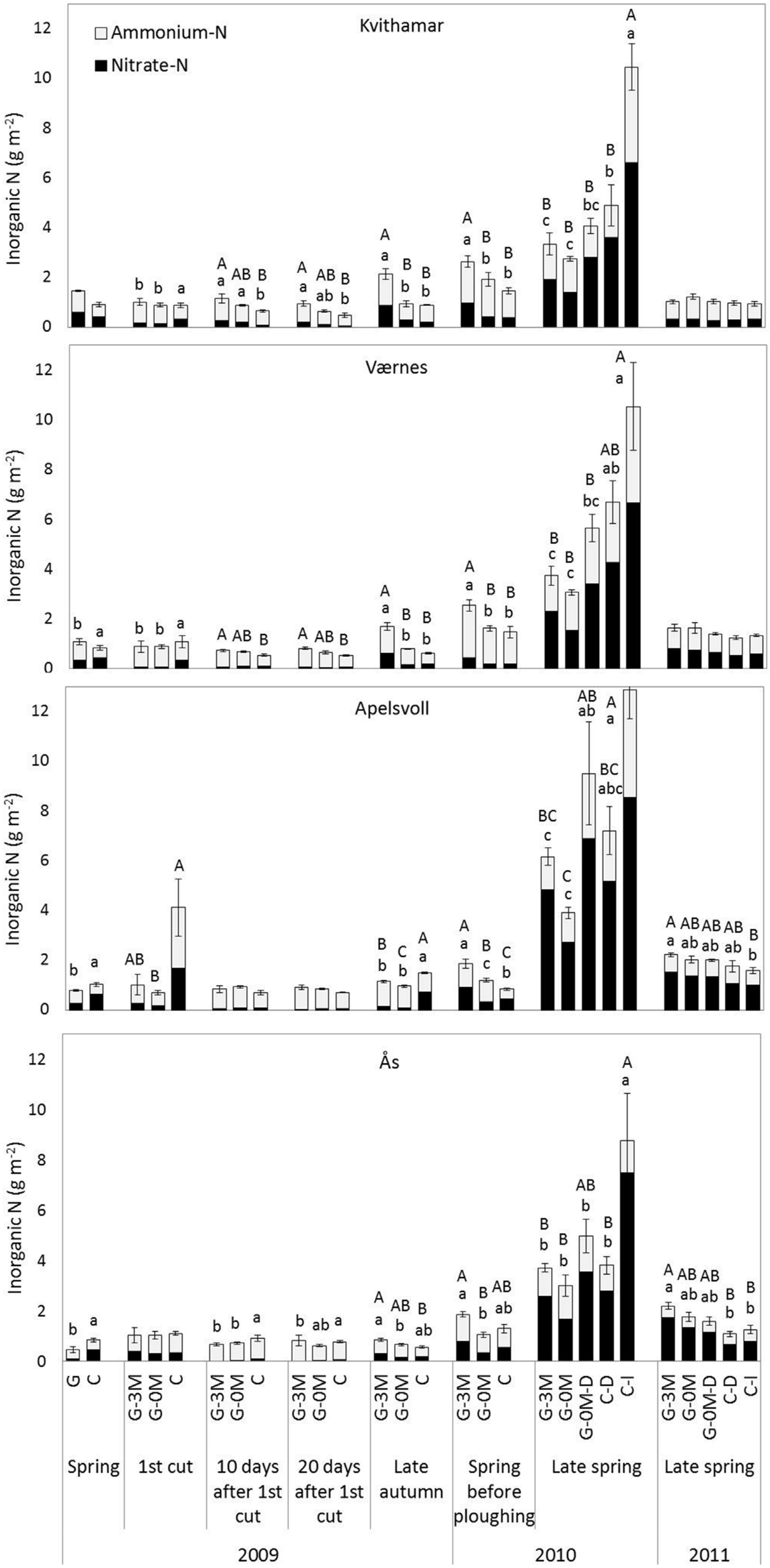

Fig. 2. Inorganic N in soil (0–20 cm depth) in spring 2009, at first cut and until 20 days after, in late autumn after the green manure treatments, in spring 2010 before ploughing and 7–10 days after the germination of the barley crop, and in May 2011. Abbreviations for the treatments are explained in Table 3. Bars (±S.E) within each sampling which do not have any letter in common are significantly different ($P < 0.05$) by Tukey HSD method. Upper case letters show inorganic N and lower case letters show nitrate-N.

Table 8
Apparent recovery (%) by spring barley (grain or above-ground biomass) of N applied as mulched herbage (G-3M or G-1M) or digestate (G-0M-D). See Eq. (1) for calculations. For digestate, the results are calculated in total N, as well as for NH_4-N. Abbreviations for the treatments are explained in Table 3. Within sites, treatment means for total N which do not share any letter in common are significantly different ($P < 0.05$) by Tukey HSD method. The highest value is shown as a.

	G-3M$_{\text{Total N}}$	G-1M$_{\text{Total N}}$	G-0M-D$_{\text{Total N}}$	G-0M-D$_{\text{NH4-N}}$
Kvithamar				
Grain	3	7	6	12
Above-ground biomass	6	11	10	18
Værnes				
Grain	9	10	18	34
Above-ground biomass	9 ab	1 b	16 a	29
Apelsvoll				
Grain	8	16	19	36
Above-ground biomass	10	16	24	44
Ås				
Grain	3	5	9	17
Above-ground biomass	2	5	15	29
All sites				
Grain	6 b	10 ab	13 a	25
Above-ground biomass	7 b	8 ab	16 a	30

After the barley crop, there was no significant difference in the level of soil inorganic N related to herbage management, but soil inorganic N was in general higher with previous green manure than without, though in most comparisons at site level it was not statistically significant.

3.4. Soil aggregate size distribution and aggregate stability

There were significant overall differences between treatments on the stability of both aggregate size groups (2–6 and 6–10 mm), with no significant interaction with site (Table 9). Treatment G-0M-D gave greater stability than did treatments G-3M and C-D, whilst the latter had in most cases similar stability. Since there was no overall effect of treatment on any of the aggregate size fractions, these results are not presented.

3.5. Earthworms

In general, higher densities and biomass of earthworms were found in G-3M, where the green manure was mulched and left on the soil surface, than in treatments where it was removed (G-0M, G-0M-D) or where only cereals were grown (C-D) (Table 10, 2009). This difference was also seen in autumn 2010, but only for the number of worms in the clay soil at Kvithamar (Table 10). No significant effects of digestate were found, when comparing treatment G-0M-D and C-D with G-0M in 2010 (Table 10).

Table 9
Stability (%) to simulated rainfall of two aggregate fractions (2–6 mm and 6–10 m), given as means of each site and of three treatments with green manure and/or digestate application. Abbreviations for the treatments are explained in Table 3.

	2–6 mm	6–10 mm
Site		
Ås	61.0	77.6
Kvithamar	59.0	69.4
Apelsvoll	78.1	85.6
Værnes	40.1	52.7
LSD, 5%	18.3	13.0
Treatment		
G-3M	55.9	70.3
G-0M-D	64.8	74.6
C-D	57.9	69.0
LSD, 5%	5.5	4.9
Mean	59.5	71.3

Fieldworm (*Aporrectodea caliginosa*) was the main species found in both soils, 63% of the individuals at Kvithamar (K) and 86% at Værnes (V). Some *Lumbricus rubellus* (15% (K) and 11% (V)) and *Aporrectodea rosea* (14%) were found, the latter only at Kvithamar, as well as a few specimens of *Lumbricus terrestris*. More *L. rubellus* ($P < 0.01$) were found in treatment G-3M at Værnes in 2009 than in the other three treatments (species data not shown). This effect was not seen in 2010. At Kvithamar, in the clay soil, more *A. caliginosa* were found in the same treatment (G-3M) only in 2010 ($P < 0.01$). No effects of digestate were found on the species composition, when comparing treatment G-0M-D and C-D with G-0M in 2010.

4. Discussion

4.1. Effect on barley yield of removal versus mulching of herbage

Contrary to the hypothesis, we found that removing green manure herbage compared to mulching affected the subsequent spring barley yields negatively. Removal of herbage increased N-deficiency in the following barley crop (Fig. 1) at all sites, and this was reflected in a consistent trend with substantial and statistically significant yield loss at harvest on two of the sites. On the sandy and loam soils, mulching increased the grain yield by 23 and 33%. This

Table 10
Biomass (g m^{-2}) and number (m^{-2}) of earthworms (0–20 cm depth) at Kvithamar (silty clay loam) and Værnes (sandy loam) in autumn 2009 and 2010 ($n = 8$). Means (±S.E.) within each site, earthworm component and year, which do not share any letter in common, are significantly different ($P < 0.05$) by Tukey comparison method. The highest value is shown as a.

	G-3M	G-0M	G-0M-D	C-D
2009				
Kvithamar				
Number	206 (48) a	84 (26) b	94 (27) ab	72 (15) b
Biomass	137 (48) a	26 (12) b	35 (13) b	36 (8) b
Værnes				
Number	266 (43) a	184 (21) ab	131 (22) b	181 (23) b
Biomass	178 (33) a	72 (12) b	64 (18) b	78 (12) b
2010				
Kvithamar				
Number	172 (33) a	78 (7) b	78 (25) b	66 (15) b
Biomass	66 (12) a	34 (4) ab	42 (17) ab	20 (4) b
Værnes				
Number	197 (45) a	172 (21) a	216 (53) a	138 (25) a
Biomass	92 (23) a	95 (14) a	125 (30) a	74 (16) a

is comparable to the 20% yield increase of spring barley following vegetables mulched with a chopped grass-clover mixture on a nearby morainic loam, reported by Riley et al. (2003).

In the case of the clay soils, results varied. At Kvithamar, with colder weather during first part of the season (on average 2 °C), all treatments were severely N deficient and the yield considerably reduced, but the relative difference between the treatments were still high. At Ås, barley grew well, but likely differences between treatments were lost due to late season lodging caused by wet weather conditions after ripening. This delayed harvesting, and led to loss of grain. From visually observations we anticipate that the grain losses were greatest on the plots with best growth.

We expected higher clover proportion in the green manure ley caused by removal of herbage, and a positive effect of this on N availability for barley the following year. However, only at Apelsvoll the removal of herbage facilitated larger regrowth and proportion of clover. Even at Apelsvoll, the larger clover content did not result in larger availability of N the year after. In general, no effect of mulch for grass and clover regrowth may indicate that N is either lost or immobilized.

The C/N ratio is found to be the most important factor determining the mineralisation from fresh plant material (Thorup-Kristensen, 1994; Marstorp and Kirchmann, 1991). Net immobilization of N is likely since the estimated average C/N ratio of the grass-clover herbage is above 15, which is found by Marstorp and Kirchmann (1991) to be a turning point for legumes. Furthermore, high herbage yields overlying and shading the stubble may also have suppressed the ley regrowth.

The decomposition rate of plant material and N mineralization from soil organic matter have both been found to be slower in clay soils than in sandy soils (Hassink et al., 1993). Shah et al. (2013) found that the total plant N recovery of applied manure in ryegrass followed the same pattern as above with regard to soil type. The limited fertilization effect of mulching in our study on the clay soils, despite signs of N-deficiency in barley in G-0M, may have been caused by slower mineralization; an effect of soil type, especially in combination with cold and wet weather conditions as for the site Kvithamar. The in general low estimated fertilizer N recovery in the barley grain, and especially for Kvithamar, indicates poor growth conditions for the barley plants in early growing season.

The progress and the level of N loss by leaching, runoff and gaseous emissions from the mulched herbage are strongly influenced by the weather conditions. Ammonia emission is more affected by precipitation than temperature or N-concentration in the herbage (Whitehead et al., 1988). Whitehead and Lockyer (1989) found that decomposing grass herbage placed on the stubble of a cut sward containing 3% N, lost 10% of its N as ammonia during 28 days with showery weather. However, emission levels twice as high as this, or even up to 39%, have also been found from grass with lower N content (Larsson et al., 1998; Whitehead et al., 1988).

At the Ås site, Nadeem et al. (2012) observed that mulching of the herbage only increased nitrous oxide (N_2O) emissions slightly. In the year with green manure, the emission was 0.037 g N_2O-N m^{-2} higher throughout the whole growing season than where herbage was removed. Some ammonia and N_2O emissions are likely to have occurred, especially after the second and third cuts at the two Northern sites, due to precipitation of 100–250 mm during the first 30 days after the cuts.

4.2. *Effect on barley yield of digestate versus mulched herbage*

When half of the N in green manure herbage was applied as biogas digestate in spring, the barley DM yields reached the same level as after mulching the herbage, and the apparent N recovery was higher, as hypothesized. The recovery in spring barley grains of NH_4-N applied in digestate on the two sites with the lightest soils, Værnes and Apelsvoll, was similar to the 29–38% recovery from manure (slurry) found by Olesen et al. (2007). In general, the digestate appeared to contribute more to the nutrient supply during early growth than did N mineralization from the green manure ley. The latter, contributed mainly later in the growing season and thus increased protein content more than the biomass. The low N harvest index in C-D indicates that an adequate early N supply to the crop was followed by a period of more severe N deficiency.

The low DM levels in the early growth stages of barley fertilized with digestate at Apelsvoll may have been a result of the DGI application technique. The row-spacing of the digestate applied with DGI was relatively large compared to the plant rows.

4.3. *Effect of herbage management on plant available nitrogen in soil*

Green manure herbage, if mulched, represents a high N input to the cropping system, but no high pulse of inorganic N caused by mulching was found in the soil on the sampling dates.

The latest measurements of soil inorganic N before barley crop nutrient uptake, 7–10 days after germination, showed a consistent trend in the order G-0M-D ≥ G-3M ≥ G-0M. Overall, only digestate application enhanced significantly the level of plant available N in soil compared to previous removal of herbage. This is in according to the hypothesis that plant available N will increase after digestate application, but not after mulching. However, in the case of mulching versus removal, on the soils most prone to leaching, we found a substantial yield response in barley from the mulched herbage. In accordance with the findings of Dahlin et al. (2011), this indicates that a considerable proportion of herbage N was incorporated into soil organic matter and mineralized during the growing season 2010.

Thorup-Kristensen and Dresbøll (2010) have recorded fast N mineralization under low temperatures after incorporation of catch crops in spring. Our results from N uptake in the barley plants indiates a slower N mineralisation from the ploughed under one year old mulched green manure ley, probably due to a higher C/N ratio of the plant material. The contribution of N from green manure root and stubble to the following grain yield was low. In general, higher levels of NO_3-N were found in the G treatments than in the C treatments in spring 2011. This indicates that N from green manure was released over a longer period.

Some mineralization in soil with and without green manure seems to have taken place during the winter of 2009 to 2010 (Fig. 2, comparing bars for inorganic N in autumn 2009 and spring 2010). This is in agreement with several studies that have shown substantial mineralisation of incorporated green manure at temperatures down to 1–3 °C (Cookson et al., 2002; Magid et al., 2001; Van Schöll et al., 1997). Our results also indicate that mineralization from stubble and below-ground green manure biomass occurs at low temperatures. The enhanced soil inorganic N content due to mulching that was found before ploughing the ley in spring 2010, corresponded to 4–7% of the total N added as mulch.

4.4. *Effect of herbage management on soil structure and earthworms*

The use of digestate improved soil aggregate stability, more than compensating for the herbage removal in treatment G-0M-D, as seen in the comparison to treatment G-3M. Further, it would seem that the use of digestate was effective in increasing the soil aggregate stability on plots previously cropped with cereals to the level found where mulching of green manure had been practiced. As found by Abiven et al. (2009), easily decomposable products are known to have an intense and transient effect on soil aggregate stability. Further, digestate of cattle slurry is found to stimulate the bacterial decomposer community more than undigested slurry, and in a similar way to that of inorganic fertilizers (Walsh et al., 2012).

In accordance to our hypothesis, a clear positive effect of mulching on the earthworm fauna was seen after only one season. It is well-known that systems with one or more years of grass and clover in the crop rotation, often host more earthworms than do all-arable systems (Edwards and Lofty, 1977; Pommeresche and Løes, 2009; Schmidt et al., 2003). In our study, the effect was consistent even the first year and was directly connected to the mulching of the plant material. One result of higher biomass of earthworms after mulching in 2009 was higher cast production. Pommeresche and Løes (2009) estimated that a density of 229 earthworms m^{-2} corresponds to 22.1 kg casts per m^{-2} $year^{-1}$. The casts contained 3 g total N kg^{-1}. Roughly estimated for our trials, this means 30 g more total N m^{-2} in casts in the mulched treatment. Almost the entire N content of the casts is organically bound and thereby protected against leaching until mineralization (Boström, 1988). In addition, dead earthworms contributes to the N mineralization as their body tissue contains 10–12% N of their dry weight and they decompose rapidly (Edwards and Bohlen, 1996).

The higher densities of *L. rubellus* and *A. caliginosa* after mulching are a response to the input of organic matter which increased both the survival of adults and juvenile recruitment. Among species, mulching gave diverging results in the two soils. At Værnes, the surface dwelling *L. rubellus* responded by increased population in the same season. Slower decomposition in the denser clay soil at Kvithamar, may explain the higher density of the soil-dwelling, soil-eating *A. caliginosa* here in 2010, compared to the lack of any effect on the earthworm parameters at Værnes in the same year.

The lack of any effects on the earthworm density or biomass after one digestate application is not in line with the hypothesis. However, one season is too short a time to conclude on possible longer term effects on earthworm parameters that may occur with repeated annual use of digestate. Ernst et al. (2008) tested effects on earthworms of cattle slurry and anaerobic digested mixture of cattle slurry and plant residue in a microcosm experiment. While the biomass of the litter-eating species (*L. terrestris* and *Apporectodea longa*) increased in both slurry treatments, that of the soil-eating species *A. caliginosa* decreased significantly in treatments with digested slurry. This is unfortunate as soil-eating species survive well with soil tillage and are the dominant species (80–100%) in arable soils in Norway (Chan, 2001; Pommeresche and Løes, 2009).

4.5. *Implications*

Our results indicate that in spring barley production under cold climatic conditions, the N supply may be limiting, even after a productive green manure ley. If the green manure herbage is removed, it can lead to further N deficiency. Mulching the last cut and removing the previous cuts will reduce the potential loss of N from the cut herbage, compared to mulching all cuts, but the subsequent barley DM yield may be also be lower.

Removing herbage from the field requires that it may be used as forage or to produce digestate in a biogas reactor. Möller and Müller (2012) concluded that biogas digestion of field residues, instead of mulching, resulted in a win-win situation, with additional energy yields, lower risk of N leaching and lower nitrous oxide emissions, although the risk of ammonia volatilization remains when applying the digestate. Halberg et al. (2008) also support this view from an energy self-reliance perspective on organic cash-crop farms.

In our trial, we applied nearly half of the N harvested in green manure herbage. At a farm scale, the surplus digestate would make it possible to fertilize other fields as well. However, running small farm-scale biogas plants solely based on green manure herbage is challenging. Cooperation with biogas plants with continuous feeding throughout the year would seem to be the best solution until new technology is developed for small farm-scale biogas plants based on grass/clover herbage.

5. Conclusions

When we evaluated the effect of various strategies for green manure management we found that both for DM yield and apparent N recovery in a subsequent barley crop, it mattered how the green manure herbage was managed. Herbage mulching compared to removal improved the barley yield, whilst herbage removal accompanied with return of about 45% of the removed N as digestate improved both yield and N recovery. The amount of N removed with the herbage was not compensated for by increased clover growth in the summer regrowth. The low effect of green manure on N supply to the following grain crop was most likely due to low net N mineralization. Lowest N recovery was found on the clay soils.

The mulched plant material gave an increase in earthworm density and biomass. Application of digestate increased aggregate stability measured shortly after application, but did not affect the biomass or density of earthworms.

Of the managements considered, the digestate strategy seems to be the most promising option as regards increasing N recovery and reducing the risk of N losses.

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Paper II

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Effect of low temperature and soil type on the decomposition rate of soil organic carbon and clover leaves, and related priming effect

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ABSTRACT

The purpose of this study was to improve the temperature response function to be used in models of soil organic carbon (SOC) and litter mineralisation. A clay soil and a sandy soil with equivalent weather and cultivation history were incubated for 142 days at 0, 4, 8.5 or 15 °C, which is representative for the natural temperature range above 0 °C of these soils. The soils were incubated with or without ^{13}C labelled clover leaves in gas tight chambers. In absence of added plant litter, the decomposition rate [mol CO_2 (mol substrate-C)$^{-1}$ day^{-1}] of SOC followed a first order reaction and it was twice as fast in the sandy soil as in the clay soil. Contrary to our hypothesis, the relative response of SOC mineralisation rate to temperature was the same in both soils; it was well described by an Arrhenius function and it could also be approximated as a linear function of temperature. The mineralisation of clover leaves was affected by soil type, and was slower in the clay than in the sandy soil. Also the initial temperature sensitivity of the clover decomposition (to 18% decomposed) could be approximated by a linear function. SOC mineralisation was enhanced (priming effect) by the presence of clover; the relative increase was most conspicuous at 0 °C (150–250% over 142 days, depending on the soil) and decreased with temperature (+40% at 15 °C). At the start of the incubation and up to 52 days of incubation the priming effect was correlated with the amount of CO_2 derived from mineralisation of clover leaves. We suggest that the effect of soil type on the diffusivity of enzymes could be an important mechanism affecting the decomposition rate and probably also the volume of soil exposed to priming around decomposing litter.

In conclusion, the temperature sensitivity of the decomposition was in the order: priming < plant litter < sandy soil SOC = clay soil SOC. For the purpose of modelling, we present parameterised equations for mineralisation rates of SOC and clover leaves as function of soil temperature range 0–15 °C. Regarding modelling of priming, there is scope for relating it to litter decomposition and the influence of soil type on the diffusion of enzymes from microorganisms around the litter surface. The effect of soil type on plant litter decomposition and soil priming should be considered in models that predict nitrogen mineralisation based on the C/N stoichiometry of substrates and decomposition products.

1. Introduction

In farming systems, plant residues are incorporated into the soil for the purpose of preparing a new seedbed. In addition, some crops, e.g. green manure and catch crops, are grown and incorporated into the soil for the purpose of increasing soil fertility and thereby enhance the yield of subsequent crops. In cold temperate regions, the incorporation is often done in the autumn or spring, and the decomposing organic matter in the soil is exposed to low temperatures before a new crop is established or when nutrient uptake by plants is still small. Several studies have shown substantial carbon (C) and nitrogen mineralisation of incorporated green manure at temperatures down to 1–3 °C (Breland, 1994; Van Schöll et al., 1997; Cookson et al., 2002). Thus, the incorporation of crops grown for enhanced C-sequestration can increase the risk of nitrogen losses as gaseous emissions, surface runoff and leaching of nitrate or soluble organic nitrogen. As the input of fresh plant material from leys, and in particular from green manure crops can be considerable, an improved modelling of C mineralisation during

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the cold season is a valuable tool for predicting nitrogen mineralisation, and thus for planning a better crop management with higher nitrogen use efficiency and reduced environmental risk.

Most models of soil organic matter (SOM) decomposition assume that SOM can be divided into a number of more and less decomposable pools and that their decomposition is proportional to the amount of substrate, i.e. that it follows first order kinetics (Manzoni and Porporato, 2009). Thus the amount C of an initial pool C_0 remaining after time t can be expressed as.

$$C = C_0 e^{-kt} \tag{1}$$

where k is the decay rate constant. One of the most commonly used functions for describing the dependency of decomposition on temperature is the Arrhenius equation (Davidson and Janssens, 2006):

$$k = Ae^{-E_a/(RT)} \tag{2}$$

where A is the pre-exponential factor, E_a is the required activation energy, R is the universal gas constant and T is the temperature in Kelvin. For the purpose of modelling, the temperature sensitivity can be expressed as a temperature function $f(T)$ that modifies a decay rate k_{ref} at a reference temperature (Kutsch et al., 2009; Moyano et al., 2009; Davidson et al., 2012):

$$k = k_{\text{ref}} f\left(T\right) \tag{3}$$

Thus, the sensitivity to temperature can be seen as the change in decay rate relative to the decay rate at another temperature (Reichstein and Janssens, 2009). The most commonly used temperature factor is the Q_{10} which expresses the relative change in k when temperature increases by 10 °C (Kirschbaum, 2006):

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \tag{4}$$

where k_2 and k_1 are respiration rates observed at temperatures T_2 and T_1. In their review of 25 incubation studies of soil, straw and soil with different substrates, Kätterer et al. (1998) found Q_{10} of 2 to be adequate for organic matter decomposition in the temperature range 5–35 °C. However, Q_{10} decreases with increasing temperature, a fact which is also predicted by the Arrhenius equation (e.g. Davidson and Janssens, 2006), and for the lower temperature range (<10 °C) Q_{10} is found to be greater and almost 8 at 0 °C (Kirschbaum, 1995; Leifeld and Fuhrer, 2005; Farrar et al., 2012). This complicates the use of Q_{10} as factor for modelling the decomposition of organic matter (Reichstein and Janssens, 2009).

How readily the organic matter in the soil is decomposed depends on its chemical quality and different abiotic constraints, which are likely to affect the response to temperature (Davidson and Janssens, 2006). Kinetic theory predicts that the temperature sensitivity of SOM decomposition should increase as the degree of substrate complexity increases (Bosatta and Ågren, 1999). Therefore the decomposition of older SOM, consisting of more complex, recalcitrant organic molecules, should be affected more by temperature than new SOM. However, results regarding the temperature sensitivity of different organic matter fractions are contradictory (Kirschbaum, 1995; Kätterer et al., 1998; Liski et al., 1999; Giardina and Ryan, 2000; Fang et al., 2005; Fierer et al., 2005; Lefèvre el al., 2014). In modelling, the same modifying temperature function $f(T)$ is often applied to all substrates in a soil, mainly as a precautious conservative rule since there is a lack of sufficient empirical evidences that can be transposed to field conditions (Coleman and Jenkinson, 1999; Hansen, 2002; Jansson and Karlberg, 2004).

Soil type is among the abiotic factors that influences decomposition. The binding of organic matter to mineral surfaces and occlusion in soil aggregates acts as chemical and physical protection of organic substrates and microbial biomass (Van Veen et al., 1985; Gregorich et al., 1991; Saggar et al., 1996; Müller and Höper, 2004; Davidson and Janssens, 2006). The adsorption of soil organic carbon (SOC) to mineral particles is expected to increase the effect of temperature on k, since higher activation energy is needed to break the bonds between the organic matter and the mineral particles (Conant et al., 2011).

The decomposition of SOM can be influenced by the addition of readily decomposable plant residues and root exudates (e.g. Löhnis (1926) referred by Kuzyakov et al., 2000; De Graaff et al., 2010; Lukas et al., 2013). Kuzyakov et al. (2000) has defined the priming effect (PE) as short term increased or decreased turnover of SOM caused by any soil treatment. Reports of direct effects of temperature on priming are also rare and cannot be generalized (Kuzyakov, 2010). Recently, PE was observed at 4 °C (Farrar et al., 2012) and around freezing point (Lukas et al., 2013). Farrar et al. (2012) and Ghee et al. (2013) found that priming caused by the addition of easily degradable substrates (glucose/amino-acids) was not altered by temperature. Thiessen et al. (2013) studied the decomposition during 199 days of fresh plant material in soil at two diurnal temperature treatments (5–15 °C, 15–25 °C) and found that the PE relative to the mineralisation of the non-amended soil was similar at the two temperature treatments.

The aim of our study was to estimate the effect low temperature and soil type have on the decomposition of SOC and of newly incorporated clover leaves. We used two soils, a sandy and a clay soil, with similar cultivation history and environmental conditions to avoid confounding of soil type with these factors. The temperature range was that normally experienced in the plough layer of Norwegian soils, when they are not frozen, with temperature intervals between treatments unevenly distributed (i.e. 0, 4, 8.5, 15 °C), in order increase the power of the statistical estimates at the lower temperatures (Kirschbaum, 2006).

The following hypothesis were tested: i) The sensitivity to temperature of decomposition is inversely proportional to the decomposition rate, and in the order plant litter << sandy soil SOC < clay soil SOC, ii) Neither chemical binding, nor adsorption or occlusion affect the decomposition of newly incorporated plant residues, therefore soil type does not affect the initial decomposition rate of plant litter, and iii) During decomposition of plant litter, a positive PE (including microbial biomass turnover) occurs, and its response to temperature is more similar to that of litter rather than to that of SOC mineralisation. The last hypothesis is based on the consideration that PE is likely to be the result of increased exo-enzymes production from microbial activity stimulated by easily decomposable plant residues.

2. Materials and methods

2.1. Experimental design

The incubation experiment ran for 142 days with two soil types in gas tight glass chambers, with or without ^{13}C labelled clover leaves, at 0, 4, 8.5 or 15 °C. The soils were taken from two arable irregular cereal-leys rotation fields, about 4 km apart, of the Bioforsk research center Kvithamar in Central Norway (63°29′N, 10°52′E). In this humid costal climate the normal value (1961–1990) for annual precipitation is 896 mm and the mean monthly soil temperature at 10 cm depth is between −1 and 15 °C (Appendix A).

The soils were a silty clay loam (clay soil) classified as Mollic Gleysol and a sandy loam (sandy soil) classified as Arenic Fluvisol

(IUSS Working Group WRB, 2006), which are representative for two of the three most common cultivated soil types in this region (Vatn et al., 2006). The silty clay loam had a higher total C content than the sandy loam (Table 1), and both soils had some carbonate (1.7% and 4% of total C, respectively) as a consequence of liming practice. Further soil characteristics are available in Table 1.

Bulk samples of the soils from 0 to 20 cm depth were collected in spring, sieved through a 2 mm mesh while moist and pre-incubated in the dark for 4½ months at about 15 °C in aerobic and moist condition. Two and a half weeks prior to incubation the soil was further moistened to 75% of pore volume at field bulk density, which was about the highest moist content that allowed handling and compaction of the soil without collapse of the small soil aggregates. Before preparing the glass chambers, the soil was kept at the final temperature for three days. Moist soil equivalent to 400 g dry soil per chamber was gently mixed with 1.6 g red clover leaves (*Trifolium pratense* L.), where relevant, filled in 1 L DURAN® glass bottles and compacted to nearly the bulk density observed in the field.

The clover leaves had a C/N ratio of 9.8 and were highly enriched in ^{13}C (Table 2). They were taken from the last harvest of a previous field experiment which had been pulse-labelled weekly (12 times) with 99% ^{13}C enriched CO_2 (Bakke, 2010). The leaves had previously been dried at 60 °C, were cut into pieces and sieved through a 2 mm mesh. The amount corresponded to about 900–1200 g DM m^{-2} to 20 cm depth, which is about the expected total above-ground DM production of a green manure ley during a growing season (Frøseth et al., 2014).

The bottles were closed with airtight corks fitted with two PTFE pipes (PRENART Equipment ApS, Denmark). One pipe was used for sampling the atmosphere of the chamber and adding oxygen, the other for sampling and replacing the CO_2 trap, which consisted of 10 mL 2 M NaOH, placed in a plastic tube in the middle of the chamber. The chambers were equally distributed between 4 incubators regulated at 0°C (Refritherm 200, Denmark), 4 °C (Termaks KB 8182, Norway), 8.5 °C (Termaks KBP 6151, Norway) and 15 °C (Termaks KBP 6151, Norway). The standard error for the incubator temperatures was ±0.2–0.3 °C. Each treatment was replicated 3 times, thus altogether there were 48 chambers. In addition, two chambers without soil, one at 0 °C and one at 15 °C, were used as blanks.

2.2. Sampling and analysis

The CO_2 trap was exchanged on day 0, 3, 8, 15, 30, 52, 80, 134 and 142. The lye was collected in 20 mL pre-evacuated glass vials and 1 mL of it was transferred to a 10 mL glass vial prefilled with 1.5 mL 2 M H_2SO_4. This was used for determination of CO_2 amount on a gas chromatograph (GC) (Model 7890A, Agilent, Santa Clara, CA, US), and the fractional abundances of ^{13}C on a gas chromatograph isotope ratio mass spectrometer (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany).

At adequate intervals for maintaining oxic conditions (day 3, 7, 14, 28, 45, 79, 116 and 142), the atmosphere of the chambers was sampled (5 mL injected into pre-evacuated 10 mL glass vials) and immediately analysed on the GC to monitor the O_2 content, and oxygen was added to raise the level to around 20% volume. Oxygen content was usually above 12%, but for a few exceptions at the beginning of the trial. By default also CO_2, N_2O and CH_4 where analysed. This provided a double check that the atmosphere remained sufficiently oxygenated, as methane remained always below 6.2 ppm, N_2O remained low, except for one episode at 15 °C in soil with clover, and CO_2 was, with the exception of a few episodes, below 2000 ppm.

The work flow in all procedures was from soil alone to soil added with labelled plant material, and within these two groups from low to higher temperature, in order to minimize contamination. All vials were He-rinsed and evacuated before use. A small amount of CO_2 was found in the samples of the blank chambers, due to small impurity in the NaOH used to prepare the lye. This was subtracted from the other measurements.

The amount of total C and nitrogen in the soil and clover leaves was analysed using the Dumas combustion method (Bremmer and Mulvaney, 1982) on a Leco CHN 1000 analyzer (LECO Corp., St. Joseph, MI, USA).

2.3. Calculations and statistical analysis

The fractional abundance of ^{13}C of the soils, the plant material and in the CO_2 evolved in the chambers was measured. Since the plant material was artificially labelled, we used the equation for mass-balance to estimate the plant-derived and the soil-derived CO_2 (Hayes, 2004):

$$m_{\Sigma}{}^{13}F_{\Sigma} = m_{s}{}^{13}F_{s} + m_{p}{}^{13}F_{p} \tag{5}$$

where m represents molar quantities of CO_2 and ^{13}F represents fractional abundance of ^{13}C ($^{13}F = {}^{13}C/({}^{13}C + {}^{12}C)$) (Table 2). The subscript p, s and $\sum$ refer to the plant derived CO_2, the soil derived CO_2 and the sum of both, respectively.

In the case of chambers with plant material added, we calculated the soil derived CO_2 by solving Eq. (5) for m_s, after substitution of m_p with $m_{\sum} - m_s$ (since $m_{\sum} = m_s + m_p$):

$$m_s = m_{\Sigma}(F_{\Sigma} - F_p)/(F_s - F_p) \tag{6}$$

At each temperature and sampling time, the PE in chambers with plant material was calculated as the soil derived CO_2 estimated by isotopic signature (Eq. (6)) minus CO_2 from soil without plant material (average of three replicates).

The remaining substrate at each time was estimated as the initial substrate minus the cumulative CO_2 evolved (both in mol C). The decomposition rate k in Eq. (1) was expressed in mol CO_2 (mol substrate C)$^{-1}$ day^{-1} and it was calculated as $k = -\ln([C]/$

Table 1
Soil particle size distribution, bulk density, air capacity and moisture retention capacity in undisturbed soil, 0–20 cm.

Soil type	Sand	Silt	Clay	Gravel in whole sample	Bulk density	Available water[a]	Wilting point[b]	pH[c]	Total C[c]	C/N[c]
	% of fine earth			%	g cm^{-3}	vol%	vol%	_	%	_
Silty clay loam[d]	3	70	27	3	1.13	36	15	6.0	4.45	11.4
Sandy loam[e]	51	43	6	0	1.53	27	6	6.2	1.30	11.8

[a] 10–1500 kPa.
[b] >1500 kPa.
[c] Analysed in new samples.
[d] From profile no. 6 in Sveistrup et al. (1994).
[e] From profile 20177 in Solbakken (1987).

Table 2
Initial C amount and CO_2 evolved from each substrate (soil or clover) after 142 days of incubation. The ^{13}C atom percentage of the substrates is also shown. Bottles contained 400 g dry soil and 1.6 g dried clover leaves. Numbers in parenthesis indicate ± standard deviation.

Substrate	Atom percent ^{13}C	Initial C (mmol g^{-1} soil)	Evolved C (% of initial C)			
			0 °C	4 °C	8.5 °C	15 °C
SOC without clover						
Silty clay loam	1.09 (0.02)	3.70 (0.03)	0.11 (0.01)	0.21 (0.01)	0.38 (0.01)	0.65 (0.17)
Sandy loam	1.09 (0.01)	1.08 (0.01)	0.22 (0.01)	0.47 (0.02)	0.73 (0.02)	1.43 (0.01)
SOC in presence of clover						
Silty clay loam		3.70 (0.03)	0.28 (0.02)	0.40 (0.03)	0.60 (0.01)	0.94 (0.21)
Sandy loam		1.08 (0.01)	0.76 (0.11)	1.03 (0.04)	1.38 (0.04)	1.95 (0.03)
Clover leaves						
in silty clay loam	2.29 (0.09)	0.156 (<0.001)	22.0 (0.4)	26.2 (0.2)	30.7 (0.9)	31.4 (0.4)
in sandy loam	2.29 (0.09)	0.156 (<0.001)	26.7 (0.8)	31.9 (0.4)	37.9 (0.4)	39.0 (1.6)

$[C_0])/t$ for time intervals when the slope of the decay curve was close to a straight line ($R^2 \geq 0.98$, selected values are shown in the inset in Figs. 1c and 2d), that is when first order decay could be assumed. Multiple regression analysis was used to estimate k with $\ln([C]/[C_0])$ as dependent variable and incubation time as independent continuous variables. The modifying temperature function (Eq. (3)) for different intervals was found regressing the relative decay rate estimates (k/k_{ref}, where k_{ref} is the decay rate estimated at 15 °C), against temperature in Celsius. The parameters of the Arrhenius equation (Eq. (2)) were found by regressing $\ln(k)$ versus $1/T$, with temperature T given in Kelvin. In all models the effect of soil type was introduced as dummy variable [0, 1], and either additive or multiplicative effect were tested. The effect of incubation time was also introduced as dummy variable, when relevant. The validity of the modifying temperature functions and of the Arrhenius models where tested by using the predicted k values to estimated substrate decay, and plotting them against the observed data.

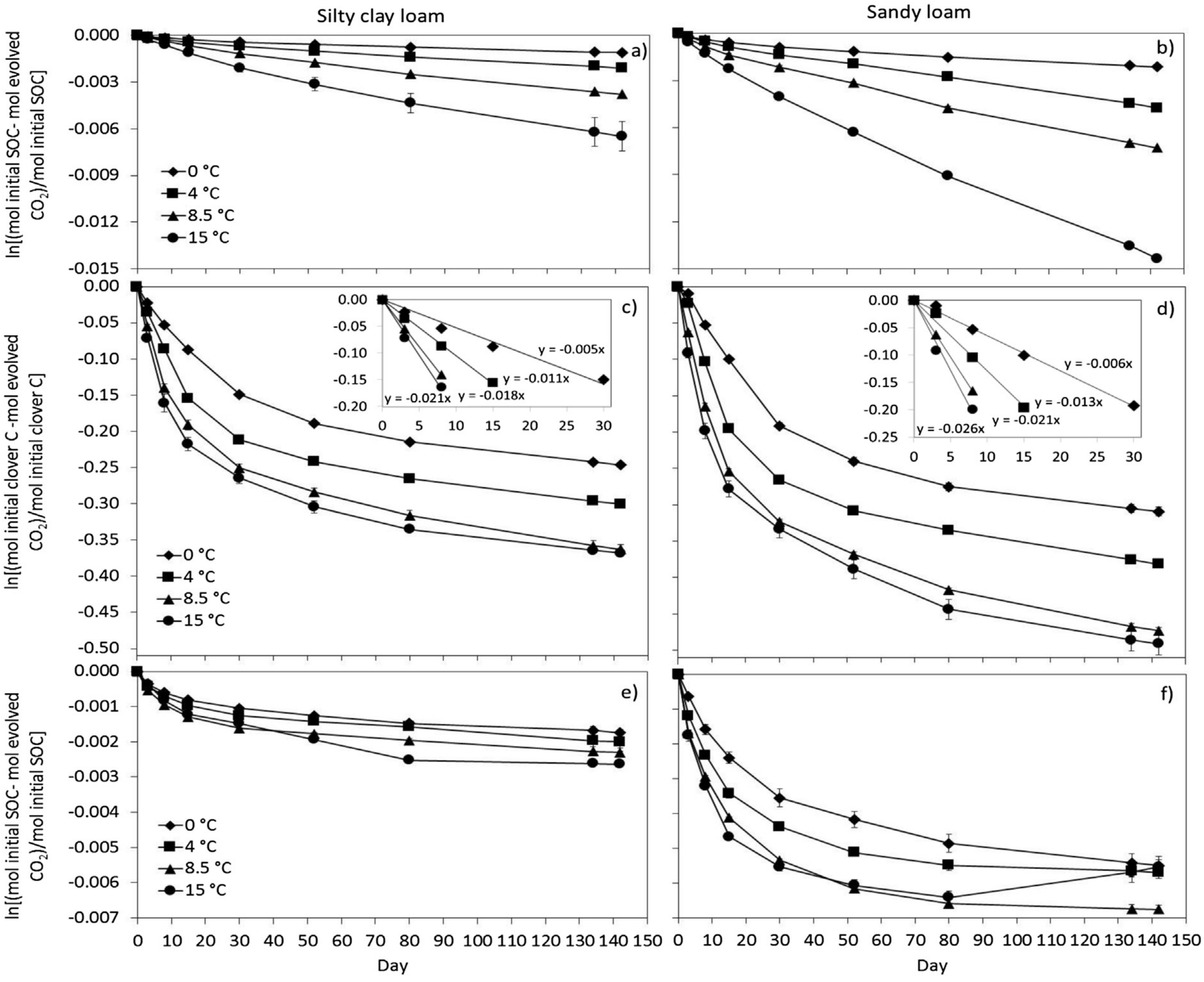

Fig. 1. Decay [ln(fraction of remaining substrate C)] of SOC without clover (a and b), clover C (c and d) and priming measured as the extra release of CO_2 from SOC caused by clover addition (e and f), versus incubation time as affected by temperature in a silty clay loam and a sandy loam. Line bars indicate standard error. The inset in c) and d) shows the slope of the decay curve when it was close to a straight line ($R^2 \geq 0.98$).

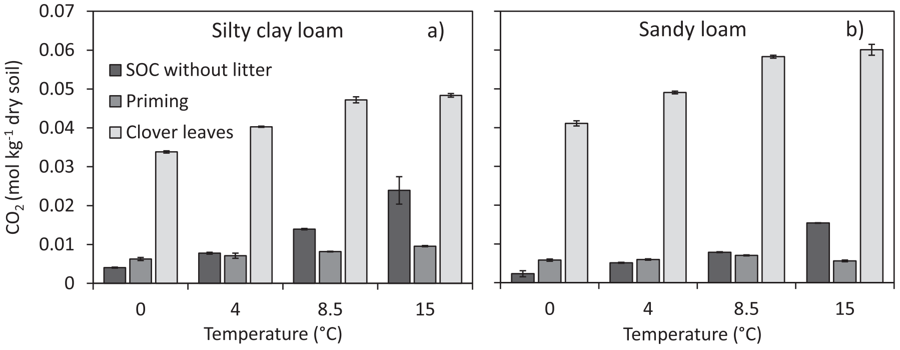

Fig. 2. Accumulated CO_2 from SOC, priming and clover after 142 days of incubation of a) a silty clay loam and b) sandy loam, with and without clover leaves at different temperatures. Line bars indicate standard error.

The statistical software package R (R Core Team, 2012) and SAS (SAS 9.3, SAS Institute Inc., Cary, NC, USA) were used for these calculations. In all tests, significance was assumed at *P*-levels <0.05.

3. Results

3.1. Total C mineralisation

In total, after 142 days and in absence of plant litter, only 0.1–0.6% of the initial SOC was mineralised in the clay soil and twice as much in the sandy soil (0.2–1.4%) (Table 2). However, the total amount SOC mineralised from the sandy soil was less than from the clay soil, because of the bigger SOC content in the latter. In the presence of litter, an additional 0.2–0.3% and 0.5–0.7% of SOC was mineralised in the clay and in the sandy soil, respectively (Table 2). The clover litter decomposed much more rapidly, and 22–39% of initial clover C was mineralised at the end of the experiment (Table 2). Neglecting the PE, as done when the difference between CO_2 evolution from soil with and without litter is used to determine the litter decay, would overestimate the clover leaves decomposition by 9–20% (Table 2 and Fig. 2). In total, depending on temperature, SOC derived CO_2, included PE, was 23–41 and 17–26% of the total amount of CO_2–C evolved in the clay soil and the sandy soil, while the amount of initial SOC was 25 and 7 fold the amount of litter C, respectively.

3.2. General decomposition pattern

Both the decomposition of soil alone and labelled litter followed the expected pattern. The plots of the natural logarithm of fraction of substrate remaining versus incubation time were used to explore the presence of linear phases, i.e. following first order kinetics, which indicated when the decomposition of a relatively homogenous pool led the mineralisation (Fig. 1). In absence of litter, the initial period of more rapid decomposition ceased when about 0.3‰ (clay soil) or 0.6‰ (sandy soil) of the initial SOC was mineralised, after about 3–18 incubation days, depending on the temperature (Figs. 1a and 2b). Thus, although gentle, handling the soil for filling the incubation chambers had raised the microbial activity, the pool involved was small. After this initial period, the SOC decomposition also followed a straight line, thus most of the SOC decomposition could be adequately described by a single pool with a first order decay at all temperatures considered.

The decomposition of plant litter showed an initial rapid phase following first order decay that lasted 8–30 days, depending on temperature (Figs. 1c and 3d). In this phase, 13–18% of the initial labelled litter was mineralised. Thereafter, the decomposition rate, as indicated by the slope of the graph, changed gradually.

The PE showed a marked phase change with increasing incubation time. It gradually decreased with incubation time, as indicated by gradually flatter slope of the decay curves in Figs. 1e and 2f.

3.3. Effect of soil type on decomposition

Both SOC and clover leaves decomposition, as well as PE, were faster in the sandy soil then in the clay soil. The mineralisation rate of the SOC in the sandy soil was twice as fast as in the clay soil, both during the initial more rapid mineralisation phase, and in the following period (see k_{15} for SOC in Table 3 and Table 2). The total CO_2 evolved per kg of sandy soil was, however, only about 60% that from the clay soil, due to lower SOC content of the sandy soil (Fig. 2). This illustrates that in soil comparisons, it matters whether mineralised C refers to SOC or to soil mass.

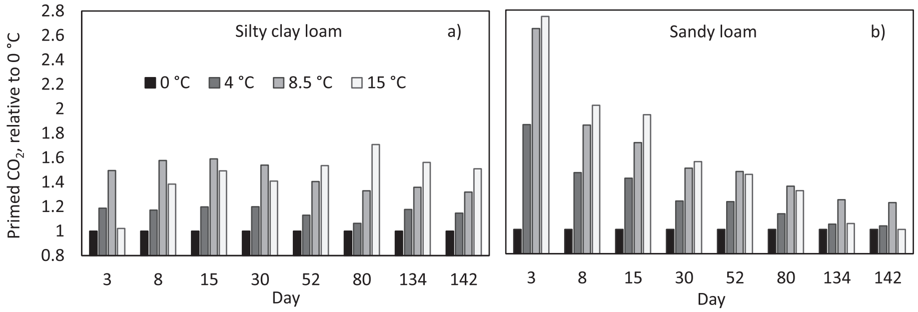

Fig. 3. Effect of temperature on the cumulated priming effect, measured as the additional mineralisation of SOC due to the presence of clover leaves, relative to the priming effect at 0 °C, during 142 days of incubation of a) sandy clay loam and b) sandy loam.

Table 3
Alternative functions for estimating the decomposition rates [mol (mol substrate C)$^{-1}$ day^{-1}] at different temperatures (T) of SOC and of the most labile pool of clover leaves in a silty clay loam ($S = 0$) and a sandy loam ($S = 1$). In the case of SOC, the PE is not included, and two periods, first with a faster then with a slower decomposition rate are identified: first 15 days of incubation ($P = 0$) and the following 127 days ($P = 1$). In the modifying equation Eq. (3), k_{15} is the substrate/soil specific decomposition rate at the reference temperature 15 °C, which values are shown in the lower part of the table. Parameter values are presented with ±standard error.

Function	Equation	R^2
SOC, in absence of clover, $n = 16$		
Arrhenius I[b]	$k = e^{[23.0(\pm 1.98) + 0.730(\pm 0.0783) \cdot S - 0.674(\pm 0.0783) \cdot P]} \cdot e^{-9331(\pm 553)/T}$	0.99
Arrhenius II[b]	$k = e^{23.0(\pm 1.94)} \cdot e^{[-9338(\pm 542) + 204 \pm (21.5) \cdot S - 189 \pm (21.5) \cdot P]/T}$	0.98
Eq. (3),[a]	$k = k_{15} \cdot [0.135(\pm 0.0174) + 0.0557(\pm 0.00197) \cdot T]$	0.98
Clover leaves, $n = 8$		
Arrhenius[b]	$k = e^{[17.09(\pm 3.86) + 0.317(\pm 0.153) \cdot S]} \cdot e^{-6079(\pm 1240) \cdot 1/T}$	0.76
Exponential[a]	$k = e^{-3.76(\pm 4.66) + 0.317(\pm 0.081) \cdot S} \cdot e^{-7.99(\pm 0.713) \cdot 1/(T+5)}$	0.97
Eq. (3),[a]	$k = k_{15} \cdot [0.00486(\pm 0.00106) + 0.000876(\pm 0.00010) \cdot S + 0.00367(\pm 0.0011) \cdot T]$	0.94
Substrate	k_{15}	
SOC, up to 16 d, clay soil	0.0000746	
SOC, up to 16 d, sandy soil	0.000142	
SOC, after 16 d, clay soil	0.0000404	
SOC, after to 16 d, sandy soil	0.0000905	
Clover leaves, in clay soil	0.0180	
Clover leaves, in sandy soil	0.0215	

[a] Temperature in Celsius.
[b] Temperature in Kelvin.

There was also a clear effect of soil type on the decomposition of clover leaves (Figs. 1c and 2d). Depending on temperature, in total 22–24% more litter was mineralised in the sandy than in the clay soil (Table 2). This soil type effect was statistically significant ($P < 0.001$) from the beginning of the incubation, when the initial rapid decomposition rate was 40–50% larger in the sandy than in the clay soil (Figs. 1c and 2d). As mentioned, this phase could be described by first order decay of a single easily decomposable pool.

The PE was also affected by soil type. After 142 incubation days the additional CO_2 mineralisation per mol SOC ascribed to the PE was larger by a factor of 3 in the sandy soil as in the clay soil (Figs. 1e and 2f). However, expressed in mol per kg soil, the total PE in the sandy soil was 80% of that in the clay soil (Fig. 2). This was true at 0–8.5 °C. At 15 °C the difference between soils was smaller due to that PE sustained for a longer period in the clay than in the sandy soil (Fig. 3).

3.4. *Effect of temperature on decomposition, as modified by soil type*

For each temperature interval, Q_{10} and the activation energy E_a were calculated for comparison with results in other studies for SOC alone and for the initial clover decay (Table 4). For the interval 4–15 °C, Q_{10} values for SOC alone were in the expected range (2.2–4.1), and considerably higher (4.1–11.8) at lower temperature. The activation energy for the interval 0–15 °C was in the range 67–86 kJ. For initial clover leaves decomposition in the interval 0–15 °C, the Q_{10} was 0.6–0.7 and E_a was 27 kJ lower than for SOC decomposition.

For modelling application, we are interested in functional relationships valid for a wider temperature range than the intervals in Table 4. For this purpose we also estimated both the modifying functions relative to 15 °C (Eq. (3)) and the Arrhenius function for the situations where reliable first order decay rates k had been found, that is for both the earlier and later incubation period (0–15 and 15–142 days) of SOC in absence of clover (Table 3). The effect of soil type and period were tested by means of dummy variable, and both additive effects and interactions with temperature were considered in regression models. Neither soil type ($P = 0.99$) nor period ($P = 0.10$) affected the modifying temperature function, which was well approximated by a straight line (Table 3 and Fig. 4).

In the case of the Arrhenius function, a model where soil type and period modified the pre-exponential factor (equation Arrhenius I in Table 3) fitted the data equally as well as a model where soil type and period affected the temperature factor (equation Arrhenius II in Table 3). We checked further the validity of the k values predicted by the two models with constant temperature response irrespective of soil type and incubation period (Eq. (3) and Arrhenius I in Table 3) by using them to estimate SOC decomposition during the incubation experiment. The predicted residual substrates were thus plotted against the observed values. The modifying temperature function (Eq. (3)) was about as good predictor of the observed SOC decomposition as the Arrhenius I function, as illustrated for the second mineralisation period in Appendix B. There were some systematic deviations from the observed values, but they were small. According to these two models, the effect of temperature on the decay rate was the same for both soils and both incubation periods.

We applied the same procedure to litter decay in the initial rapid phase (up to 18% litter decomposed) and in the late period (80–142 days) (Figs. 1c and 2d). There was a clear increase with temperature of the initial clover decay rate and the response was similar in both soils, but the pattern was irregular and the standard error large (Fig. 5a, Figs. 1c and 2d). Both the modifying function and the Arrhenius function showed a moderately lower effect of temperature on litter decomposition than in the case of SOC (Table 3, compare estimates of temperature parameters, and also Table 4 where temperature parameter in the Arrhenius functions are converted to E_a). The large variation of the k estimates and the irregular response pattern contributes to some uncertainty on the precise temperature effect and on whether this depends on soil type as indicated by Eq. (3), but not by the Arrhenius model in Table 3. An exponential model similar to the Arrhenius function, but with temperature expressed in Celsius and basis temperature −5 °C

Table 4
Activation energy, E_a, and Q_{10} estimated for SOC in two soil types (without clover leaves, earlier and later incubation period estimated separately), and for clover leaves (earlier incubation period, up to 18% decomposed) in these soils, calculated for different temperature intervals. The Arrhenius I function for SOC decomposition and the Arrhenius function for clover decomposition presented in Table 3 was used for calculating E_a for 0–15 °C.

Substrate	Period	E_a (kJ)				Q_{10}			
		0–4 °C	4–8.5 °C	8.5–15 °C	0–15 °C	0–4 °C	4–8.5 °C	8.5–15 °C	0–15 °C
Silty clay loam	0–15	119	66	57	78	6.5	2.8	2.3	3.2
	15–142	11	90	58	78	5.9	4.1	2.4	3.5
Clover in silty clay loam	Up to 18% dec.	12	17	50	51	6.9	1.3	2.1	2.5
Sandy loam	0–15	89	70	53	78	4.1	3.0	2.2	2.8
	15–142	16	59	71	78	11.8	2.5	2.8	4.0
Clover in sandy loam	Up to 18% dec.	12	14	32	51	6.8	1.2	1.6	2.2

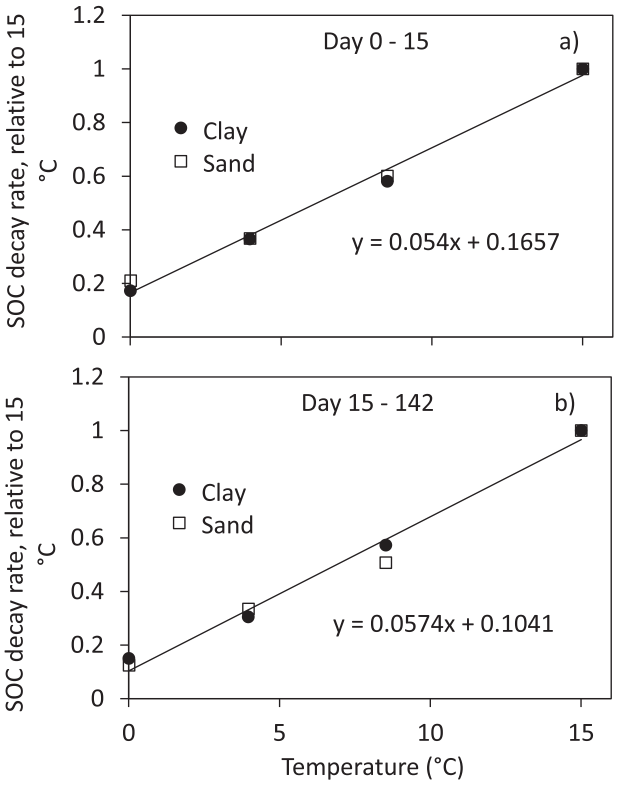

Fig. 4. The modifying temperature function for the decay of SOC (without clover addition), based on the estimated decay rate at each temperature relative to the decay at reference temperature 15 °C, at the a) initial phase and b) the rest of the incubation period. The effect of incubation period was not significant. See Table 3 for common estimates for the equation.

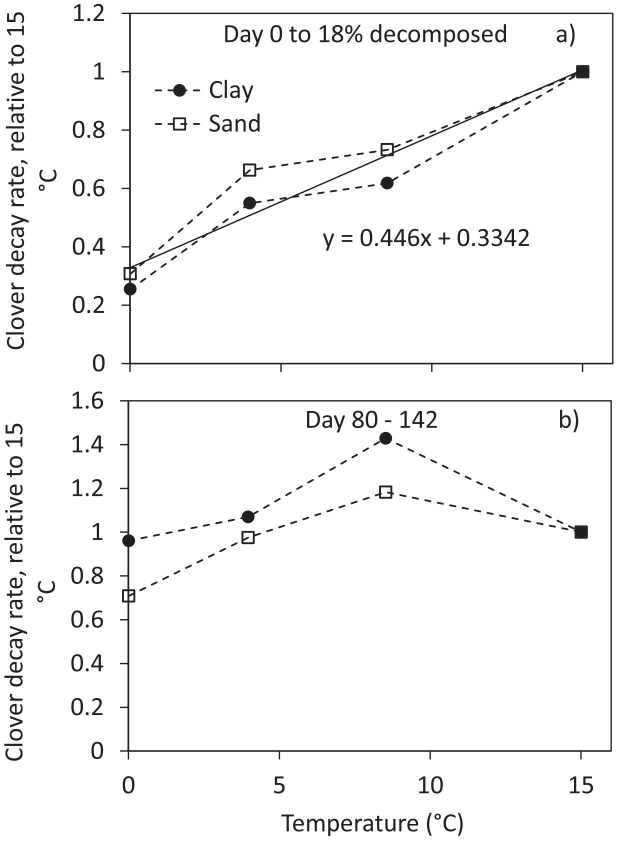

Fig. 5. The modifying temperature function for the decay of clover leaves, based on the estimated decay rate at each temperature relative to the decay at reference temperature 15 °C, during the a) initial phase and b) the last 62 days of the incubation period.

rather than in Kelvin (Table 3), gave a better fit to the observed data by larger coefficient of determination and more even distribution of the residuals, and confirmed that when the soil type was included as a general effect (in the pre-exponential factor) on litter decay, the response to temperature was similar in both soils (no soil effect on the exponent).

For the late period of litter decomposition, it was not possible to approximate the modifying temperature effect by a straight line or an Arrhenius function over the whole temperature range 0–15 °C (Fig. 5b). However, the pattern of the temperature effect on the decomposition rate of litter was similar in both soils, in spite of slower decomposition rate in the clay soil.

The effect of temperature on the PE was more complex. Since the PE changed rapidly, we were not able to calculate comparable decay rates for longer periods. Therefore, we investigated the temperature effect on the additional cumulative CO_2 release, relative to PE at 0 °C (Fig. 3). Initially there was a significant ($P < 0.05$) and roughly linear effect of temperature in both soils up to 8.5 °C. In the clay soil, the effect of temperature on cumulative PE persisted during the experiment (Fig. 3a). In the sandy soil, the effect of temperature on cumulative PE decreased gradually and was no longer present at the end of the trial (Fig. 3b). The effect of temperature on total PE was considerably lower than for SOC and litter decomposition.

3.5. Relationship between PE and SOC decomposition or litter decomposition

The magnitude of the PE relative to SOC mineralisation in unamended soil was large, particularly at the lower temperatures (+150% in the clay soil and 250% in the sandy soil at 0 °C, Table 2). As temperature influenced SOC mineralisation in absence of plant litter more than the PE, the relative enhancement by litter was smaller but still conspicuous at 15 °C (nearly +40% in both soils). At the first sampling (3rd day) the PE was particularly large and strongly related to plant decomposition, which increased with temperature. A linear regression model showed that litter derived CO_2 (mol CO_2 bottle^{-1} day^{-1}) accounted for most of the variation in PE (Fig. 6) from the third to the 52nd incubation day. Soil type had a significant but small effect which accounted for less than 2% of the regression sum of squares, modifying the slope and the intercept of the line (Fig. 6). There was also a residual significant effect of temperature on the intercept alone; however it accounted for less than 0.5% of sum of squares and did not improve the distribution of the residuals (data not shown). There was no correlation between PE and SOC mineralisation. In the second half of the incubation period, when litter decay was slow, the PE was small. At the two last samplings it was even slightly negative in the sandy soil, with no or even a loose negative relationship to litter decomposition (data not shown). A similar correlation to SOC decomposition of non-amended soil was not found.

4. Discussion

4.1. Total C mineralisation and general decomposition pattern

The general decomposition pattern shown by the decay curves were similar to those reported in similar incubation studies (Farrar et al., 2012). The fact that most of the SOC decomposition, in

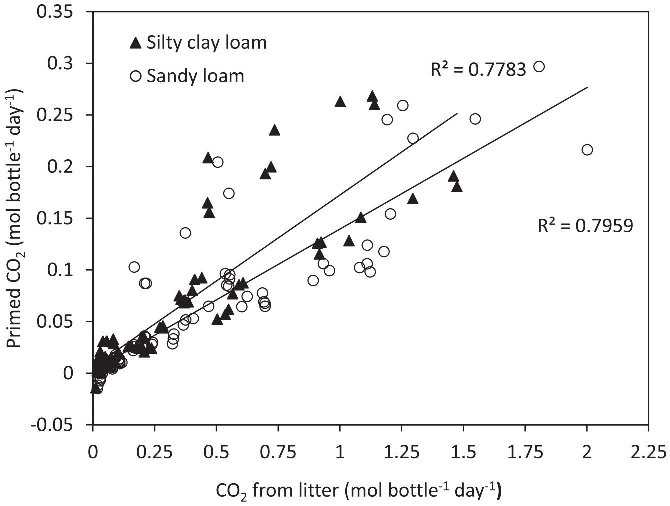

Fig. 6. Primed CO_2 plotted vs. litter derived CO_2 for the period 3–52 incubation day.

absence of plant litter, could be modelled as a single pool following first order decay shows that the pre-incubation had effectively eliminated the more easily decomposable residues, and reduced the active microbial population. The pre-incubation period was therefore sufficient to ensure that effects of soil disturbance did not confound the results. The markedly two-phase decay curve of clover litter reflects the chemical composition of the litter, which corresponds to minimum two pools, a slow and a fast decaying one, for a satisfactorily model reproduction of the litter decomposition.

4.2. *Soil type*

In line with Van Veen et al. (1985) we found the SOC mineralisation rates to be twice as fast in the sandy loam as in the clay loam soil. However, the soil type effect on initial mineralisation of plant litter was in contrast to our hypothesis which state that chemical binding, adsorption or occlusion would not affect the decomposition of newly incorporated plant residues. A rate-decreasing effect of fine mineral particles has been reported in many works, and several mechanisms have been proposed (Sollins et al., 1996). Mainly, the reported effects are on the mineralisation of organic matter already present in the soil and of added soluble substrates as e.g. sucrose, which are likely to establish a more close contact with soil minerals than newly added plant litter. We found only two previous studies on the effect of soil type on the decomposition of plant residues incorporated in the soil. Sørensen et al. (1996) found no or marginal differences between litter mineralisation in a clay soil and in a loamy sand. Saggar et al. (1996), on the contrary, found that the decomposition rate was negatively correlated with the specific surface of the soil minerals in four contrasting soils, rather than with the clay content as such. The soils used had similar cultivation history in the last decades and experienced the same weather, so the differences are most likely a main effect of soil type rather than of the quality of previous litter inputs.

Bacteria produce exo-enzymes that must reach the substrate, and the product of decomposition must diffuse ‘back’ to the bacteria. Thus diffusion could be an important mechanism limiting the rate of decomposition by microbial activity (Poll et al., 2006). It is known that clay content decreases diffusion by increasing the tortuosity factor, which depends exponentially on the pore-size distribution index, Campbell b, that corresponds to the slope of the soil water retention curve in a log–log coordinate system (Olesen et al., 1996). Moldrup et al. (2003) found that the tortuosity factor is more related to the soil specific surface than to clay content. Thus, our results are in agreement with Saggar et al. (1996), and a tentative explanation of our result could be reduced diffusion of enzymes and other solutes related to the high specific surface of the clay soil.

Although priming correlated well with litter-derived CO_2, priming-derived CO_2 from the clay soil was always about 80% of that from the sandy soil (data not shown). This ratio was remarkably constant, except for the first sampling date. Since the clay content and the SOC content in the two soils are correlated, it is not possible to draw a conclusion about the effect of soil type alone. However, mineral-organic linkage, physical occlusion and not least lower diffusion rates in clay soil suggest less priming and a slower mineralisation rate, while higher C concentration increases the amount of substrate available for priming, leading to the opposite effect.

4.3. *Sensitivity to temperature of the decay rate*

Decomposition of SOC, clover leaves and the PE were active at 0 °C. This is in line with Schütt et al. (2014) who reported SOC mineralisation at −1 °C and Lukas et al. (2013) who reported mineralisation of plant residue and PE around freezing point.

A correct estimate of the modifying temperature function depends on the ability to assess reliable temperature-specific decay rates. This was the case for SOC in absence of plant litter, both in the more rapid initial phase and the successive long period, and for the early decomposition (up to 18% decomposed) of added litter. In particular, the second phase of SOC decomposition which lasted nearly 130 days provided reliable k estimates. For these cases, we calculated Q_{10} for each temperature interval, and found them to vary with temperature in the range observed in other studies (e.g. Schütt et al., 2014). However, a temperature response factor that varies with temperature is of difficult application for predicting decomposition. Both an Arrhenius and a modifying linear model (Eq. (3)) adequately described the effect of temperature on the decay rates using a single factor for the whole temperature interval 0–15 °C. The intervals between the lowest temperatures were intentionally shorter in order to improve the leverage of lower temperatures in the regression analysis, as recommended by Kirschbaum (2006). The weak exponential and the alternative linear response are of course limited to the temperature range tested, which however is representative of the normal range observed in the region these soils were taken from (Appendix A). A check showed that the decay rates predicted by these two models indeed estimated litter mineralisation correctly (Appendix B). A closer look to reported data in the literature show that a straight line could approximate the response at temperature below 15 °C also in other studies, for example of the soil heterotrophic respiration in a beech forest (Epron, 2009). Beyond 15 °C, an exponential response, and at even higher temperature, a negative temperature response is expected (Lloyd and Taylor, 1994; Moyano et al., 2009).

The Arrhenius function uses two parameters less than the straight modifying function to predict the effect of temperature both in the earlier and in the later decomposition period of both soils, and is therefore preferable with respect to the principle of parsimony. The straight line function is a practical simplification. Compared to the Arrhenius function, it proved to be an adequate predictor and it is easily applicable into many field models of C and nitrogen mineralisation, where other important variables, not least the modelling of soil temperature, are subject to a larger degree of approximation.

A shift to slower decaying pools was the likely reason why we could not estimate temperature response for intermediate period of the litter decay, and from 8.5 to 15 °C in the last period (80–142 days) of litter decay. We can notice that the observed changes in apparent temperature sensitivity gave different results to the same question, depending on the time interval used. Further investigations require the simultaneous optimisation of several parameters in a mechanistic decomposition model (Reichstein and Janssens, 2009), including the quantification of the pools (Borgen et al., 2011) and the microbial yield in addition to the decay rates.

Also the initial priming effect (3rd and 8th incubation day) showed a clear increase with temperature (by about 150% and 250% in the clay and in the sandy soil from 0 to 8.5 °C) which gradually decreased. Thus this study supports that the priming by clover litter is modified by temperature. At somewhat higher temperatures, Thiessen et al. (2013) studied decomposition in 199 days of fresh plant material in soil during two diurnal temperature treatments (5–15 °C, 15–25 °C) to avoid changing substrate availability. They found larger absolute PE in the warm treatment than in the cold treatment, and the temperature sensitivity for PE was the same as for the respiration in the non-amended soil.

4.4. *Soil type and response to temperature*

In both the Arrhenius and the modifying linear equation, a temperature dependent term multiplies a substrate specific constant (A and k_{ref}) which is a measure of the susceptibility of the substrate to microbial decomposition. Thus, the sensitivity to temperature in this study is perceived as a relative change in the reaction rate (Reichstein and Janssens, 2009). According to this definition, in the case of SOC with same response to temperature, a rise in temperature will increase the mineralisation of the labile substrates more than that of the recalcitrant ones, when measured in absolute values of decay rates. In an analysis of variance model where temperature is introduced as an additive factor, this will appear as a significant interaction between substrate and temperature, leading to the conclusion that the substrates differ in their sensitivity to temperature. How the temperature effect is defined and analysed may therefore lead to different conclusions. This should be considered when comparing contrasting reports about the effect of temperature on the decomposition rate.

Regarding SOC, the effect of temperature (range 0–15 °C) on k was the same in both response models, in spite of strong differences in the decay rates of the substrates. This is in contrast with our hypothesis, with several reported studies (e.g. Knorr et al., 2005; Hartley and Ineson, 2008; Lefèvre et al., 2014) and with the general expectation that the decomposition of more stable SOC is more susceptible to temperature than less stable SOC (Bosatta and Ågren, 1999; Davidson and Janssens, 2006; Hartley and Ineson, 2008). It is, however, in agreement with several other results (e.g. Fang et al., 2005; Reichstein et al., 2005; Conen et al., 2008). There are several possible reasons for these contrasting results. First, that there is indeed a large variability in the substrate response to temperature increase. Second, the concept of temperature sensitivity can be perceived differently, as an additive rather than a multiplicative factor, as already mentioned. Third, that decay rates have been assessed on a mixture of non-homogeneous pools in term of decomposition rate, and a rapid consumption of the more labile pools in the samples kept at higher temperature has led to underestimation of the response to temperature (Reichstein and Janssens, 2009). Further, Conen et al. (2008) have shown that details in the experimental setting may lead to opposite conclusions. Finally, because of the multiplicative nature of the Arrhenius model the quality of the substrate can be reflected by a change of the activation energy E_a as well as by a change of the pre-exponential factor A. This was exemplified in this study by the Arrhenius model I and Arrhenius model II for SOC (Table 3) which were equipollent. We have chosen to give weight to the Arrhenius model I (effect of soil type or incubation period included in the pre-exponential factor, effect of temperature in the exponent) since this conforms to the fact that the ratio of the CO_2 evolved in the two soils was the same at all temperatures (Table 2), and with the results of modifying temperature model. Unfortunately many studies report only activation energies, while the effective change in decay rate due to temperature depends both on the pre-exponential factor and on the activation energy. Therefore, when studying differences in substrate susceptibility of decay rate to temperature, all estimates of the parameters in the Arrhenius functions should be reported, not just the activation energy. Furthermore, multiple linear regression or other adequate statistical methods should be used to test whether differences between substrates can be well described by general differences in susceptibility to microbial degradation (pre-exponential factor), or whether it is really necessary to advocate a different susceptibility to temperature changes (activation energy).

4.5. *Priming: increased SOC decomposition induced by litter*

The enhanced release of CO_2 from soils induced by litter could originate from other sources than SOC, namely from pool substitution in carbonates or in the microbial biomass (Kuzyakov et al., 2000). Carbonate-C in soil can be exchanged by CO_2 from ^{13}C labelled clover leaves. The amount of carbonate-C present in the soil was only 1.7% and 4% of total C for the clay soil and the sandy soil. The amount of primed CO_2 was about 16% relative to carbonate-C at 15 °C. The PE effect was immediate and increased with temperature at the beginning of the experiment, while the solubility of CO_2 in the soil solution is reduced by temperature increase, and this reduces the possibility of a reaction with carbonates. In addition, the pH of the soils was lower than 6.8, and therefore we consider the effect of solubility of CO_2 in the soil solution, and pool substitution, to be negligible (Blagodatskaya and Kuzyakov, 2008).

The PE correlated with litter decomposition, but the decay curves for PE flattens earlier than the litter decay curves (Fig. 1). This indicates that the substrate exposed to priming was depleted earlier than the initial clover decomposition. Blagodatskaya and Kuzyakov (2008) distinguish between enhanced degradation of SOM by microbial activity after addition of an easily degradable compound (real PE) and a temporary increase in CO_2 emission derived from enhanced microbial turnover (apparent PE). We estimated the amount of soil microbial C that could contribute to pool exchange, assuming that the microbial C accounted for 2% of the total SOC (Kutsch et al., 2009), and that the volume reached by diffusion of exo-enzymes from microbial activity on the surface of plant litter was 4.3% and 5% of the bulk volume, for the clay and the sandy soil respectively (calculated based on a specific surface area of the clover litter of 100 cm^2 g^{-1}, a depth of diffusion of the enzyme of 2×3 mm = 6 mm (Poll et al., 2006; and ignoring the effect of clay content on diffusivity), and disregarding any physical occlusion). Based on these assumptions, the cumulative priming was more than twice (clay soil) and four times (sandy soil) the microbial C in reach of the exo-enzymes, and it accounted for 13–31% (clay and sandy soil, respectively) of the microbial biomass in the whole soil. Thus, it is unlikely that an exchange of microbial C alone accounted for the whole increase in soil respiration caused by addition of plant litter that. It seems reasonable to assume that the increased bacterial activity caused by the addition of clover also acted on SOC, depending on the affinity between enzymes and substrates.

5. Conclusions

We studied the decay rates in a clay soil and a sandy soil in their natural temperature range from 15 °C down to 0 °C. There was a noteworthy decomposition of plant material and SOC, also at 0 °C.

In presence of plant material, we found a positive priming effect that correlated with decomposition of plant litter, but responded to temperature less than litter decomposition did. In the lower temperature treatments (0 and 4 °C), the PE more than doubled the total mineralisation of SOC. Thus, the effect of plant litter on soil priming should be considered in C and nitrogen mineralisation models as it can have an important effect on the amount and on the C/N stoichiometry of the decomposition products. A tentative modelling approach could be to relate it to the rate of litter decay, possibly in combination with a constrained volume of influence around the litter included in the soil.

In the absence of added plant material, SOC decomposition followed a first order reaction which was twice as fast in the sandy soil as in the clay soil. The decomposition rate of clover leaves was also higher in the sandy soil than in the clay soil. However, the influence of temperature on SOC and on clover decomposition was the same in both soils. We provide parameterised Arrhenius and modifying linear temperature functions that together with decay rates at reference temperature can be used for predicting decay rates of SOC *per se* and of the labile pool of clover leaves, in a temperature range which is most common in cultivated Norwegian soils (0–15 °C, Table 3).

The litter decomposition was lesser reduced by lower temperature than SOC decomposition. The temperature sensitivity of the decomposition was in the order: PE $<$ plant litter $<$ sandy soil SOC $=$ clay soil SOC.

Many mechanisms have been proposed for the effect of soil type on SOC decomposition. We recommend considering also the influence on soil texture and mineral contents on the diffusion of exoenzymes, as diffusion could be a mechanism behind the observed effect of soil type on litter mineralisation, and it also conforms to a common temperature response irrespective of soil type.

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Appendix I. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.10.004.

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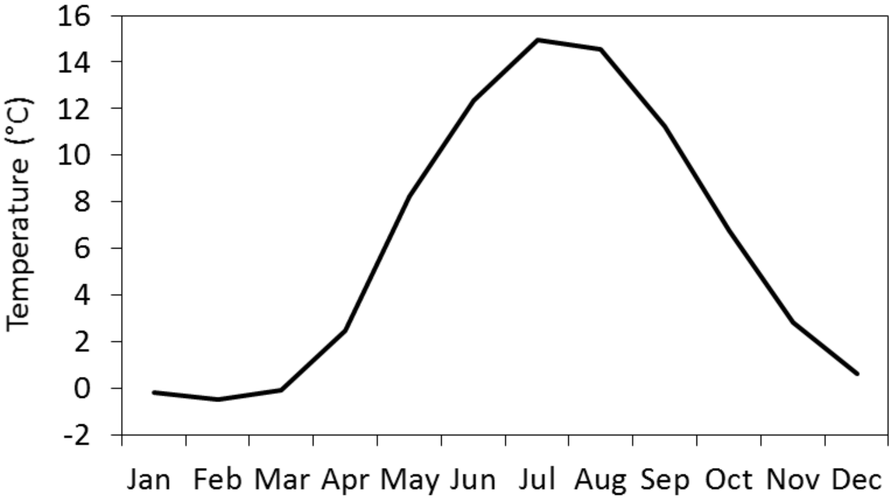
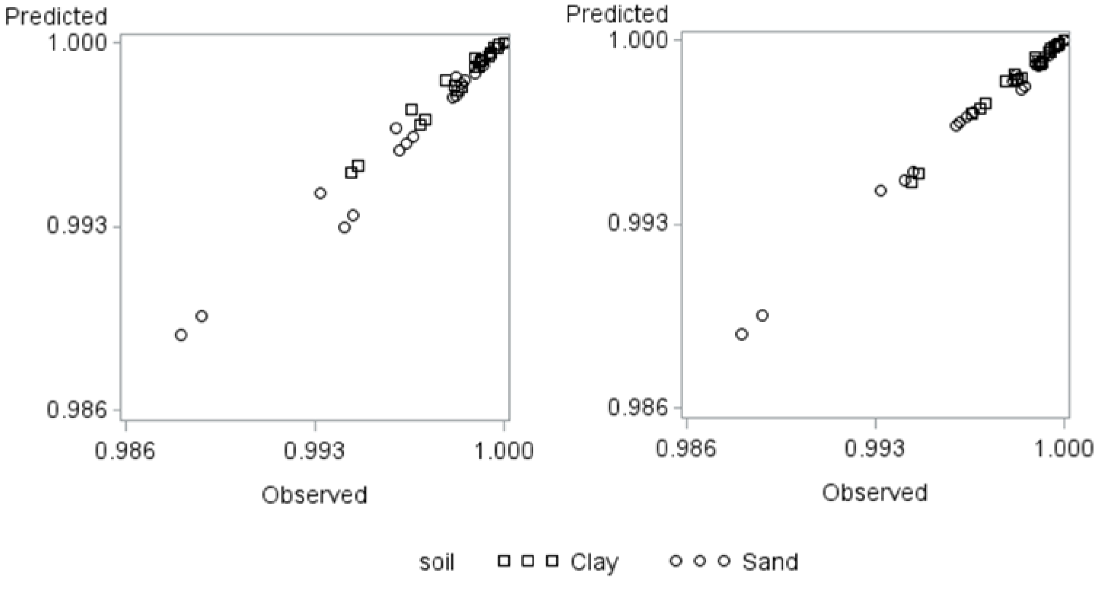
Supplementary data related to Paper II



Appendix A. Average monthly soil temperature at 10 cm depth for the years 2006-2013 at Kvithamar meteorological station.



Appendix B. Predicted vs. observed values for SOC decay at incubation day 15 to 142 by prediction with a) the modifying temperature function (Eq. (3)) and b) Arrhenius I function (Table 3).

Paper III

Frøseth R B, Thorup-Kristensen K, Hansen S, Bleken M A. **Mineralization of clover leaves at low temperatures in contrasting soil types.** Manuscript.

Mineralization of clover leaves at low temperatures in contrasting soil types

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Abstract

Aims To provide empirical data to improve model prediction of N mineralization of N-rich plant material at low temperatures in different soil types.

Methods A silty clay loam and a sandy loam were incubated with or without clover leaves for 80 days at 0, 4, 8.5 or 15 °C. The EU-Rotate_N model was tested and improvements were attempted by raising the decomposition rate of the fast decomposing litter pool at low temperature.

Results A substantial N mineralization already on 3rd day after clover incorporation, unaffected by temperature, was followed by net N immobilization for about 4 weeks in clay soil, with similar tendencies in sandy soil, and more severely at the higher than the lower temperatures. During this phase, the ratio of net mineralized N to C was higher at lower than at higher temperatures and higher in the sandy soil than in the clay soil. Thereafter, mineralization increased, positively related to temperature, and the N/C ratio became constant. The model could not mimic the initial N mineralization after clover incorporation or the following immobilization period.

Conclusions More knowledge of how temperature affects microbial growth is needed for improving prediction of net N mineralization of newly incorporated N-rich plant material.

Keywords: litter decomposition, green manure, cold climate, soil type, N dynamics, nitrogen mineralization

Introduction

In the absence of animal manure in stockless organic farming, green manure is grown to improve soil fertility, and thereby enhance the yield of subsequent cash crops. A wide range of legumes and non-legumes are used as green manure crops, for their nutritional value or other benefits (Cherr et al. 2006). In cold temperate regions, clover is commonly used as green manure, often in mixture with grasses, cultivated for a whole growing season, during which it is cut several times. The chopped herbage is left on-site as mulch. Then, the green manure ley is usually incorporated in the soil by ploughing in late autumn or in the following spring. The decomposing nitrogen (N)-rich plant material is thus exposed to low temperatures during its initial turnover, before the establishment of the following crop. Despite the low temperatures, field studies have shown rapid N mineralization from such plant material (Breland 1994; Thorup-Kristensen and Dresbøll 2010).
In spring cereals, the presence of a sufficient amount of plant available N early in the growing season is a key factor for grain yield. However, the inorganic N present in the soil in the late autumn, winter and spring is, especially when the soil it not frozen, at risk of being lost through nitrate leaching, gaseous losses or runoff (Chantigny et al. 2002). The understanding of N mineralization during the cold season is therefore instrumental for predicting the fate and value of N mineralizing from newly incorporated plant material, and thus for planning better crop management with higher N use efficiency and reduced risks of N losses to the environment.

The decomposition of organic matter and the mineralization of its N content depend on the substrate and the environmental conditions, including the soil organisms (Swift et al. 1979). In general, decomposition is found to be more affected by temperature in the lower temperature range (<10 ºC) than at higher temperatures (Kirschbaum 1995; Kätterer et al. 1998). According to kinetic theory, the decomposition of substrate with high molecular complexity, i.e. soil organic matter, is more sensitive to temperature than is substrate with low molecular complexity, i.e. fresh plant material (Davidson and Janssens 2006). Several studies confirm this, but contradictory results are also found (Giardina and Ryan 2000; Fang et al. 2005). The variability of the response to temperature of different substrates is large, but differences in the definition of the temperature effect, the experimental setting and the method of analysis may also affect the conclusions (Conen et al. 2008; Frøseth and Bleken 2015).

Different soil types provide contrasting environments for decomposition of organic matter. Mineral particles and soil aggregates act as chemical and physical protection of organic substrates and microorganisms, and clay soils show slower decomposition rates of both soil organic matter and added readily decomposable substrate than do sandy or silty soils (Van Veen et al. 1985; Saggar et al. 1996; Frøseth and Bleken 2015). There is little knowledge about the effect of soil type on the N dynamics of decomposing N-rich plant residue, but it might be expected that N mineralization, similar to carbon (C) mineralization, is affected by soil type. In a field trial, however, Müller (1988) found that soil type varying from sandy loam to clay only slightly affected the release of clover N from mesh bags during Finnish winter conditions.

A broad group of microorganisms take part in the decomposition and ammonification (mineralization) processes, while a much smaller group of microorganisms oxidizes the ammonium to nitrite and to further to nitrate (nitrification). Nitrification more inhibited by low temperature than is ammonification (e.g. Schütt et al.

2014). N mineralization is closely linked to C mineralization, but at low temperature, the ratio of these processes has been found to be altered; C mineralization is depressed more than net N mineralization (Kirschbaum 1995; Magid et al. 2001; Schütt et al 2014). The suggested reason is that gross microbial growth (immobilization) is restricted more by low temperature than is gross N mineralization (Andersen and Jensen 2001; Magid et al. 2001).

Dynamic soil-crop models are widely used tools in predicting N pre-crop effects and related environmental impacts of different agronomic measures. A single modifying temperature function for the decay rate is often applied to all organic substrates in a soil, whether they are fresh plant material or native soil organic matter. This is due to the lack of sufficient empirical evidence for differentiating temperature functions which can be transposed to field conditions (Coleman and Jenkinson 1999; Hansen 2002; Jansson and Karlberg 2004). Such models are therefore not able to simulate rapid N mineralization in cold soil from easily decomposable plant material, and data suitable for improving the temperature response functions are lacking (Müller 2006).

With the aim of acquiring empirical data about the response of decomposition and mineralization to low temperature, to be used for improving the simulation of this process, we investigated decomposition of clover leaves at low temperatures down to 0 °C. The effects of soil type and different temperatures on the net N mineralization and the relationship between N and C mineralization from decomposing soil organic matter and N-rich plant material were studied. Our hypotheses were:

- Rapid N mineralization from N-rich plant material occurs even at 0 °C.
- The ratio of net mineralized N to mineralized C from N-rich plant material is larger at lower than at higher temperature.
- The ratio of net mineralized N to mineralized C from N-rich plant material is not affected by soil type

A further objective was to investigate whether it was possible to improve the ability of a soil-crop model to simulate the N mineralization of freshly added plant material at low temperatures, by altering the turnover rates of the rapidly decomposable organic matter pools in order to make them less inhibited by low temperature.

Materials and methods

Incubation experiment

Experimental design

The incubation experiment for measuring C and N mineralization ran for 80 days with two soil types with or without clover leaves, in chambers maintained at constant temperatures of 0, 4, 8.5 or 15 °C. The soils were taken from two arable fields, about 4 km apart, of the Bioforsk research center Kvithamar in Central Norway (63°29’N, 10°52’E). At this site, the normal (1961–1990) annual precipitation is 896 mm and the mean monthly soil temperature at 10 cm depth through the year (2006–2013) varies from –1 °C to 15 °C. The soils were a silty clay loam classified as Mollic Gleysol and a sandy loam classified as Arenic Fluvisol (IUSS Working Group WRB, 2006). Further soil characteristics are available in Table 1.

Bulk samples of the soils were collected in spring 2011 from 0–20 cm depth, sieved through a 2 mm mesh while moist and pre-incubated in the dark for 4 ½ months at about 15 °C under aerobic and moist conditions. Two and a half weeks prior to incubation the soil was further moistened to 75% of pore volume at field bulk density, which was about the highest moisture content that allowed handling and compaction of the soil without destroying of the small aggregates. Before starting the incubation, the soil was kept at the final temperature for three days, then the soil was filled in the incubation chambers, red clover leaves (*Trifolium pratense* L.), corresponding to 4 g dry matter kg^{-1} dry soil, were gently mixed in half part of the chambers and the soil was compacted to nearly the bulk density observed in the field (Table 1). The clover leaves consisted of 46.3% C and 4.8% N (C/N = 9.8). The amount corresponded to about 43–58 g N m^{-2} and 900–1200 g dry matter m^{-2} to 20 cm depth, which is about the expected total above-ground dry matter production of a green manure ley during a growing season (Frøseth et al. 2014). Prior to mixing, the plant material was dried at 60 °C, cut into pieces and sieved through a 2 mm mesh. The leaves were enriched in ^{13}C. Details about this enrichment are given by Frøseth and Bleken (2015).

There were two sets of incubation chambers. One set for the analysis of mineralized N (NO_3–N and NH_4–N), consisted of 50 g soil (dry weight) samples in 200 ml plastic cups, with lids open to allow some aeration.

A second set, for the analysis of CO_2 release, consisted of 400 g soil (dry weight) samples kept in 1 l DURAN® glass bottles with 2M NaOH lye traps for collecting CO_2 (see Frøseth and Bleken 2015).

The chambers were equally distributed between four incubators regulated at 0 °C (Refritherm 200, Denmark), 4 °C (Termaks KB 8182, Norway), 8.5 °C (Termaks KBP 6151, Norway) and 15 °C (Termaks KBP 6151, Norway). Temperature data loggers monitored the incubator temperature. The standard error for the incubator temperatures was ±0.2–0.3 °C. Within each temperature, soil samples with and without plant material, were replicated three times for gas analyses and four times for mineral N analyses.

Sampling and analysis

Sampling was performed after 24 hours (day 0), and on days 3, 8, 15, 30, 52 and 80 after incubation started.

The chambers for inorganic N analysis were sampled destructively. The whole sample was extracted with 130 ml 2M KCl, and analysed by spectrophotometry on a FIAstar^{TM} 5000 Autoanalyser (Foss Tecator AB, Höganäs, Sweden, Application Notes 5232 and 5226 (2001) for NO_3–N and NH_4–N, respectively). Four samples of clover leaves without soil were also extracted and analysed by the same method to find the amount of inorganic N released from the leaves.

The lye in the CO_2 trap was collected in 20 ml pre-evacuated glass vials, from which 1 ml was transferred to a 10 ml glass vial prefilled with 1.5 ml 2M H_2SO_4. This was used for determination of CO_2 on a gas chromatograph (GC) (Model 7890A, Agilent, Santa Clara, CA, US). The fractional abundance of ^{13}C was analysed with a gas chromatograph isotope ratio mass spectrometer (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany). In addition, at adequate intervals, the atmosphere of the gas tight glass chambers was sampled (5 ml injected into pre-evacuated 10 ml glass vials) and immediately analysed on the GC to monitor its O_2 content. Oxygen was added when necessary to increase the level to around 20% volume. The oxygen content was usually above 12%, with a few exceptions at the beginning of the trial.

The amount of total C and N in the soil and clover leaves was analysed using the Dumas combustion method (Bremmer and Mulvaney 1982) on a Leco CHN 1000 analyzer (LECO Corp., St. Joseph, MI, USA).

The results were expressed on a dry weight basis, and for comparison with model simulations, also converted to area units using appropriate bulk density values for 0–20 cm depth (Table 1). The net mineralized N was expressed as the change in inorganic N since the start of the experiment, i.e. the value at day 0 was subtracted. The net mineralized N from the clover leaves was calculated by the difference method; subtracting inorganic N in soil without clover leaves from inorganic N in soil with clover leaves added. The mineralized C was calculated based on the measured fractional abundance of ^{13}C in the soils, the ^{13}C-labelled clover leaves and in the CO_2 evolved in the chambers. More details about the calculations for estimating plant-derived and soil-derived CO_2 are given by Frøseth and Bleken (2015). For best visualisation of the relationship between N and C mineralization, we present the N/C ratio, rather than the C/N ratio.

Statistical analysis

Analysis of variance (ANOVA) was performed using a general linear model (GLM) for soil inorganic N data and CO_2–C evolved as input data for each soil type and in total. Analyses were performed for all sampling occasions for each temperature and soil type using recordings for single chambers as input data. For multiple comparisons tests, Tukey HSD procedure was used. A regression analysis was performed of net mineralized N vs. mineralized C on the two last sampling days, to check whether there was an effect of soil type on the N/C ratio of the mineralized products. The statistical software packages R (R Core Team 2012) and SAS (SAS 9.3, SAS Institute Inc., Cary, NC, USA) were used for these calculations. In all tests, significance was assumed at P-levels ≤ 0.05.

Simulations with the EU-Rotate_N model

The field-scale soil-crop model EU-Rotate_N model (version 1.8, http://www2.warwick.ac.uk/fac/sci/lifesci/wcc/research/nutrition/eurotaten/) was used in the simulations. The model was developed as a tool for assessing the effects of different fertilizers and crop rotation practices on N losses to the environment, across Europe (Rahn et al. 2010). The mineralization module is based on routines used in the Daisy model (Hansen 2002). The soil organic matter is divided into three main pools; dead native soil organic matter (SOM), microbial biomass (SMB) and added organic matter (AOM). Each pool is divided into two sub pools: one with slow turnover and one with faster turnover. The decay rate for each pool at the actual situation is calculated by multiplying a reference decay rate by factors for abiotic conditions; soil temperature, soil water content and clay content (for SMB). The same temperature function is applied to all organic matter pools.

We adapted the model to allow variable temperature functions for the different organic matter pools, where the decline in turnover rates at low temperatures can be reduced by enhancing a parameter value for temperature response (Tr) given in the model runfile. The Tr parameter was used to calculate a temperature response factor (Rf) at four specific temperatures:
–2 °C: Rf = 0
0 °C: Rf = 0.1*(Tr–1)
8 °C: Rf = 0.5*Tr

20 °C: Rf = 2.0

Between these temperatures, Rf was calculated by linear interpolation. The value of the Tr parameter could vary between 1 and 4. At a value of 1.5 for the AOM fast pool and 1 for the other pools, the temperature response was very close to the original temperature response in the EU-Rotate_N model, whereas at a value of 4 for all pools, the Rf reached its maximum already at 8 °C, and throughout most of the temperature range from 0 to 8 °C it was 4 to 7 times higher than the original temperature response.

A crop sequence resembling the actual field history was simulated for the two years preceding the experiment, to initiate organic pools (SOM, SMB and AOM) in the soil. Then the incubation conditions were imitated with constant temperature and clover leaves added in the amount and quality per kg dry soil as used in the incubation study. Because of different bulk densities, these amounts corresponded to 11300 and 15300 kg dry matter per ha for the silty clay loam and the sandy loam, respectively.

We tested the Tr values only for the AOM fast pool, affecting the early turnover of easily decomposable fractions of the clover leaves. The EU-Rotate_N model was first run as in its original version with Tr=1.5 for the AOM fast pool, and then compared with output from simulations made with values of Tr up to 4. We evaluated the N mineralization performance of the different combinations values of Tr for the faster organic matter pools by using the temperature response pattern relative to 15 °C. Model performance criteria based on absolute values was not useful since the simulated inorganic N level was far higher than that which was measured.

Results

Inorganic N

Soil organic matter

During 80 days of incubation at 15 °C, the level of inorganic N in the soils without clover leaves increased with 7.4 mg kg^{-1} in the silty clay loam and 7.5 mg kg^{-1}in the sandy loam (Fig. 1). It was significantly reduced by lower temperatures, and at 0 °C there was a net decrease in inorganic N, suggesting net immobilization. The decrease was larger in the sandy loam (4.0 mg kg^{-1}) than in the silty clay loam (1.1 mg kg^{-1}). Nitrate amounted to 89–100 % of the inorganic N throughout the incubation, with highest levels in the silty clay loam. The ammonium content decreased or did not change during the incubation (Fig. 2). In general, the lowest temperature treatments had the highest ammonium levels in the soil during the incubation period, but there was no significant difference between the treatments at the end of the incubation.

The effect of clover leaves

Incorporation of clover leaves led to a rapid and non-temperature dependent increase in the content of inorganic N in both soils during the first three days of incubation (Fig. 1). Then, in the silty clay loam, a significant net immobilization occurred when incubated at the two highest temperatures. In this period, the highest inorganic N content was found in soil incubated at the two lowest temperatures. In the sandy loam, there were similar tendencies, though the net immobilization was not significant; the inorganic N content was similar (day 14) or higher (day 30) when incubated at 4 °C compared to 8.5 °C or 15 °C. From day 52 onwards, the inorganic N content in both soils was highest in the highest temperature treatments. This situation lasted until the end of the incubation, when the inorganic N content at 0 °C compared to 15 °C was 40% and 28% lower in the silty clay loam and the sandy loam, respectively. However, at the end of the incubation, the relative effect of temperature on net N mineralization of clover leaves was weaker than that of the soil organic matter alone (Table 2).

After 80 days of incubation, clover leaves increased the inorganic N content by 25–42 mg kg^{-1} in the silty clay loam and 30–42 mg kg^{-1} in the sandy loam. This corresponded to 13–22% of the clover N in the silty clay loam and 16–22% in the sandy loam (Table 2). Almost half of the increase in the inorganic N content caused by decomposition of clover leaves was achieved within the first week of incubation. This substantial increase in inorganic N was almost exclusively due to mineralization of organic N from the clover leaves, as the measurement of nitrate-N and ammonium-N released from clover leaves not mixed with soil amounted to only 2% of the inorganic N observed at day 3, or 0.1–0.2% of the clover N (data not shown). After the rapid increase in inorganic N within the first days of incubation, the amount of ammonium decreased and the nitrate level increased. The decrease in ammonium was faster with high than with low temperature, and faster in the silty clay loam than in the sandy loam (Fig. 2). At the end of the incubation, the enhanced ammonium level caused by incorporation of clover leaves had in most cases disappeared, and there was no significant difference between the temperature treatments in the soil ammonium content. An exception was found at 0 °C in the sandy loam, where the ammonium content was still substantially higher than at the higher temperatures.

C mineralization

The C mineralization from the soil C after 80 days of incubation was twice as high in the sandy loam as in the silty clay loam (Table 2). In both soils, the C mineralization at 0 °C was 82% lower than at 15 °C. The C mineralization of the clover leaves was also affected of temperature, but the effect was much smaller than for soil C (Fig. 3). Depending on temperature, 19–29% and 24–36% of clover C was mineralized after 80 days of incubation in the sandy loam and the silty clay loam, respectively (Table 2). More details about C mineralization in this experiment are given in Frøseth and Bleken (2015). The data presented is for comparison with the net N mineralization. The plot of net mineralized N vs. mineralized C shows a positive relationship, except for the period when net N immobilization occurred, when the correlation was negative (see black symbols in Fig. 4).

N/C ratio

In the first weeks of incubation of clover leaves, there was a markedly higher ratio of net mineralized N to mineralized C (Fig. 5). This high N relative to C mineralization was stronger at 4 °C and especially at 0 °C than at higher temperatures, and stronger in the sandy loam than in the silty clay loam. During the incubation the N/C ratios of the N and C mineralized from the clover leaves incubated at different temperatures converged to the same ratio (0.075 for the silty clay loam and 0.063 for the sandy loam), at though the difference between the soil types was not significant (Fig. 4c and d). When applying the difference method, instead of the ^{13}C-labelling method, on calculating the mineralized C from the clover leaves, the N/C-ratio became lower, 0.054 for the clay and 0.052 for the sandy loam (Appendix A). In soil without clover leaves, the ratio of mineralized N to mineralized C fluctuated with no systematic effect of temperature in the first part of the incubation (data not shown). However, in soil with clover added, the effect of clover leaves dominated the N/C ratio, which converged to 0.057 for the silty clay loam and 0.052 for the sandy loam (Fig. 5a and b).

Modelling

It was not possible to make good simulations of the N dynamics measured during the first 30 days simply increasing the decomposition rate at low temperature for the fast decomposing pool of added organic matter (AOM2) in the EU-Rotate_N model. We observed a very fast mineralization during the first three days, then slow mineralization or even immobilization, followed by faster mineralization again. In the end, net mineralization of the N added with clover was only 13–22 %. Even though the model included a total of six organic matter pools, including two pools of AOM, with different C/N ratios and different decomposition rates, the model was not able to simulate a temporal pattern of N mineralization resembling that which was measured. The simulated N mineralized from clover N was also twice as high as that measured.

While it was not possible to make simulations which fitted the data from the first 30 days of the incubation experiment, we were able to make better simulations for the two latest sampling days (results for day 80 are shown in Fig. 6). Thus for these two dates, we compared the measured temperature responses of N mineralization to the simulated results using the original EU-Rotate_N temperature response and the modified temperature responses, which allowed higher turnover rates at low temperatures for AOM2. We found the best fit for the added clover material when we used the original temperature function for all organic matter pools in the model, except for the AOM2 pool (Fig. 6). For this pool, we found that using a Tr value of 3.5, allowing much faster decomposition of the organic matter at low temperatures, gave the best fit. This will increase the turnover rate of AOM2 by factors of 5, 2.5, 2.3 and 1.3 at 0, 4, 8 and 15 °C respectively, compared to the original EU_Rotate-N temperature response.

Discussion

The net N mineralization from the two soil types in the absence of clover leaves was similar after 80 days of incubation when measured per kg soil, but when measured per g soil N it was 3.6 times higher in the sandy loam than in the silty clay loam. The observed significant temperature effect on net N mineralization in unamended soil is in line with several other studies (Cookson et al. 2002; Clark et al. 2009). The pattern in net N mineralization at the beginning of the trial may have been an effect of the initial soil disturbance. However, when studying the C release, we found the effect of soil disturbance did not confound the results (Frøseth and Bleken 2015).

In line with our hypotheses, we found a rapid initial release of ammonium-N on the 3rd day after incorporation of the clover leaves, even at 0 °C. This corresponded to about 7% of the added N, and was independent of temperature in the tested range (0 to 15 °C). The rapid increase in net mineralized N due to the addition of clover leaves could also partly originate from an increased turnover of the soil organic matter (a real priming effect) or an increased turnover of the soil microbial biomass (an apparent priming effect) (Blagodatskaya and Kuzyakow 2008; Kuzyakow et al. 2010). Labelling of the clover C in the present experiment showed a substantial priming effect (Frøseth and Bleken 2015), but in lack of a labelled N pool we could not quantify a priming effect on soil N mineralization. Since the C/N ratio of the bulk soils and of clover leaves was almost similar, close to 10, the stoichiometry of the mineralized C and N would not change much if all organic components were decomposed at a similar rate. If we estimate primed N based on the amount of primed C on the 3rd incubation day and assume a C/N ratio of 10, the amount of primed N constituted 4–6% of the mineralized N of the clover leaves on this day. However, the observed priming effect on soil C at the beginning of the incubation period was positively correlated with temperature, particularly in the sandy loam (on the 3rd day at 8.5 °C priming was 50% and 160% more than at 0 °C, in the clay and sandy loam respectively), while the early N mineralization was not affected by temperature. Thus priming effect alone does not provide a plausible explanation for the early N mineralization, and the rapid increase in mineral N seems to have been mainly caused by a substantial mineralization of the clover leaves.

After the initial burst of mineralization, there was a shift to net immobilization in some of the treatments. This was most pronounced in the silty clay loam, and the immobilization increased with higher temperatures. The observed shift between net mineralization and net immobilization is in line with several other studies, and it seems to take place between 3–5 °C and 8.5–11 °C (Andersen and Jensen 2001; Magid et al. 2004; He et al. 2014). In other studies, this pattern has also been found for plant material with higher C/N ratio than in our experiment. The results indicate that N mineralization of plant material at low temperatures can supply plants with N early in growing season, in consistence with findings of He et al. (2014), but also that a period with immobilization may occur in clay soils at somewhat higher temperatures. During the immobilization period, the net N mineralization was negatively correlated with the C mineralization. This may be explained by stronger enhancement with temperature of microbial biomass (with a low C/N ratio) than of net C decomposition, which is also supported by the N/C ratio of the mineralized products, as discussed later. The immobilization phase can be interpreted as strong microbial growth during turnover of the fast decaying pool of the clover leaves, which in this experiment was found to last until 13–18% of the clover C was decomposed, which occurred after 8–30 days (Frøseth and Bleken 2015). A net immobilization in the silty clay loam in late spring after ploughing the green manure ley may also partly explain the low barley yields on this soil compared to those on the sandy loam, which we found under field conditions (Frøseth et al. 2014). Cookson et al. (2002) found comparable mineralization pattern when using constant temperatures (2, 5, 10 and 15 °C). Fluctuating temperatures occur in the field, and these may have different effects on N transformations than those of constant temperatures (Campbell and Biederbeck 1972; Cookson et al. 2002). Cookson et al. (2002) found that immobilisation was the dominant process when the temperature decreased from 15 to 2 °C.

Clover leaves have a lower C/N ratio and contain more easily degradable compounds than the rest of the clover plant, such as stems and roots, which are commonly incorporated into the soil. Cookson et al. (2002) added clover material consisting of both shoots and roots and observed that the peak in ammonium-N, similar to that which we found after 3 days at all temperatures, in their experiment occurred later at lower temperatures (2 and 5 °C) than at higher temperatures (10 and 15 °C).

Our experiment was not designed to study the temperature sensitivity of nitrification. However, the results conform to results by e.g. Andersen and Jensen (2001) and Cookson et al. (2002), who found that nitrification is more sensitive to low temperature than is ammonification. Delayed nitrification at low temperatures can be positive for reducing the risk of N leaching, as ammonium is less prone to leaching.

Despite N-rich plant material, the total amount of N mineralized after 80 days of incubation at 15 °C was low (ca 22%) compared to that found in other studies. Cookson et al. (2002) found after 161 days of incubation at 15 °C that 60% of clover N had been mineralized, while 22% was mineralized when incubated at 2 °C. Van Schöll et al. (1997) found that 39% of rye shoot N was mineralized after 70 days of incubation at 15 °C. However, when we studied the soils under field conditions, the apparent N recovery from mulched green manure in spring barley was also low, i.e. around 7% (Frøseth et al. 2014). The low net N mineralization and N recovery may indicate a longer turnover time of organic matter by soil microorganisms, adapted to the cold climate.

In line with our hypothesis, we found that the ratio of net mineralized N to mineralized C from easily decomposable plant material was larger at lower than at higher temperature. An interpretation can be that the gross mineralization of plant material rich in easily degradable substances was affected less by low temperature than was gross immobilization, as found by Magid et al. (2001). Low temperature creates stronger separation of the decomposition of the substrate components over time than do higher temperatures (Magid et al. 2004).

We expected that the ratio of net mineralization of N to mineralization of C from the clover leaves would not be affected by soil type. Contrary to our hypothesis, we found that the N/C ratio during the first weeks of incubation was higher in the organic matter of the sandy loam than in that of the silty clay loam, and that it was especially higher at the lower temperatures. However, by the end of the incubation, the N/C ratios had converged to the same ratio in both soils and at all temperatures.

Inorganic N dynamics are the outcome of many different processes that take place during the decomposition of plant material in soil. Our results show that it was a correct assumption that the temperature response for the fast part of the AOM pool was less affected by low temperature than was the case for the other pools. Though we could not simulate the short term mineralization correctly, the simulation of temperature responses at the end of the incubation was improved, i.e. it showed more N to be mineralized from incorporated plant residue at low temperature than did simulations with the original temperature response.

In spite of the improvement achieved, it is also evident that the model is far from able to simulate the short-term dynamics of N mineralization from easily decomposable plant material under low temperatures. This can be important when trying to simulate the early N mineralization of crop residues incorporated in cold soil in autumn or spring, and can lead to erroneous estimates of their effect on N leaching risk as well as on early N supply to the succeeding crop. The original temperature response of the model was based on studies of the decay of bulk organic matter (Stanford et al. 1973; Campbell et al. 1981; Addiscott 1983; Nordmeyer and Richter 1985), and N mineralization is calculated as a function of the C turnover. Our results support several other studies, which show that the effect of low temperature on organic matter turnover and mineralization is not simply that the same processes proceed at a reduced rate (Kirschbaum 1995; Magid et al. 2001; Schütt et al

2014). Different temperature regimes may affect the community composition of soil biology in long term, but temperature may also affect which organisms are active in the early phases of the decomposition process (Schmidt and Lipson 2004). Furthermore, at temperatures close to 0 °C, the physiology of the microorganisms may change, for instance because they need to produce osmotica as frost protection, altering their C to N relations (Schimel et al. 2007). For modelling purposes, we suggest to make a temperature function for microbial growth, which is more reduced by low temperature than the decay rate of added plant residues.

Conclusions

We studied the effect of low temperature and soil type on mineralized N and C from decomposing soil organic matter and N-rich plant material in an incubation experiment. There was an initial rapid and substantial net N mineralization from the plant material, which was not reduced by low temperature. The ratio of inorganic N to mineralized C was higher at low temperatures than at high temperatures during early decomposition, showing that N mineralization is not simply a function of C mineralization. Later in the incubation, the C to N ratio converged to the same value for all temperature treatments.

It was not possible to simulate the processes that took place in the early phase of decomposition simply by changing the temperature function in the EU-Rotate_N model. It appears that more fundamental model changes are needed for simulating the effects of low temperatures on N mineralization from fresh plant material. We suggest altering the ratio between net N and C mineralization by including a temperature response function for microbial growth, as the latter is more inhibited by low temperature than is the decomposition of plant material.

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Figures

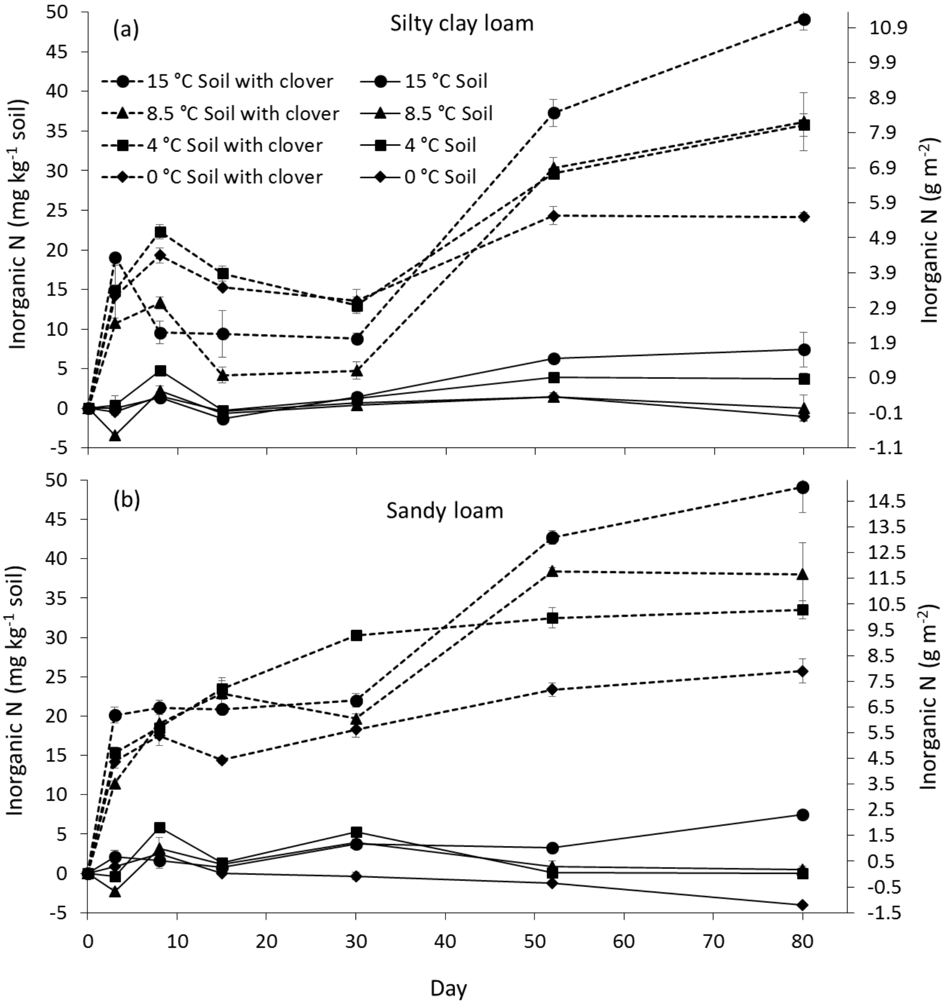


Fig 1 Net inorganic N (ammonium-N and nitrate-N) during 80 days of incubation at 0, 4, 8.5 and 15 °C in (a) a silty clay loam and (b) a sandy loam with clover leaves (dotted lines) and without (lines). Inorganic N at the start of the experiment is subtracted. Line bars indicate standard error for mg kg^{-1} soil. The right-hand vertical axis shows the N content in a 20 cm soil layer assuming the soil original bulk density in situ (Table 1).

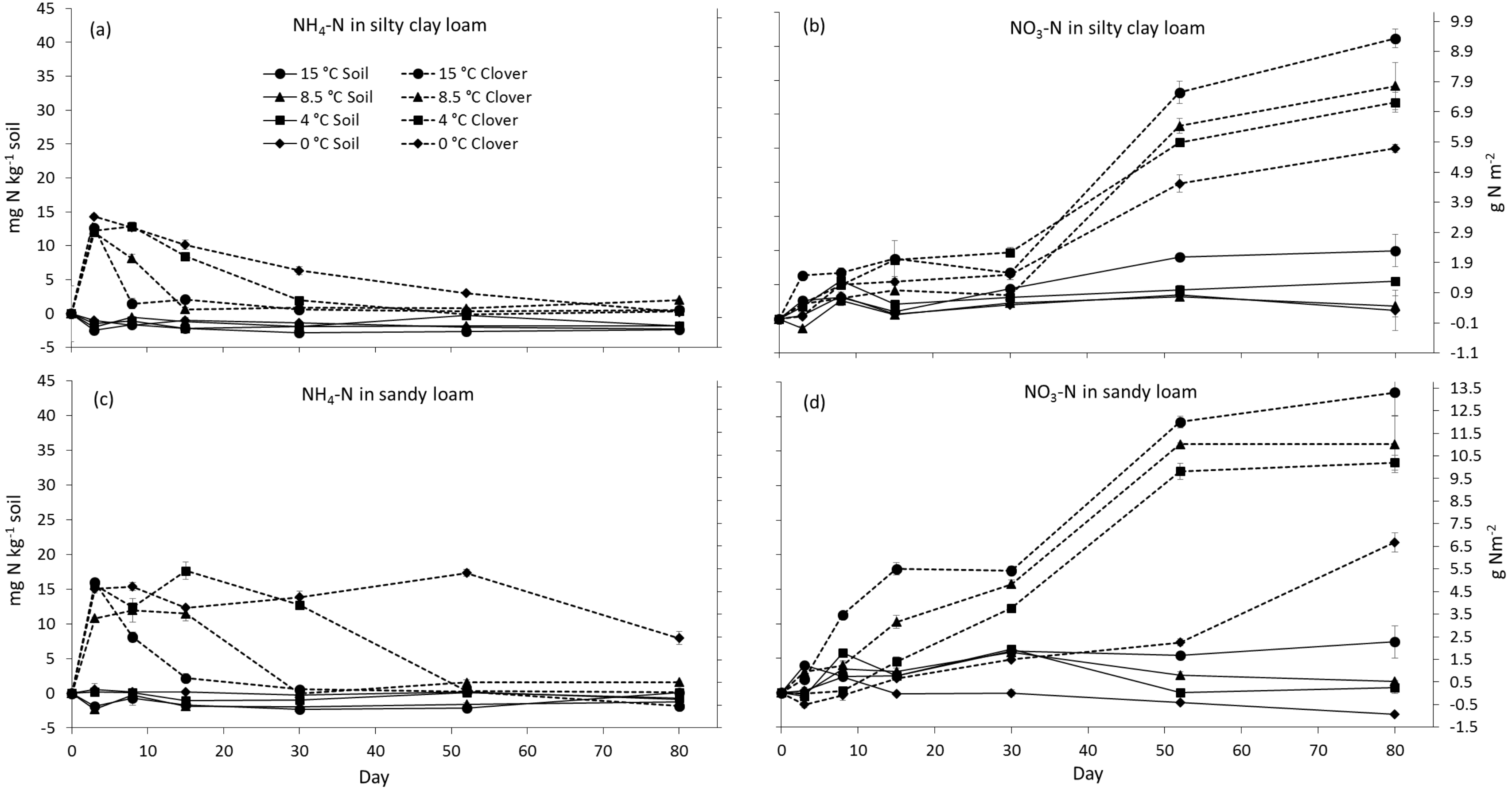


Fig 2 Net ammonium-N and nitrate-N measured during 80 days of incubation at 0, 4, 8.5 and 15 °C in a silty clay loam (a and b) and a sandy loam (c and d) without clover leaves (lines) and as the additional effect of clover leaves added (dotted lines). Inorganic N at the start of the experiment is subtracted. Line bars indicate standard error for mg kg^{-1} soil. The right-hand vertical axis as in Fig. 1.

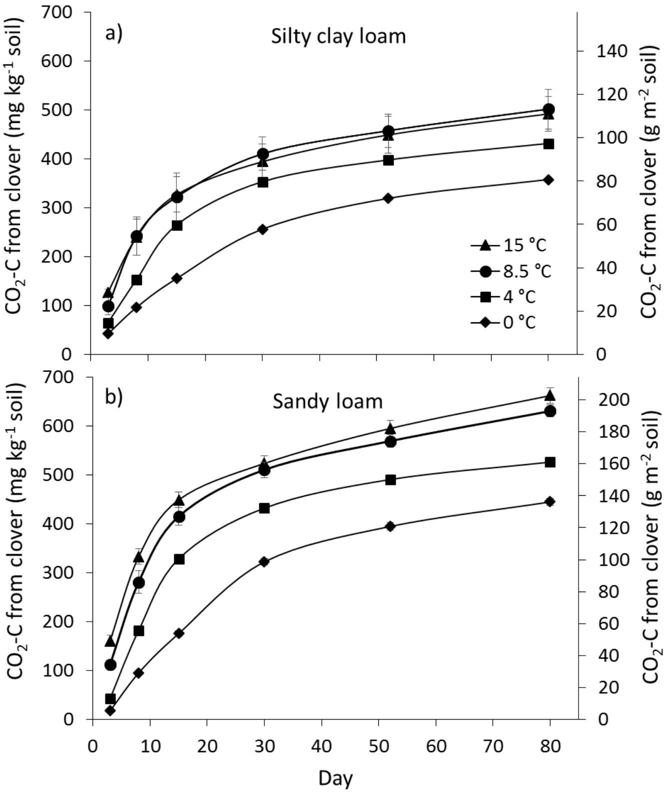


Fig 3 Mineralized C from clover leaves during 80 days of incubation in (a) a silty clay loam and (b) a sandy loam, measured as accumulated CO_2-C per kg dry soil and per m^2 for 0–20 cm depth. Line bars indicate standard error for mg kg^{-1} soil. The right-hand vertical axis as in Fig. 1.

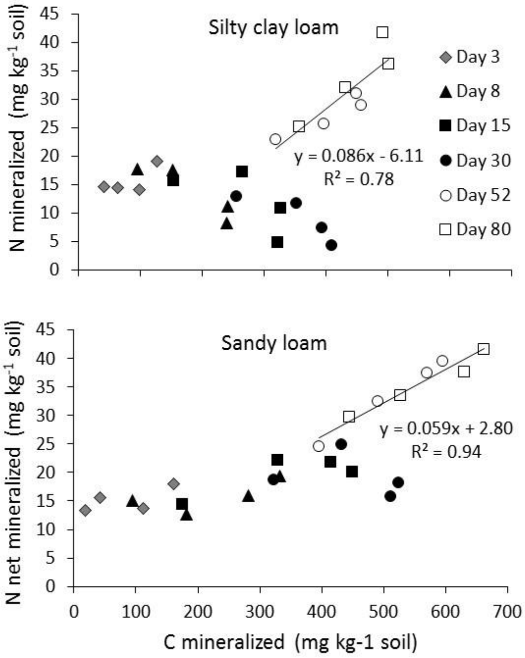


Fig 4 Net mineralized N (ammonium-N + nitrate-N) vs. mineralized C from clover leaves during 80 days of incubation in (a) silty clay loam and (b) sandy loam. The net mineralized N from the clover leaves was estimated by the difference method, while mineralized C was estimated by ^{13}C-labelled clover.

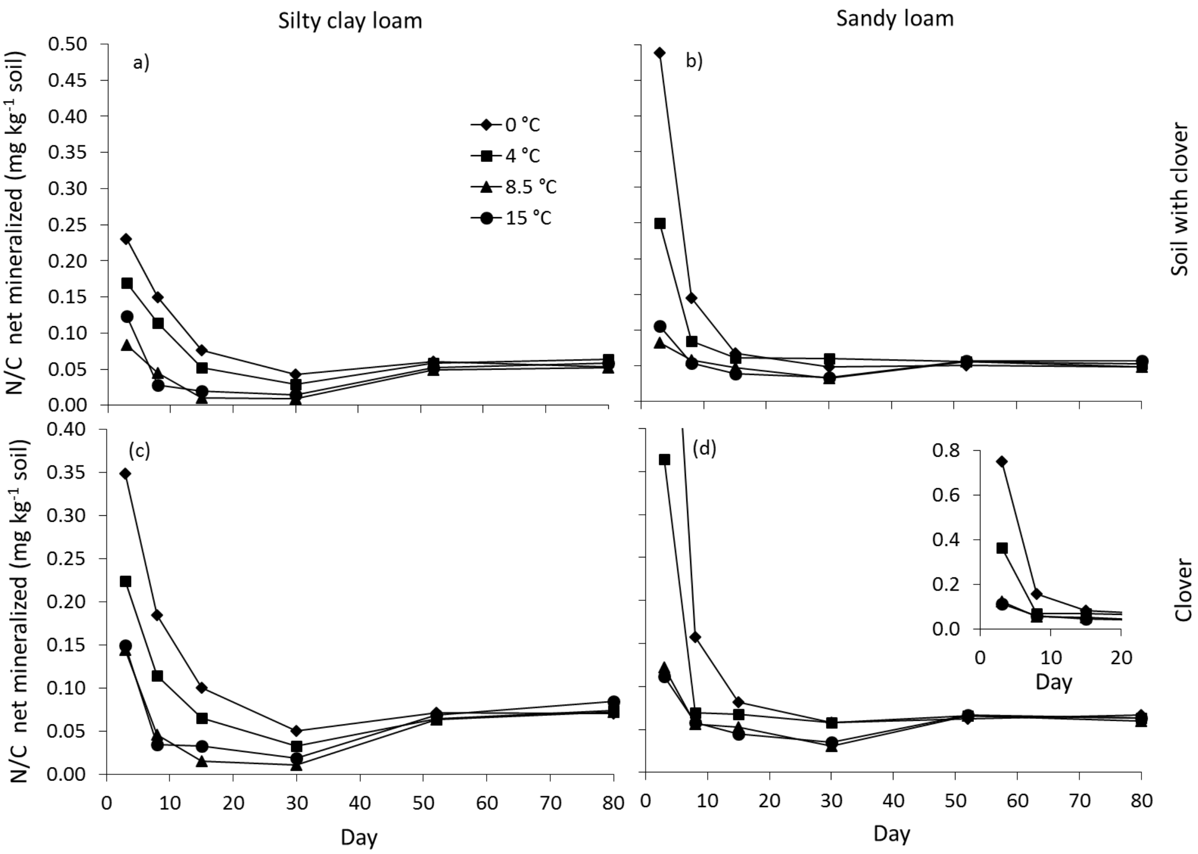


Fig 5 The ratio of net mineralized N (ammonium-N and nitrate-N) to mineralized C from soil and clover (a and b) and clover (c and d) in silty clay loam and sandy loam during 80 days of incubation at different temperatures. The net mineralized N from the clover leaves (c and d) was estimated by the difference method, while net mineralized C was estimated by ^{13}C-labelled clover.

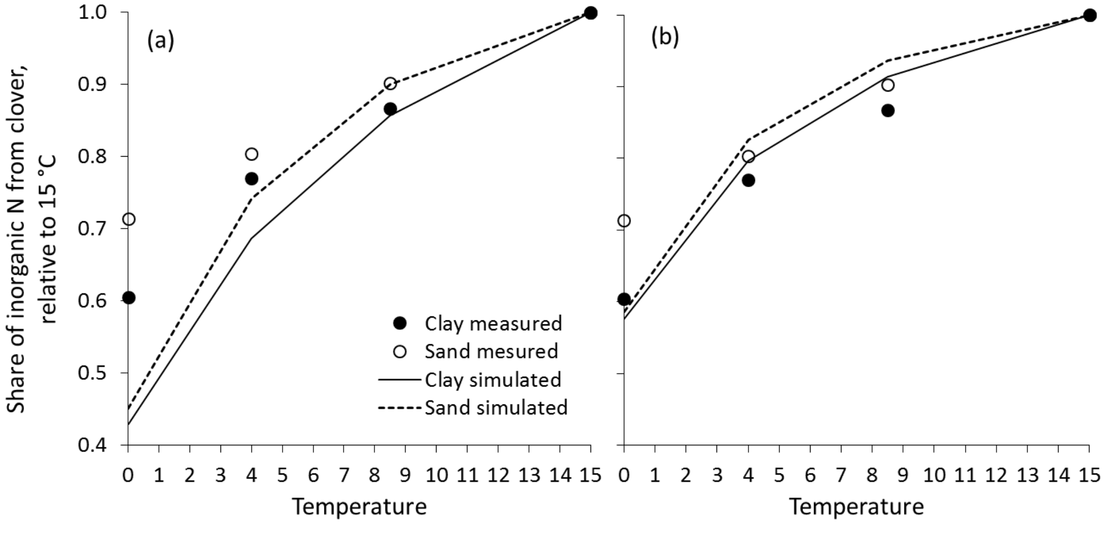


Fig 6 The temperature response for net N mineralization caused by clover leaves after 80 days of incubation, presented as measured and simulated by (a) the original EU-Rotate_N temperature response parameter for the AOM1 (Tr=1.5) and (b) the best fit after adjusting this parameter value (Tr=3.5).

Tables

Table 1 Soil particle size distribution, bulk density, available water (10-1500 kPa), wilting point (>1500 kPa), pH, total C and N in undisturbed soil, 0–20 cm.

Soil type	Sand	Silt	Clay	Bulk density	Available water	Wilting point	pH[a]	Total C[a]	C/N[a]
	% of fine earth			$g\ cm^{-3}$	vol%	vol%	_	%	_
Silty clay loam[b]	3	70	27	1.13	36	15	6.0	4.45	11.4
Sandy loam[c]	51	43	6	1.53	27	6	6.2	1.30	11.8

[a] analysed in new samples, [b] from profile no. 6 in Sveistrup et al. (1994), [c] from profile 20177 in Solbakken (1987).

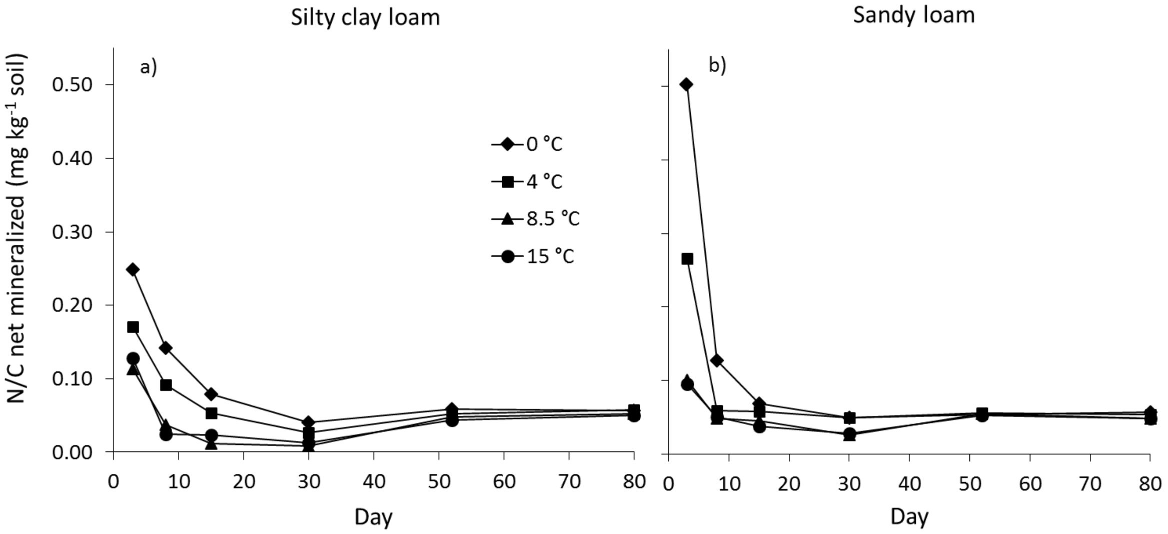
Table 2 Mineralized N (nitrate-N and ammonium-N) and C from clover leaves and soil organic matter as percent of its total N and C. Mean ± standard error are presented.

		N mineralized (% of initial clover N or soil N)				C mineralized (% of initial clover C or soil C)			
	Day	0 °C	4 °C	8.5 °C	15 °C	0 °C	4 °C	8.5 °C	15 °C
Clover leaves*									
Silty clay loam	3	7.7 ±4.1	7.6 ±0.7	7.5 ±0.6	10.0 ±0.4	2.3 ±0.0	3.5 ±0.1	5.3 ±0.2	6.9 ±0.3
	8	9.4 ±1.0	9.2 ±1.0	5.9 ±0.8	4.3 ±1.5	5.2 ±0.1	8.3 ±0.1	13.1 ±0.5	14.9 ±1.1
	15	8.2 ±0.4	9.1 ±1.0	2.5 ±1.1	5.7 ±2.7	8.4 ±0.1	14.3 ±0.0	17.4 ±0.6	19.6 ±0.7
	30	6.8 ±1.5	6.1 ±1.1	2.3 ±1.1	3.9 ±0.7	13.8 ±0.3	19.1 ±0.1	22.2 ±0.4	23.2 ±0.6
	52	12.0 ±1.2	13.5 ±0.6	15.2 ±1.2	16.3 ±1.8	17.2 ±0.2	21.5 ±0.2	24.7 ±0.4	26.2 ±0.6
	80	13.3 ±0.5	16.9 ±1.5	19.0 ±3.8	21.9 ±1.4	19.3 ±0.2	23.3 ±0.2	27.1 ±0.5	28.5 ±0.3
Sandy loam	3	7.0 ±0.9	8.3 ±0.7	7.3 ±0.3	9.5 ±1.0	1.0 ±0.1	2.3 ±0.3	6.1 ±0.1	8.7 ±0.6
	8	7.9 ±1.3	6.7 ±0.5	8.4 ±1.8	10.2 ±1.0	5.2 ±0.2	9.9 ±0.2	15.2 ±0.4	18.0 ±0.9
	15	7.6 ±0.4	11.6 ±1.5	11.4 ±1.4	10.6 ±0.5	9.5 ±0.3	17.4 ±0.2	22.5 ±0.3	24.3 ±0.9
	30	9.8 ±1.0	13.1 ±0.6	8.3 ±0.6	9.6 ±0.9	17.4 ±0.3	23.4 ±0.2	27.6 ±0.3	28.3 ±0.9
	52	13.0 ±0.9	17.0 ±1.4	19.8 ±0.5	20.8 ±0.9	21.4 ±0.4	26.5 ±0.1	30.8 ±0.2	32.2 ±0.9
	80	15.7 ±1.6	17.6 ±1.2	19.8 ±3.7	21.9 ±3.5	24.1 ±0.4	28.5 ±0.2	34.1 ±0.2	35.8 ±0.9
Soil**									
Silty clay loam	3	–0.012 ±0.022	0.010 ±0.062	–0.087 ±0.012	0.001 ±0.014	0.008 ±0.001	0.009 ±0.001	0.014 ±0.002	0.025±0.002
	8	0.039 ±0.025	0.122 ±0.015	0.056 ±0.035	0.034 ±0.026	0.014 ±0.002	0.024 ±0.001	0.035 ±0.002	0.060±0.006
	15	–0.008 ±0.023	–0.007 ±0.018	–0.016 ±0.026	–0.034 ±0.014	0.024 ±0.002	0.042 ±0.001	0.065 ±0.003	0.110±0.013
	30	0.018 ±0.029	0.033 ±0.015	0.011 ±0.023	0.036 ±0.028	0.041 ±0.002	0.069 ±0.003	0.115 ±0.004	0.208±0.027
	52	0.037 ±0.009	0.101 ±0.028	0.037 ±0.028	0.162 ±0.016	0.057 ±0.003	0.099 ±0.005	0.174 ±0.005	0.312±0.044
	80	–0.027 ±0.033	0.095 ±0.032	0.001 ±0.083	0.190 ±0.112	0.074 ±0.002	0.138 ±0.003	0.248 ±0.005	0.433±0.063
Sandy loam	3	0.080 ±0.197	–0.033 ±0.027	–0.212 ±0.086	0.195 ±0.136	0.023 ±0.002	0.023 ±0.002	0.036 ±0.000	0.055 ±0.000
	8	0.228 ±0.053	0.532 ±0.040	0.286 ±0.249	0.146 ±0.186	0.041 ±0.003	0.051 ±0.004	0.084 ±0.003	0.128 ±0.004
	15	0.004 ±0.036	0.124 ±0.070	0.105 ±0.078	0.070 ±0.051	0.059 ±0.003	0.086 ±0.002	0.139 ±0.001	0.226 ±0.002
	30	–0.030 ±0.088	0.483 ±0.052	0.358 ±0.137	0.340 ±0.105	0.089 ±0.004	0.137 ±0.002	0.215 ±0.010	0.402 ±0.002
	52	–0.115 ±0.061	0.010 ±0.068	0.082 ±0.122	0.293 ±0.034	0.115 ±0.003	0.195 ±0.002	0.315 ±0.009	0.628 ±0.004
	80	–0.365 ±0.037	0.004 ±0.131	0.040 ±0.087	0.679 ±0.067	0.152 ±0.003	0.277 ±0.006	0.476 ±0.010	0.904 ±0.005

*Clover leaves: For N: Soil with clover minus soil without clover; For C: Soil with clover minus soil without clover and minus priming effect.

** Soil without clover leaves added

Supplementary data



Appendix A The ratio of net mineralized N (ammonium-N and nitrate-N) to mineralized C from clover leaves in (a) silty clay loam and (b) sandy loam during 80 days of incubation at different temperatures, estimated by the difference method